

3rd INTERNATIONAL CONGRESS ON ENVIRONMENTAL HEALTH 2014

PORTO, 24th to 26th SEPTEMBER 2014

PROCEEDINGS BOOK

EMERGING RISKS AND CHALLENGES ON ENVIRONMENT, HEALTH AND SAFETY

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Vieira da Silva, Manuela; Oliveira, Rui; Rodrigues, Matilde; Nunes, Mafalda;
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(ESTSP-IPP)
Scientific Area of Environmental Health of
Allied Health Sciences School of Polytechnic Institute of Porto

Rua de Valente Perfeito, 322
4400-330 Vila Nova de Gaia
Porto - Portugal
t. +351 222 061 000
f. +351 222 061 001
e. geral@estsp.ipp.pt
w. www.estsp.ipp.pt

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PREFACE



"Letter from President of the Organising Committee of ICEH 2014 and Coordinator of the Scientific Area of Environmental Health of ESTSP-IPP"

Dear Colleagues,

On behalf of the Organising Committee, welcome to International Congress on Environmental Health 2014 to be held in the beautiful city of Porto, Portugal.

We would like to refer the auspices of the Environmental Health area of the Allied Health Sciences School of Polytechnic Institute of Porto by the organization of ICEH2014, promoting an excellent moment to scientific communities, both national and international participants. We are privileged this edition by the special participation as co-organizer of the European Agency for Safety and Health at Work (EU-OSHA) and as regular co-organizers, the Health schools of Lisbon, Coimbra and Beja.

This third edition addresses to **'Emerging risks and challenges for the environment, health and safety'** and intended as a guide to the various symposiums and workshops, to provide all present the most recent scientific and technological advances in the areas: Occupational Health and Toxicology; Exposure to Nanoparticles; Assessment and Risk Management; Occupational Safety; Exposure to Bioaerosols; Environment and Sustainability; Food Safety and Public Health.

We would like to thank all sponsors and the institutional sponsors, particularly the Porto City Council for facilities, the International Federation of Environmental Health, the Taylor & Francis Group, the National Focal Group for Health and Safety at work and the Polytechnic Institute of Porto.

We hope that this conference has a special impact, confirming the importance of research and training for prevention of risks on occupational health and environment.

Thanks, and enjoy the conference.

Manuela Vieira da Silva
President of the Organising Committee of the ICEH2014
Allied Health Sciences School of Polytechnic Institute of Porto

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PLENARY LECTURES



"International Co-operation on Environmental Health in the Frame of IFEH"

Henning I. Hansen

Project Manager at Aarhus Municipality, Denmark; President of International Federation of Environmental Health (IFEH); Chairman of the European Federation of Environmental Health (EFEH)



"Air Quality Towards Better Health"

Carlos Borrego

Institute of Environment and Development (IDAD)/CESAM and Department of Environment and Planning, University of Aveiro; Full Professor of Environmental Engineering and Director of Department of Environment and Planning at University of Aveiro; Director of the Institute of Environment and Development (IDAD).



"A Historical Perspective of Occupational Safety"

Sérgio Miguel

President at Sociedade Portuguesa de Segurança e Higiene Ocupacionais (SPOSHO); Invited full Professor, as retired, at Universidade do Minho (UM) and at Faculdade de Engenharia da Universidade do Porto (FEUP-UP), Portugal.



"Emerging Risks, Monitoring and Detection of Foodborne Pathogens"

Paul Gibbs

Principal Consultant at Leatherhead Food Research Association, United Kingdom



“Evolution of the European Standardization in the Area of Nanotechnology, with Special Emphasis on Occupational Exposure”

Luis Almeida
Full Professor at Departamento de Engenharia Textil - Universidade do Minho (UM), Portugal



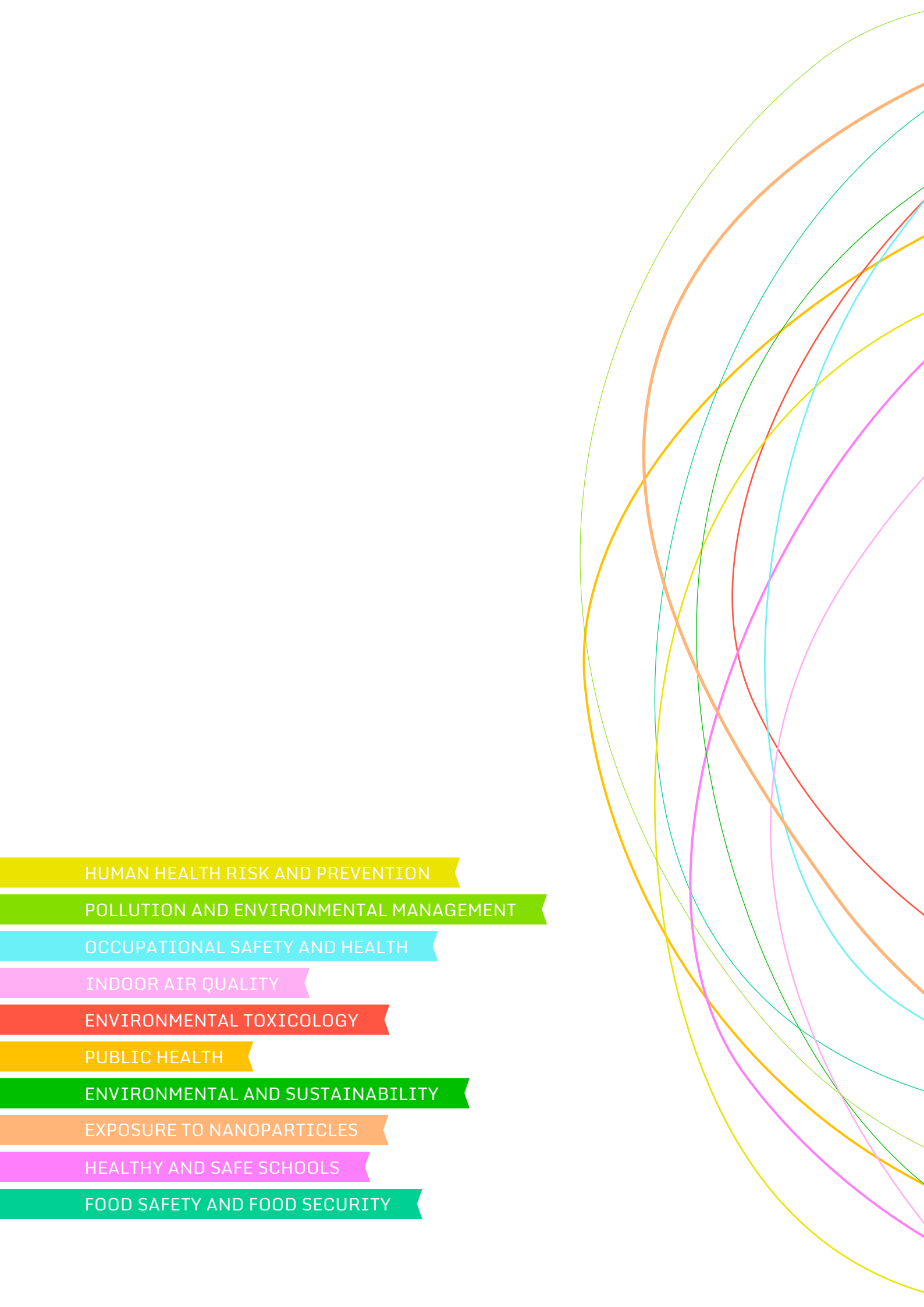
“Microbial Degradation of Fluorinated Compounds: Environmental Significance and Metabolic Processes”

Fátima Carvalho
Researcher at the Interdisciplinary Centre of Marine and Environmental Research - University of Porto (CIIMAR/CIMAR-UP), Portugal



“Health Impact Assessment”

Miguel Coutinho
Executive Director Of IDAD - Institute of Environment and Development, University of Aveiro, Portugal


HUMAN HEALTH RISK AND PREVENTION
POLLUTION AND ENVIRONMENTAL MANAGEMENT
OCCUPATIONAL SAFETY AND HEALTH
INDOOR AIR QUALITY
ENVIRONMENTAL TOXICOLOGY
PUBLIC HEALTH
ENVIRONMENTAL AND SUSTAINABILITY
EXPOSURE TO NANOPARTICLES
HEALTHY AND SAFE SCHOOLS
FOOD SAFETY AND FOOD SECURITY

ABSTRACTS

HUMAN HEALTH RISK AND PREVENTION ABSTRACTS

Social support and the locus of control of the health of elderly people

Authors: Mariola Janiszewska, Teresa Kulik, Dorota Żołnierczuk - Kieliszek, Ewa Kawiak - Jawor, Agnieszka Barańska

Chair of Public Health Medical University of Lublin, Poland

Presenting Author: Email: mariola.janiszewska@gmail.com | Tel.: +48 509 394 035 | Fax: +48 817 423 712

INTRODUCTION:

Elderly people require diverse forms of social support, which may play an important role in maintaining the health of the elderly, prevent disease and support the process of recovering. The effective social support regards interpersonal contacts and the role they perform which by the update and supporting the good self-assessment and reducing the social isolation. Social contacts have direct and indirect effect on the good functioning of elderly individuals. Searching the level and network of social support and the locus of control of the health of elderly people were the underlying purpose of this study.

OBJECTIVES:

The aim of this study was to establish the level and the network of social support and the locus of control of the health of elderly people - the students of University of the Third Age.

MATERIALS AND METHODS:

The research was conducted in the period from April to July 2010 amongst students of University of the Third Age in the Lublin macroregion (southeastern Poland).

762 individuals participated in the examination, 581 filled in sets of questionnaires were received (77 from them were rejected as incorrectly or partially filled in). After all 504 sets of questionnaires were analysed.

In order to obtain the research material a method of the diagnostic survey was applied. The following standard research tools were used:

1. The Need of Support and Service Questionnaire (NSSQ) by J. S. Norbeck in the adaptation J. Kirenko. The questionnaire measures the network of social support and the intensity (level) of received support in its individual dimensions: emotions, affirmation and the help.

2. Multidimensional Health Locus of Control (MHLC) Scale by K. A. Wallston, B. S. Wallston, R. DeVellis in the adaptation of Juczyński containing 18 statements and is including beliefs examined, determining feeling the locus of control enables the health in three dimensions: (internal - showing that the control over the own health depends on me; influence other - dimension showing that the own health is an influence of others, of especially medical staff, coincidence - coincidence or other extrinsic factors decide on the medical condition).

Original questionnaire consisting of 14 questions was also used. These questions concerned demographic data and the self-assessment of the medical condition of the examined. The obtained research material was subjected to a statistical analysis with the help of Statistica programs in version 9 and SPSS Statistics IBM in version 19. The empirical material was subjected to descriptive and statistical analysis. Tukey's test, t-Student test and Pearson coefficient of correlation, Spearman's coefficient of correlation were all applied. A p materiality level was accepted <0.05.

The age of respondents varied from 54 to 85 years. For scientific purposes and statistical analysis examined

population of elderly people were grouped according to classification of D. B. Bromley into group of up to 65 (time before the retirement) constituting I group and individuals above 65 years (the retirement pensioners and the senile age) forming the II group. Amongst examined individuals the majority were women (85.1%) with men in minority (14.9%). In the examined population the majority were individuals living in matrimony - 46.6% (in group I - 53.2%, in group II - 41.0%). The characteristics of the group was also made, taking into consideration the education of the examined. The University of the Third Age consists largely of respondents having higher education - 50.2% and secondary education- 45.8%. Only a 3.2% examined had a vocational education. The characteristics of the examined was also made on account of the domicile, 492 individuals (97.6%) lived in a city, 7 respondents (1.4%) indicated as the domicile - the settlement, and 5 (1.0%) were country dwellers.

RESULTS AND DISCUSSION:

Group diversity at the examined individuals was described on account of the age and determined with the t-Student test for independent data. Basing on statistical analyses conducted one should state that there exist statistically essential differences in the locus of control of the health at both examined groups both in the dimension of the internal locus of control of the health and in the dimension announcing the influence of others to the health of the examined individuals. However substantial differences weren' t stated statistically in the dimension of the random locus of control of the health amongst the examined.

According to the obtained results of the persons from group II in indeed large degree they are indicating, distinguishing to group I internal dimension of locus of control of the health, that is considerably more often that the control over health depending on subjects alone. Individuals displaying the dominance of the internal control of the health are usually more independent in undertaking favourable behaviours for health, of course the more they are conscious, that their health to a large extent depends on them alone they more often abide by recommendations of health service employees. These data are presented in table 1.

Table 1 - Age and the locus of control of the health of elderly people.

Dimension of health	group I (up to 65 year of life)		group II (above 65 year of life)		t-Student test	
	M	SD	M	SD	t	P
Internal	22.38	4.83	26.00	5.47	1.36	0.005*
Influence of others	21.23	5.50	25.68	5.42	1.13	0.003*
Coincidence	21.68	4.98	21.11	5.07	1.27	0.203

*P< 0.05; ˜ close to statistical significance

As a result of conducted analyses one should stat that the level of social support received by students the University of the Third Age from the group I differs indeed statistically from the level of support for group II. According to the received results, individuals of up to 65 are characterised by the highest level of social support in its all dimensions of the support (emotional, affirmation and practical), differences are on the significant statistically level which signifies that the examined of up to 65 of year of age will both sustain bigger feeling of social support, as well as strong declare its need.

They are also characterized by a bigger feeling of certainty as for the fact that they are granted with trust from their network of support, in the matter of abilities and competences necessary in taking action. In comparing to individuals above 65 they also considerably receive the highest level of the practical support, in the financial or functional help.

Amongst respondents from groups I and II distinguished on account of the age there exists statistically essential strong correlation between the internal locus of control of the health and the indicator of the affirmation support ($p < 0.01$; r Pearson = 0.49; r Pearson = 0.45). Additionally from group II, a statistical relation is characterizing examined individuals between the internal locus of control of the health and the emotional support ($p < 0.01$; r Pearson = 0.49). Along with increasing support of the examined above 65 year of age in their taken actions, competences, ability and growing with feeling of approval and the admiration,

there raises the conviction of seniors that their health depends only on them .
Next negative, but also statistically essential correlation, appears between the influence of others and the practical support ($p < 0.01$; r Pearson = -0.55). It results from this that along with falling intensity of support in the financial and functional help conviction of seniors that their health is under the influence of others, especially medical staffs grows.

Research conducted by Woźniak (2007) shows that the presence of the social network provides the sense of security for the individual, the feeling of membership, approval and integrating. The research of other authors Leszczyńska- Rejchert (2005), Idzikowski (2000) also show that holding social contact, having supporting individuals considerably influences the improvement and keeping the health, the quality of life, improves cognitive functions and reduce the mortality amongst seniors.

In their examinations Tobiasz-Adamczyk and Zawisza (2008), described a positive effect of social support on the medical condition and the life span of seniors show that good social relations prolong living on average of about 2.8 of years for women and 2.3 for men.

CONCLUSION:
The researched students of the University of the Third Age receive all three types of support on similar level. In spite of minor differences it is possible to put them in order from the strongest to the weakest in the following order: AFFIRMATIVE SUPPORT, EMOTIONAL SUPPORT, PRACTICAL SUPPORT.

Seniors of up to 65 experience bigger social support than respondents above 65 of year of age, and they declare its need much stronger .The highest level of the provided support is given to, in general sense and in all dimensions, to seniors in relationship, and the least support is offered to individuals experiencing a bereavement (divorce or partner's death).

Students of the University of the Third Age are presenting the internal control of the health in the large degree. On the second place the examined seniors mentioned the influence of others, and the lowest significance in their view the notion of coincidence.

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Environmental health diagnosis of childhood population living in complex urban scenarios with integration of social, environmental and health indicators in a quantitative community environmental health index.

Authors: Domínguez-Cortinas G[1], Ortega-Elorza LE[2]

1 *Centre forApplied Research in Environment and Health, Medicine Faculty, Autonomous University of the San Luis Potosí (UASLP), Mexico*

2 *Multidisciplinary Postgraduate Program in Environmental Science, Autonomous University of the San Luis Potosí (UASLP), Mexico*

Presenting Author: Email: lau_treamont@hotmail.com | Tel.: +052 44 48262300-8465

INTRODUCTION:

The general health status of a community does not solely depend on the well-being and optimal competence condition of each separate individual, but also, on the existing prevalence within the multiple social, environmental and health factors of the group of individuals as a whole (Lee, 2006). This investigation sets forth a new methodology in the establishment of integral environmental health diagnoses, quantitatively expressed by means of a community environmental health index can serve as an objective measure by which the communities can be differentiated and systematically arranged depending on the extent to which their health is affected. Moreover, through a spatial distribution analysis, those communities having the major health problems and which represent the highly vulnerable areas that require prioritized attention, can be identified.

OBJECTIVES:

The principal objective of this investigation is to pose a new methodology in both the epidemiological and public health spheres, in order to establish integral environmental health diagnoses of childhood population living in complex urban scenarios that can be quantitatively expressed through the generation of a community environmental health index.

MATERIALS AND METHODS:

The study was conducted in the child population of three urban communities of the San Luis Potosí, S.L.P. metropolitan zone in Mexico. The community health index was constituted in three dimensions (social, environmental, health) and included a total of 31 indicators. We designed a general empirical model based on the hypothesis that environmental health is given on the basis of multiple interactions and combined effects among the factors of four general constructed concepts: morbidity (M), direct health determinants (DHD), social determinants (SD) and environmental determinants (ED).The inclusion criteria of the study sites were: a) levels of segregation and poverty; b) type and sources of contamination; and c) level of aggregation per the basic geostatistical area. We randomly selected children according to the following inclusion criteria: a) ages between 3 and 12 years, b) time of residence at the selected site, c) location of the household within the concerned geographic area. The final size of the population sample included a total of 145 children. We conducted field work and applied questionnaires (previously validated and standardized) to the parents. We obtained the information corresponding to the direct health determinants groupthrough the application of clinical and toxicological tests to each participating child. As to the morbidity indicators, the information was obtained through clinical examinations conducted by health community personnel. With the data obtained from each indicator (variables), we implemented an exploratory analysis of factors, applying the Principal Components extraction method (Johnson, 2002). The index of each community was obtained by multiplying the scores of each selected component based on their explained total variance value, adding up these new values in order to generate the total scores per site.

RESULTS AND DISCUSSION:

The variables which had the major correlation coefficients (R) in each one of the extracted components, reflecting the intensity and weight of the contribution of each. In accordance with this, the first component has a total of 9 outstanding variables, being the most important: the presence of insect infestation in dwellings (R=0.674), the burning of solid waste (R=0.671), the exposure to lead (R=0.662), a low IQ (R=0.584) and the use of firewood (R=0.529). The variables of lesser weight in this first component were: lack of sanitation infrastructure (R=-0.499), parasitic infections (R=0.489), child labor (R=0.483) and overcrowded living conditions (R=0.419). In the second component, the variables having a major weight were: malnutrition (CMI, R=0.860; low weight, R=0.772) and upper respiratory infections (R=-0.520). As to the third and fourth components, the outstanding variables were: exposure to environmental toxins, such as, POPs (R=0.749) and PAH (R=-0.766), as well as, dental fluorosis (R=-0.706), respiratory disorders (R=0.636) and tobacco consumption (R=-0.439).We obtained the R^2 values, which explain the association force of each variable with its component. This means that the observed variables (indicators)now behave as independent variables that in a given proportion, explain the dependent variable that is represented in each of the extracted components. Incorporating these results to our general model, we are able to observe more clearly those variables which are determining, with a greater specific weight, the condition or the general health status of the communities, thus constituting their main risk factors (Figure 1).

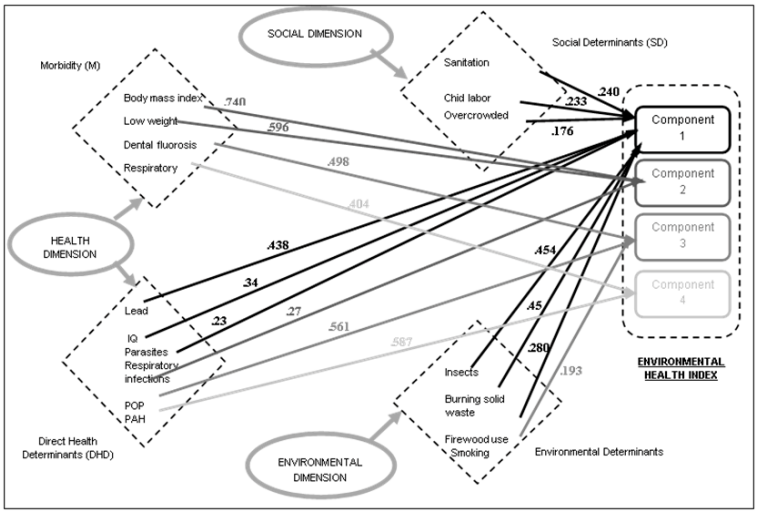


Figure 1 - Model association between weighted observed variables (indicators) and the extracted components. The numbers on the arrows are the values of R2 representing the strength of the associations and the fraction explained by each variable with respect to the component with the highest saturation.

The variables which affected a high percentage of the population were grouped together, presenting an unjust distribution standard which coincided with a segregation gradient; that is to say, that the insect infestation, the burning of waste material, the exposure to lead, the use of firewood, the low IQ, the lack of sanitation infrastructure, the parasitic infections, child labor and overcrowded living conditions mainly affected the segregated communities of Municipal Garbage Dump and Brick Producing Site. This same behavior was observed in the major weight variables of the second component (CMI, low weight, upper respiratory infections).The distribution standard progressively and subtly presented variations in the subsequent components, in such a way, that in the third, the POPs, dental fluorosis and tobacco consumption variables, presented high affectedness levels, though in less disproportion among the three communities. The contribution percentage of said variables in the conformation of the environmental health indexes showed a 35.3% corresponding to variables of toxic agents exposure, infectious agents exposure and low IQ; a 23.5% corresponding to environmental variables; another 23.5% corresponding to morbidity variables; and, a 17.7% corresponding to social variables. It is important to state that of all of these variables, 88% were the result of a segregation gradient. The media of the total scores obtained represent the community environmental health index. These indexes were: Central Zone = -0.150084, Brick Producing Site = 0.050244 and Municipal Garbage Dump = 0.071886. These indexes were analyzed applying the Dalenius and Hodges

stratification method, and results allowed us to classify the communities in one of the four obtained strata, corresponding to stratum 1 the lowest scores and the highest to stratum 4 (Table 1).

Table 1 - Strata obtained from the scores of the first component of each community, using the method of stratification Dalenius and Hodges. Interpreting the calculated layers.

Strata	Ranges Index	Environmental health índex interpretation	Classification of the study sites according to the index obtained
1	-0.75 _ -0.25	GOOD	
2	-0.25 _ 0.0	REGULAR	CENTRAL ZONE
3	0.01 _ 0.25	POOR	MUNICIPAL GARBAGE DUMP BRICK PRODUCING SITE
4	0.25 _ 0.5	VERY POOR	

CONCLUSION:

The environmental health index that we have developed in this investigation, as the quantitative expression of the integral environmental health diagnoses, can be constituted a highly useful and objective tool in the classification and localization of those communities having the major environmental health and vulnerability problems, making possible to destine resources and assistance programs to where it is most required. The solution to the serious problems faced by our society in respect to public health, is centered on the development and implementation of a methodology, sufficiently robust, that may generate integral health diagnoses and be coherent with the local realities.

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The knowledge about osteoporosis prevention in peri - and postmenopausal women

Authors: Mariola Janiszewska, Teresa Kulik, Dorota Żołnierczuk - Kieliszek, Małgorzata Dziedzic

Medical University of Lublin, Chair of Public Health

Presenting Author: Email: mariola.janiszewska@gmail.com | Tel.: +48 815 093 940 35 | Fax: +48 817 423 712

INTRODUCTION:

Osteoporosis is one of medical and social problems. In addition to extending average lifespan in the developed countries it is possible to expect the increase of osteoporosis incidence in the near future and in consequence the increase in the number of osteoporotic fractures. Especially women in the postmenopausal age are predisposed to low-energy fractures, therefore it is predicted that almost half of women, over 50, will be affected with the fracture.

Most important risk factors of osteoporosis are: age, the female sex, the ethnic group, genetic factors, an inherited conditioning, the early menopause, the slim posture and the low body mass, diseases disturbing the bone metabolism, chronic applying of some medicines, the insufficient physical activity, calcium and vit. D underconsumption, alcohol or caffeine overconsumption and cigarette smoking. Osteoporosis prevention involves lifelong action, such as healthy lifestyle in diet and physical activity. These habits play a crucial role in the normal development of the bone tissue and keeping the bone metabolism on the desirable level.

OBJECTIVES:

The aim of the study was to determine the knowledge of women in the peri- and postmenopausal age on prevention of osteoporosis which can be helpful in formulating the preventive programs concerning this disease.

MATERIALS AND METHODS:

The study was conducted in the period from 2 January to 30 March 2012 on a group of randomly selected women at the age range of 45 to 65, being patients of healthcare centres in Chełm, Lublin and of surroundings of Zamość (Lublin Province, south eastern Poland).

Osteoporosis Knowledge Test (2011) was used to own examinations. OKT is a tool consisting of 32 elements allowing evaluation of the knowledge in applying a well-balanced diet with a lot of the products rich in calcium, essential for construction and of keeping mass of the bone; the physical activity and risk factors, the screening and the treatment of osteoporosis. Total possible score for OKT was 32 points, for OKT Calcium was 26, for OKT Exercise was 20. The scale in Polish version was translated based on the processes of forward translation (into Polish), back translation (into English), and comparison (between the original English version and the back translation version). A minor modification to OKT Scale was introduced - a new subscale was added - the Knowledge about risk factors, screening tests and the treatment of osteoporosis (the total possible score for this subscale was 14). In the destination of setting the operands, enabling to present the characterization of the research group, the original questionnaire was used, containing 13 questions.

The empirical material was subjected to descriptive and statistical analysis. Tukey's test, t-student test and variance analysis (ANOVA) were all applied. A p materiality level was accepted <0.05 and $p<0.01$. Computer software IBM SPSS Statistics 19 was applied.

The examined group was constituted by women aged 45 to 65. 77% of respondents were in the age of 45 - 55 years (n = 321). Remaining women were from 56 up to 65 years of age (n = 69; 23%). Considering the level of

education it turned out that almost a 40% of the examined had a secondary education (n = 119), 43 women (14.3%) had vocational and 14% (n = 42) higher education.

As regards socio-economic conditions it was a 51.3% (n-154) of the examined women described them as good, however 30.3% (n = 91) as average. The scanty percentage complained about bad conditions (n = 6, 2.0%).

RESULTS AND DISCUSSION:

Respondents were asked about the fact of reporting for tomodensitometry. As it turned out the significant number of examined women had never performed examining the bone density (n = 208; 69.3%), and 3 individuals out of examined weren' t sure what such an examination consists in.

As regards the acquaintance of risk factors of osteoporosis amongst women in the peri- and postmenopausal period, it was shown that majority of respondents (77.6%) agreed with the fact that the calcium low diet constitutes essential risk factor of osteoporosis. Older age was indicated by 89.4% of the examined, and menopausal period at women by 85.4%. Unfortunately only a 34% and consecutively the 41.7% of respondents connected the alcohol overconsumption and the cigarette smoking with the possibility of falling ill with osteoporosis. Actually the comprehended physical activity and applying the calcium rich diet constitute fundamental links with reference to the prevention of osteoporosis.

Most respondents thought that cycling was the best way of preventing osteoporosis (61.3%), aerobics (65%), running (48.3%) and swimming (43%) and being next in turn, whereas with the best source of calcium - cheese (98%), sardine (44.7%), meat of the chicken (61%), carton of yogurts (95%) and ice-cream (48%) was stated. Majority of respondents declared that for correct absorbing of calcium vitamin D was essential (70%), which is provided by sunlight (67%). Unfortunately only the 13% of women indicated growing up as the optimum period in life for building the strong bones.

Summing up, women in the peri- and postmenopausal age presented a medium level of knowledge on the role of physical activity for osteoporosis prophylaxis (M=13,93) and the low level of knowledge related to problems connected with proper diet rich in calcium (M=9.77).Turkish studies by Gemalaza et al. (Gemalmaz, A., Oge, A. 2008) are an example of noticing the relation of the knowledge in this respect. Also studies conducted in Vietnam by Nguyen et al. (Nguyen N, Dinh T, Ngo Q., & at al. 2011) and by Costa - Paiva et al. (Costa - Paiva, L., Gomes, D., Morais, S. 2011) in San Paula in Brazil demonstrated the identical relation.

Knowledge about risk factors, screen tests and the treatment remained on medium level (M=8,00). The questioned women presented insufficient general knowledge on subject of osteoporosis (M=15.71).

Analysing the influence of education on the level the knowledge of women about osteoporosis it can be stated that the level of education was diversifying respondents in terms of the presented knowledge about the role of calcium rich diet in osteoporosis prevention. Women with the primary education and vocational one had a significantly lower knowledge on this subject from women with college and higher secondary education. The analogous situation took place in case of the influence of education on the level the knowledge about the role of physical activity, risk factors, screening and the treatment and of the general knowledge about osteoporosis.

Examining the influence of socio-economic conditions on the level of the knowledge of respondents, it was stated that individuals declaring very good socio-economic conditions had presented the highest level the knowledge indeed (M = 8.61) to the subject of risk factors, the screening and the treatment of osteoporosis as compared to individuals having average or bad socio-economic conditions.

Analysing the influence of the age on the level of the knowledge about osteoporosis one should notice that younger women of 45 to 55 years old had a significantly higher knowledge about the role of the physical

activity (M = 10.03), than older 56-65 years old women (M = 8.91). Younger women also introduced the highest level of knowledge indeed about risk factors, the screening and the treatment (M = 8.26), than older women (M = 7.12).

Examining the influence of keeping the physical activity on the level of the knowledge about osteoporosis it was pointed out that women, being active in terms of physicality were characterized highly indeed by a high level of the knowledge about the role of feeding, physical activity, risk factors and generally osteoporosis, than women, not practising sport.

Properly comprehended, everyday physical activity exerts beneficial effect on the structure and keeping bone mass in every age. Pilewska et al. (Pilewska, A., Kanadys, K., & Łepecka - Klusek, C. 2003), examining the influence of the physical activity on appearing of osteoporosis, presented data, saying that only a 17.3% of women at the age 45 - 60 confirmed performing regular physical exercises through 1 - 2 hours every week.

Szczygielska - Majewska et al. (Szczygielska - Majewska, M., Papis, E. (2003), after conducting their examinations, concerning a lifestyle in the aspect of the prevention of osteoporosis, demonstrated that as many as 65.8% of individuals examined by them didn' t carry out any form of physical exercise.

Diagnosing osteoporosis bases, among others, on the lowered BMD bone mineral density (Eng. Bone Mineral Density in g/cm 2). BMD is an indicator of the resistance of the bone structure, that is it is responsible for the 70% of its endurance. It results from our own examinations that the fact of performing the above examination had no impact on a level of the knowledge in the prevention of osteoporosis.

CONCLUSION:

Women in the peri and and postmenopausal age presented an average level of knowledge about the role of the physical activity in osteoporosis prevention and a low level of the knowledge about calcium diet. The respondents had an average, but insufficient knowledge in risk factors, the screening and the treatment and the general knowledge in the aspect of osteoporosis.

Socio-economic factors as well as some forms of behaviour, associated with a lifestyle, as constant maintaining of physical activity, declaring performing prophylactic examinations, long-term applying of pharmacological therapy potentially causing the loss of bone mass and fact of the cigarette smoking indeed influenced the step of the possessed knowledge.

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Geographic Information Systems in assessment of risk factors in cancer prevention planning

Authors: Cristina Amil-Dias[1], Paulo Veloso Gomes[1]

1 *Allied Health Sciences School of Polytechnic Institute of Porto, Portugal*

Presenting Author: Email: avd@estsp.ipp.pt | Tel.: +351 919 386 493 | Fax : + 351 222 061 001

INTRODUCTION:

Between 2002 and 2012 almost 24% of deaths registered in Portugal originated from malignant diseases (INE, 2014).

According to the International Agency for Research on Cancer in the coming years as a consequence of demographic evolution and exposure to risk factors, the incidence of oncology diseases is expected to increase. According to the WHO (2005) it is expected that 40% of cancer might be avoided if the main risk factors could be modified. The Implementation of preventive measures, most of low cost but poorly applied, depends on systematic evaluation of national risk factors (WHO, 2007).

OBJECTIVES:

This work presents a model of crossing information about incidence of cancer and risk factors using Geographic Information Systems.

MATERIALS AND METHODS:

This work proposes an experimental model of geographic mapping of data crossing information related to risk factors and geographic prevalence of oncologic disease.

RESULTS AND DISCUSSION:

Several risk factors have been identified as potential causes for neoplasia, namely smoking habits, consumption of alcohol, overweight/obesity, prolonged physical inactivity, dietary habits, hepatitis B virus, Human papiloma virus, environmental pollution and exposure to radiation, among others.

The importance of knowledge of the location of patterns of cancer is well known for the implementation of national health policies (Registo Oncológico Regional do Norte, 2013), but this analysis has not been yet crossed with local risk factors.

Crossing information of geographic distribution of cancer with that of risk factors is quite essential to define the appropriate strategic policies in Public Health. This planning depends on the existence and monitoring of complex population data, that must include smoking habits, alcohol consumption and timing of those data related to each factor on one hand and the prevalence of cancer on the other for a given region.

The value of crossing information in infectious conditions is well known, but for chronic diseases and cancer in particular it has a very relevant role to forecast epidemiologic evolution of those diseases. This process may allow planning and intervention to identify individual factors that may explain variations in the expected epidemiology and evolution of the disease.

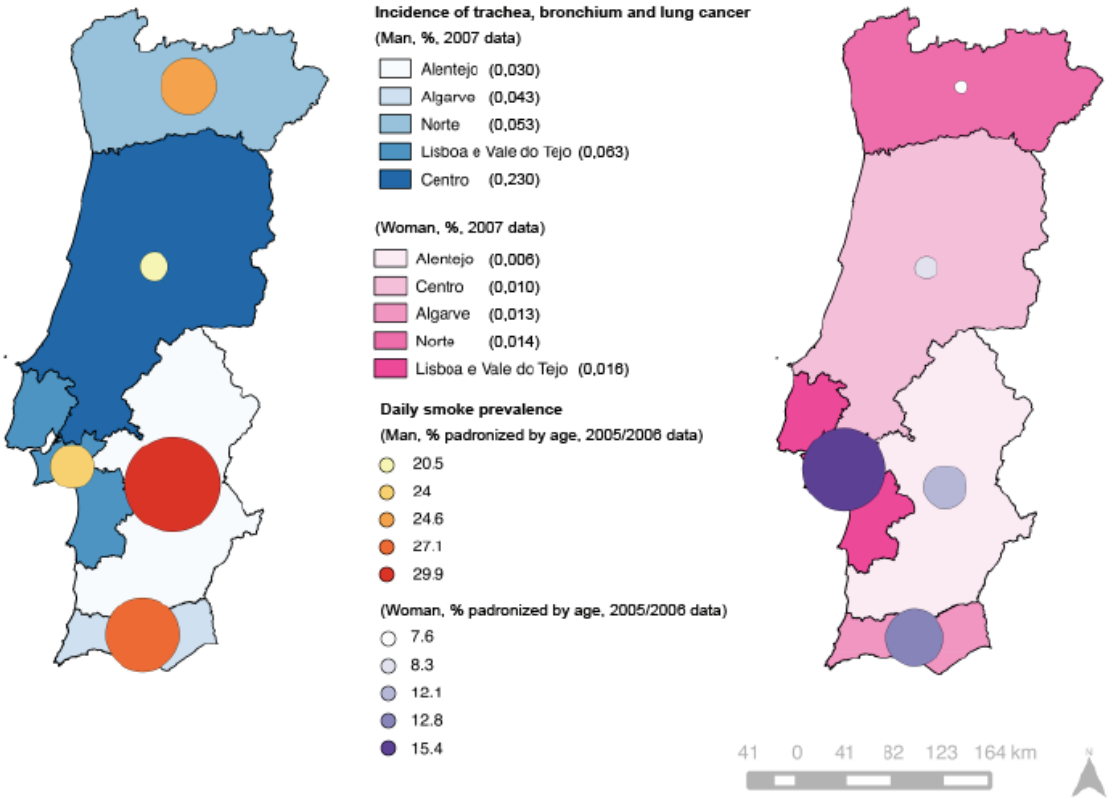


Figure 1 - Geographic distribution of the incidence of trachea, bronchial and lung cancer (%, 2007) and daily smoke prevalence (padronized %, 2005/2006), by gender in Portugal

The figure shows superimposed geographic epidemiological data of 3 variables on a reference map related to the incidence of trachea, bronchium and lung cancer (2007 data), daily smoke prevalence (2006 data) and gender. These data are provided as an example and show how geographical co-location may be used to identify potential areas of interest for subsequent individual analysis of risk factors, as well as a tool to compare risk factors in countries with the same cancer incidence or drastic difference. This type of evaluation maybe regarded as a first line of data mining in the epidemiological studies of disease.

CONCLUSION:

The model of multifactorial co-location may help identifying clusters of oncology disease, guiding strategic measures of health promotion and screening in areas of higher risk.

This type of analysis may bring additional value in research and identification of potential risks poorly characterised for hotspots of incidence above expected values for a specific region.

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Indoor Air Quality in Hospitals: Assessment for a Proper Management

Authors: Susana Marta Almeida[1], Sandra Cabo-Verde[1], João Matos[1,2],Tiago Faria[1,3], Carla Viegas[3]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Loures, Portugal*
2 *Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Monte da Caparica, Portugal*
3 *Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal*

Presenting Author: Email: smarta@ctn.ist.utl.pt | Tel.: +351 219 946 124

INTRODUCTION:
The diversity of facilities and occupants makes the hospital environment much more complex than any other commercial or industrial building. Consequently, this complexity requires special attention to ensure healthful Indoor Air Quality (IAQ) to protect both hospital staff and patients, especially immunosuppressed and immunocompromised [1]. Poor hospital IAQ may cause outbreaks of sick hospital syndrome (SHS), causing headaches, fatigue, eye and skin irritations. More seriously, improper control of hospital IAQ may cause hospital-acquired infections and occupational diseases [2].

OBJECTIVES:
The objective of this work was 1) to assess the IAQ in different areas of a Portuguese Hospital by conducting chemical, comfort and microbiological measurements, 2) to evaluate the perceived IAQ by the hospital's workers, and 3) to identify the sources associated with the poor IAQ.

MATERIALS AND METHODS:
Measurements of chemical and comfort parameters were performed with the direct reading equipments Dust Track (PM10, PM2.5 and PM1), Graywolf (CO2, CO, VOCs, O3, temperature and relative humidity) and FormaldemetherhtV-M (CH2O). Airborne microorganisms were collected by impaction method using a Microbial Air Monitoring Equipment (MAS100). After sampling, processing and culturing (culture solid media) of the samples, quantitative (colony forming units counting) and qualitative (phenotypes) results were obtained, with characterization and identification of airborne isolates.

Measurements were performed in the following places: 1) Urgencies - waiting room (WR); screening (S); medical consultation (MC); treatment room (TR); open room (OR); corridor (Cu) and observation room (SO); 2) Infirmary: rooms (R); corridor (Ci); and 3) Operating Theater (OT) and room for care after surgery (CAS). Three types of measurements were performed 1) during 24 hours to identify emission sources; 2) during 20 minutes, in different seasons, to assess the influence of the outdoor temperature and humidity and 3) sequentially in the outdoor, technical room (where the air treatment units are places) and in the spaces, in order to evaluate the maintenance and performance of the HVAC system. An evaluation of the design, performance and maintenance of the ventilation systems was carried out.

RESULTS AND DISCUSSION:
The perceived IAQ and the related symptoms were collected by means of a questionnaire survey among 70 workers in this hospital. The questionnaire not only demonstrated that the workers present symptoms associated with poor IAQ, such as dry and irritated eyes, dry and runny nose, sore throat, dry skin, headaches and fatigue, but also showed that they believe that these symptoms may be related to the environmental conditions of their workplace. Workers are unhappy with parameters of comfort and well-being mainly related to temperature, air circulation, humidity, odors, light and noise. The general perception of comfort depends greatly on the service being poor for workers from emergency and satisfying for workers from the

operating room service (Figure 1). Over 50% of employees stated that their productivity can be influenced by environmental conditions of their workplace.

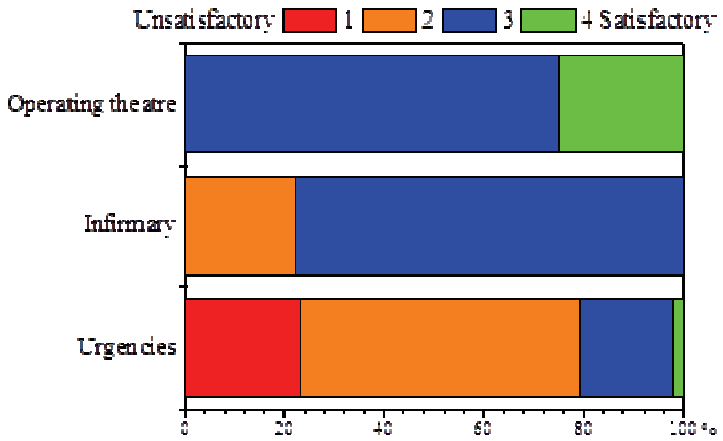


Figure 1: Global comfort assessed by the questionnaire applied to the hospital workers.

Figure 2 shows that each of the services has its particularities. The urgencies present the highest concentrations of particles exceeding the limit values set by the WHO and by the national legislation[3]. This is due not only to the high infiltration of particles from outside through open windows and doors, but also due to the high occupancy in this service that promotes the re-suspension of particles. The concentrations of VOCs and CH2O present the highest values not only in the urgencies but also in the operating theatre. Increasing concentrations of these pollutants are associated with medical procedures and with the use of disinfectants. The concentrations of these pollutants also exceed the WHO references and the national legislation. The values of microorganisms were particularly high in the urgencies. The results show that there is a strong dependence between the IAQ and the pollutant levels measured outside. This is particularly visible in the urgencies, where high levels of PM10 and VOCs measured in the outdoors in June, were reflected in a decrease of the IAQ. This direct relationship is due to the fact that this is the service where the infiltration of outdoor air is felt with greater intensity due to the opening of windows and doors.

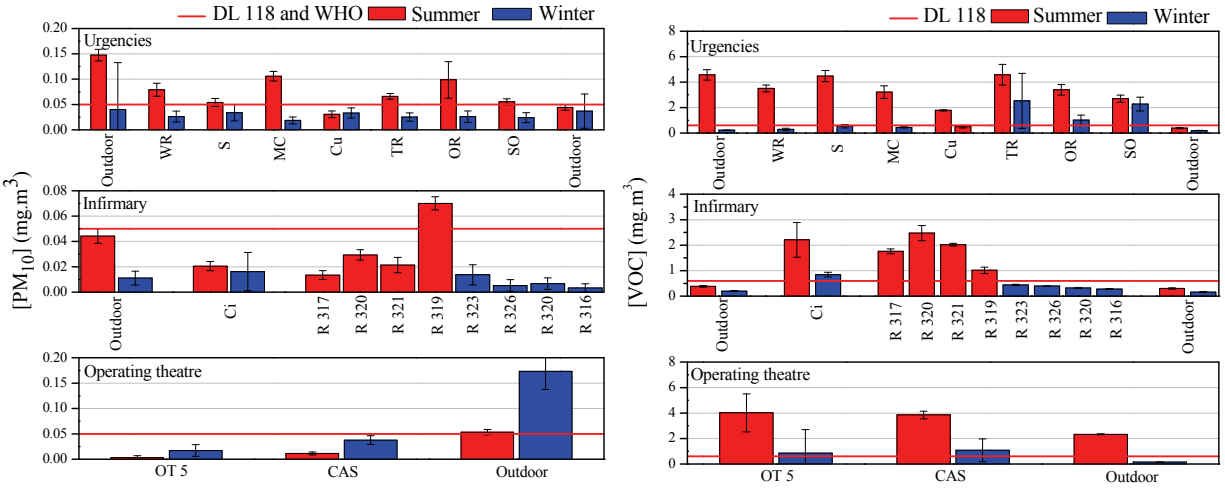


Figure 2: PM_{10} and VOC concentrations measured in the hospital.

CONCLUSION:

The assessment of the IAQ in the studied hospital showed that the concentrations of chemical and microbiological parameters exceeded the limit values defined by the WHO and by the Portuguese legislation.

The improvement of HVAC performance and maintenance, the increase of ventilation rates and the instruction of the health professionals is essential to improve the IAQ in hospitals.

ACKNOWLEDGMENTS:

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Financial and Economic Analysis of Prevention of Needlesticks in the Hospital Sector

Authors: Delfina Ramos[1], Paulo Afonso[2], Pedro Arezes[2]

1 *Polytechnic Institute of Cávado and Ave, Technology School, Barcelos, Portugal & ISLA - Polytechnic Institute of Management and Technology, Portugal*

2 *Research Centre for Industrial and Technology Management, School of Engineering, University of Minho, Guimarães, Portugal*

Presenting Author: Email: gramos@ipca.pt | Tel.: +351 253 802 260 | Fax: +351 253 823 127

INTRODUCTION:

According EU-OSHA (2014), workers in the healthcare sector are at risk from needlestick or sharp injury. Such injuries are of concern as the worker may become infected by blood-borne pathogens. The Human Immunodeficiency Virus (HIV) and Hepatitis B (HBV) or C (HCV) are the commonest risks, but there are more than 20 blood-borne diseases that may be transmitted.

In Europe, it is estimated that there are 1 million needlestick injuries annually. It is not just medical professionals who are at risk. While nurses working in acute medical situations are identified as being at the highest risk, many other workers have the potential to sustain these injuries. For example, auxiliary staff such as cleaners and laundry staff can also be at significant risk.

Prevention of needlestick accidents can be achieved by means of information and training measures, as well as by adequate organizational measures and by the implementation of safety-engineered protection mechanisms. A cost-benefit analysis model has been developed by the authors (Ramos et al, 2012). This model takes into account not only the internal benefits (financial analysis) but also the "externalities" (economic analysis).

OBJECTIVES:

This paper discusses the financial and economic analysis of prevention of needlestick accidents related to Occupational Health and Safety projects and presents the application to a case study involving a Hospital.

MATERIALS AND METHODS:

An analysis of the work accidents has been made in a Portuguese public Hospital during one year. This study concentrated on the needlestick accidents occurred in six of the services of the Hospital (three medicine services, two orthopaedic services and the emergency services).

The cost of each accident due to needlesticks has been estimated, taking into account a detailed study made at the Hospital. The direct cost is of the same order of magnitude as presented in the literature (see for instance WittmannandZylka-Menhorn, 2007).

The direct costs of the accidents have been estimated taking into account the labour cost of each injured worker, as well as the costs incurred by the Hospital for the treatment and rehabilitation. The indirect costs of the accidents have been calculated using the simple methodology proposed by Heinrich (1951), since this approach is the one used by the Hospital. According to this methodology, indirect costs can be estimated as being four times the direct costs, so the total costs are five times the direct costs.

A risk evaluation has been made in six services, using a semi-quantitative method. Following this risk assessment, a detailed plan of the preventive measures to be implemented has been designed, with an estimate of the corresponding costs.

Subsequently, the Benefit/Cost ratio (B/C) of these measures has been calculated, both in financial terms (from the organisation's perspective) and in economic terms (including the benefits for the worker and for

the Society). The economic B/C ratio has been estimated taking into account the "externalities", namely in terms of the impact of the investments not only on the Hospital but also on the workers and on the Society. To calculate the externalities, some questions from the model "Cost-Benefit Analysis in Occupational Safety and Health" of Ramos et al. (2012)have been used and new items were also added, based on the study conducted by the Health and Safety Executive (HSE, 2012).

RESULTS AND DISCUSSION:

The total number of reported accidents due to needlesticks in the Hospital has been 18 for the year studied. This corresponds to 19% of the work accidents in the Hospital.

In the second column of Table 1 are presented the costs of the accidents related to needlesticks in the six services studied. In two of the services, no accidents of this type have been reported in the year studied.
The second column presents the annual costs of the designed preventive measures.

The fourth column presents the financial Benefit/cost ratio (B/C), estimating that a reduction of 80% of the needlestick accidents will be avoided if all the preventive measures have been well designed and will be successfully implemented.

Table 1 - Cost of accidents, cost of preventive measures and financial B/C ratio for the six hospital services

Service	Cost of accidents (€)	Annual cost of preventive measures (€)	Financial B/C ratio
Medicine A	0	1072	0
Medicine B	7500	1264	4.81
Medicine C	4500	1211	2.97
Orthopaedic A	3000	911	2.63
Orthopaedic B	0	777	0
Emergency	12000	5932	1.62
TOTAL	27000	11148	1.94

If the B/C ratio is higher than 1, the preventive measures are effective, because the benefits outweigh the costs. Thus, high values of the B/C ratio correspond to very effective preventive measures.

Considering this, it is possible to see that in all the services in which accidents have occurred, there is a financial advantage of implementing the measures related with needlesticks, as the B/C ratio is higher than 1. In the two services where no accidents occurred in the year studied, although B/C ratio is zero, potential benefits can nevertheless be foreseen, as the probability of occurring an accident will be reduced.

The benefits for the worker and for the society have been estimated taking into account the statistics and estimations made by Health and Safety Executive (HSE, 2012), by means of relationships of the external costs to the company (externalities related to the worker and to the Society) from the computed internal cost of the accidents (from the perspective of the company). Estimations are presented in Table 2.

Table 2 - Externalities involved in the reduction of one accident per needlestick

Externalities	Related with	Benefit (€)
Implications in terms of family stability, including pain and suffering	Worker	1005
Implications in terms of productivity and competitiveness for the economy	Society	7995
Prevention campaigns related to needlesticks, extending to other entities in the health system	Society	7500
TOTAL		16500

Taking into account that 18 accidents have occurred during the studied year and that 14 of them can be avoided, the estimated external benefit is 231000 €, which should be added to the internal benefit (80% of 27000).

The global economic B/C ratio is 22.7, amply justifying the investment in economic terms, i.e. if the social impact of these measures are considered.

CONCLUSION:

The analysis of the financial Benefit/Cost ratio showed that, for the Hospital considered in this case study, the preventive measures defined in the risk assessment related to the prevention of needlesticks are cost effective. The economic B/C ratio is much higher, fully justifying the investments. This fact should be taken into account by the administration of the Hospital in the moment of deciding to implement the preventive measures resulting from the risk assessment. Indeed, as the Hospital studied is public, it is important to consider the effect of the preventive measures on the workers and on the Society.

ACKNOWLEDGMENTS:

The authors would like to acknowledge the Hospital which supplied the data for the case study.

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Health risks following wheat dust exposure during agricultural work - Focus on *Fusarium* spp

Authors: Gaëlle Vacher[1], Hélène Niculita-Hirzel[1], Thierry Roger[2]

1 *Institute for Work and Health, University of Lausanne and Geneva, Epalinges-Lausanne, Switzerland*
2 *Infectious Diseases Service, CHUV and University of Lausanne, Lausanne, Switzerland*

Presenting Author: Email: gaelle.vacher@chuv.ch | Tel.: +41 213 144 774 | Fax: +41 213 147 430

INTRODUCTION:

Although inflammatory and innate responses are essential for protective immunity against infection, persistent inflammation in the lungs promotes respiratory diseases. Chronic exposure to high concentration of organic dust can promote such respiratory pathologies. One of the most concerned occupational populations is that of agricultural workers, in particular grain workers who are highly exposed to organic dust during their activities. This population is known to develop complex respiratory diseases due to exposure to grain dust, a complex mixture of fungal particles, bacteria, insect compounds, animal wastes, inorganic compounds(silicates), chemicals, gases and fumes (Linaker & Smedley, 2002).The grain dust components responsible of these pathologies are still unknown. However, *Fusarium* spp., particularly *Fusariumculmorum* is one of the most predominant fungal species present in grain dust. *Fusarium* spp. are responsible for infectious, allergic and toxic effects in humans. Yet, the innate immune response to these species is poorly characterized.

Innate immune cells such as alveolar macrophages lining the respiratory tract and dendritic cells (DCs) located within the epithelium and interstitium of the lung, form the primary line of defense against invading microorganisms. These cells sense microorganisms via pattern recognition receptors (PRRs), which are specialized in the recognition of conserved microbial structures called microbial associated molecular patterns (MAMPs). Upon microbial sensing, innate immune cells produce a panel of cytokines and chemokines that play a key role in the initiation, amplification and regulation of the inflammatory and innate and adaptive immune responses.

OBJECTIVES:

Our objective is to characterize the response of innate immune cells to *Fusariumculmorum* and to identify the PRRS involved in host-fungus interaction.

MATERIALS AND METHODS:

Fusariumculmorum 2156 (thereafter called *Fc*2156) was isolated by Agroscope from Swiss wheat samples and characterised in our lab.*Fc*2156 was grown on potato dextrose agar plates. Spores were collected and stored for 18 hours in PBS at 4°C or 22°C before usage. *Fc*2156was used as total preparation or centrifuged to isolate spores and supernatant. Bone marrow precursors were cultured for 7 days with granulocyte-macrophage colony-stimulating factor (GM-CSF) to obtain bone marrow-derived dendritic cells(BMDC). BMDCs ($2.5.10^6$cells/ml in96-well plates) were exposed for 24 hours to lipopolysaccharide (LPS, 10 ng/ml) as control and*Fc*2156preparations (total preparation, spores and supernatant) at an equivalent multiplicity of infection (MOI, *i.e.* a cell/spore ratio) of 0.01, 0.1 and 1. The concentrations of tumor necrosis factor (TNF), interleukin (IL)-6, IL-12p40, macrophage inflammatory protein (MIP)-1α and MIP-1βin cell culture supernatants were quantified by Luminex.

RESULTS AND DISCUSSION:

Total preparation and spores, but not supernatant, of *Fc*2156 induced vigorous production of TNF, IL-12p40, MIP-1α, MIP-1β and, to a lesser extent, IL-6 (Table 1 and data not shown). The stimulatory effect followed a bell-shaped response curve, with the highest concentrations of cytokines and chemokines obtained using

*Fc*2156at MOI 0.1. The lowest concentrations of mediators were measured using *Fc*2156 at MOI 1, due to cytotoxic effects.The differential effects of *Fc*2156 preparations were also observed using primary macrophages (data not shown).

Table 1 - Cytokine and chemokine release (in pg/ml) by BMDCsexposed to *Fusarium* spores

	TNF	IL-6	IL-12p40	MIP-1α	MIP-1β
Medium	7	3	19	4	23
LPS 10 ng/ml	52	200	975	323	910
***F.c* 2156MOI 0.01**	1188	81	857	946	3115
***F.c*2156 MOI 0.1**	5026	206	1478	7105	6594
***F.c*2156 MOI 1**	213	10	323	770	976

CONCLUSION:

F. culmorum spores strongly activate innate immune cells causing them to release cytokines and chemokines. It is well known that *Fusarium* spp. produce toxic secondary metabolites such as mycotoxins when exposed to stress conditions. This may account for the observed cytotoxicity and reduced production of inflammatory mediators (Stockmann-Juvala, Alenius, & Savolainen, 2008)when using high loads of *F. culmorum*. Work is in progress to identify the PRRs implicated in the sensing of *F. culmorum spores*.

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Self-reported health symptoms associated with pesticide use among rice farmers in Zanzibar, Tanzania

Authors: Marisa da Silva[1], Nadja Stadlinger[2], Gaetano Marrone[1], Aviti J. Mmochi[3], Cecilia Stålsby Lundborg[1]

1 *Department of Public Health Sciences, Global Health (IHCAR), Karolinska Institutet, Stockholm, Sweden*
2 *Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm, Sweden*
3 *Institute of Marine Sciences, University of Dar es Salaam, Zanzibar Town, Tanzania*

Presenting Author: Email: mrsadasilva@gmail.com | Tel.: +46 762 944 441 | Fax: +46 (0) 831 1590

INTRODUCTION:

Agriculture is the most important economic sector and livelihood worldwide; this is especially true for low and middle-income countries (LMIC) (FAO, 2011). The agrarian population in LMIC suffers from a number of adverse health effects due to pesticide exposure such as neurological abnormalities, respiratory illnesses, and reproductive, endocrinological, and dermal problems. Acute pesticide poisoning (APP) is globally estimated to cause over 300 000 deaths per year (Kesavachandran et al., 2009).In Zanzibar, the government subsidizes pesticides to enhance local rice production by making pesticides affordable for an increased number of farmers (R. S. Mberek [Director at the Department of Agriculture, Zanzibar Ministry of Agriculture and Natural Resources],personal communication, April 9, 2013).

OBJECTIVES:

The study aim was to assess Zanzibar small holder rice farmers' pesticide use and self-reported health symptoms, in relation to training and protective measures under the current pesticide subsidies. The pragmatic aim of the study was to raise awareness of the problem situation for future local policy formulations.

MATERIALS AND METHODS:

An exploratory cross-sectional, interviewer-administrated study was conducted between February 27 and April 5, 2013, in seven villages with high levels of rice production and many households producing rice. The villages were situated on Unguja Island, the largest island of the two main islands of Zanzibar. Study participants were based on a purposive sample to include three categories of rice farmers: (1) pesticide users who personally applied pesticides, (2) pesticide users who did not personally apply pesticides, and (3) non-users. Local agricultural officers as key informants administrated the recruitment. The inclusion criteria were farmers cultivating rice and living at the study site. The additional sampling instruction the agricultural officers received were to recruit rice farmers of all ages and both sexes. A shortened and adapted questionnaire on pesticide use and health from a study in Vietnam by the World Bank Group (Dagsupta, Meisner, Wheeler, Xuyen&Thi Lam, 2007) was used. The adapted questionnaire included questions on socio-demographics, farming characteristics, pesticide use and exposure, knowledge, general health, and health symptoms directly related to pesticide use. The pesticide-related health symptoms included were skin irritation, eye irritation, headache, dizziness, convulsion, shortness of breath, fever, diarrhea and vomiting. The symptoms were compared between the three categories of rice farmers based on their expected exposure (users personally applying pesticides, users not personally applying pesticides, and non-users). Health symptoms and the three categories of farmers were further analyzed in relation to training and protective measures. Descriptive statistics were performed: mean, standard deviation and median were used for summarizing numerical variables, and frequencies and percentages for the categorical variables. Bivariate analyses with Chi-Square or Fisher´s exact test were conducted to compare two categorical variables and to examine significant differences. P-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION:

A total of 99 rice farmers between 23 and 88 years old with a mean age of 48 years were interviewed. Refusal rates were not collected. The majority of the farmers had attended school (81%), of which 46% had secondary school as their highest completed education. Most rice farmers (81%) used pesticides. The main reasons for not using pesticides among non-users were: lack of money, lack of availability of pesticides, and for having enough manpower to do weeding without pesticides. Half of the pesticide users personally applied the pesticides, and the other half did not personally apply. Yet, 70% of the pesticide users that did not personally apply pesticides were present during application, and the difference in self-reported health symptoms between the two categories of pesticide users was marginal. The most commonly self-reported symptoms among pesticide users in the acute phase were skin irritation (23%), eye irritation (21%) and headache (21%). On weekly basis pesticide users personally applying pesticides suffered more from pesticide-related symptoms than pesticide users not personally applying pesticides, and the non-users suffered the least from the same symptoms. Pesticide users who had received training in safe handling and application displayed a higher use of protective measures, yet only 50% of pesticide users had received such training. The most frequently used protective measures were full-length trousers, full sleeve shirt, hat/head cover and shoes/rain boots. Among less commonly used protective measures were gloves, mask and glasses. The use of protective measures (specifically gloves) had a significant protective effect on skin irritation and headache, which were among the most common self-reported symptoms related to pesticide use, however only 43% of pesticide users personally applying pesticides used gloves. This study indicates a positive relationship between training and the use of protective measures, and between the use of protective measures and health symptoms. The positive relationship between protective measures and health symptoms has previously been shown significant (Dagsupta et al., 2007; Jensen, Konradsen, Jørs, Petersen & Dalsgaard, 2011). All pesticide users were using herbicides for rice production. This perceived need of herbicides for weed management as a labor substitute for small-scale farmers is arguable (Sanderson, 2010). The government of Zanzibar is encouraging farmers to return to manual weeding by restricting the import of pesticides for agricultural use, simultaneously, the government is subsidizing the pesticides (R. S. Mberek [Director at the Department of Agriculture, Zanzibar Ministry of Agriculture and Natural Resources], personal communication, April 9, 2013). These contradictive policies are problematic in effort to change farmers' weed management and to become less dependent on pesticides. The study by Dagsupta et al. (2007) showed a weak association between self-reported symptoms and actual poisoning when using a medical test[acetyl cholinesterase enzyme (AChE) blood test]. This suggests a necessary use of medical tests as control measures in future research studying pesticides and adverse health effects.

CONCLUSION:

To elucidate the extent and severity of pesticide health effects in Zanzibar further studies also including medical tests are needed. This study highlights prevention of symptoms of pesticide use and policy implications. Based on the study results, it is strongly recommended to offer training in safe handling and application of pesticides, and to increase the availability and use of protective equipment for rice farmers in Zanzibar, particularly since the government currently subsidizes pesticides.

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Typification of respiratory diseases, exceedances of troposphere ozone and particulate matter (PM10) through latent class models

Authors: Maria Neves Ameida[1], Paula Vaz-Fernandes[2,3], Jaime Fonseca[3,4], Carla Martinho[5], Fernando Caetano[2,6]

1 *Public Health Unit ACES-GL-III, Regional Health Administration of Lisbon and Tagus Valley, Portugal;*
2 *Department of Science and Technology (DCeT), Universidade Aberta, Portugal;*
3 *Center of Public Policy and Management/Institute of Social and Politics Science (CAPP/ISCSP), Portugal;*
4 *University of Lisbon, CAPP, Institute of Social and Political Sciences, Portugal;*
5 *Institute of Accounting and Administration of Lisbon / Lisbon Polytechnic Institute;*
6 *Center of Structural Chemistry (CQE), IST-UTL, Portugal.*

Presenting Author: Email: paulavaz@uab.pt | Tel.: +351 213 916 300

INTRODUCTION:

The exposure to urban air pollutants continues to be a public health issue causing illness and death. Breathing small amounts of pollutants over many years is considered hazardous as well as breathing high concentrations of air pollutants. Traditionally measured pollutants are the fine particulates matter and tropospheric ozone. For these reasons, whenever the value of 180 g/m3 is exceeded, it is required to alert the population. There is currently no study made to assess the relationship between those pollutants and the frequency of respiratory diseases in the so called big Lisbon area of Amadora and Sintra which is the second highest density populated region in Portugal. The elder are the most susceptible to air pollution exposure and those with inflammatory disorders of the respiratory airways, such as asthma or severe bronchitis. Children are known as at greater risk because their lungs are still developing.

OBJECTIVES:

This work aims to study the relationship of the exceeding quantities occurred in the air pollutant (PM10) and tropospheric ozone (O3) in the Amadora and Sintra municipalities' inhabitants and the episodes of hospital admissions due to respiratory causes in all age groups. The data used relates to the period between 2004 and 2009.

MATERIALS AND METHODS:

We appealed to the records of Hospital Diagnostic Groups (GDH), which provided some data on the hospitalization for respiratory pathologies classified according to the Disease International Classification (ICD-9). We have analyzed this data of respiratory diseases occurred between the years 2004 and 2009, for users whose home is located in the municipalities of Amadora and Sintra and went to the three Hospitals from the National Health Service (NHS) - Central, West and North Lisbon Hospitals Centers, and one referral Hospital (Fernando da Fonseca). The data corresponding to GDH respiratory diseases were provided by the Central Administration of Health Services (ACSS) and the data relative to the two pollutants (ozone and particulates) were provided by the online database of Portuguese Environmental Agency, Department of Atmospheric Air Quality. The analysis was made for the complete years and all seasons. Concerning the optimal way to present research evidence for the episodes of hospital admissions due to respiratory causes, we argue that a simplified conceptual model is given by Latent Class Model multivariate statistical methods (Fonseca 2013a; Fonseca2013b; Fonseca 2008).

RESULTS AND DISCUSSION:

Regarding the referral Hospital our findings show that we have a cluster1 with 91 per cent of cases, characterized by PM10 (Mean) = 1.49, Ozone (Mean) = 0, and CID9: Acute respiratory infections; Pneumonia and influenza; other diseases of the respiratory tract; Pneumoconiosis and other pulmonary diseases. They

are aged up to 20 and more than 69 years old, months: January, February, March, April, May, October, November, December, years: 2004, 2007, 2008. A cluster 2 with 9 per cent of cases, with PM10 (Mean) = 1.93, Ozone (Mean) = 4.59, and CID9: Other respiratory diseases; chronic obstructive pulmonary disease and similar. They are aged between 20 and 69 years old, months: June, July, August, and September, years: 2005, 2006, 2009. Furthermore, we found that ozone is correlated with both PM10 and month, PM10 is correlated with CID9, parish and month. Regarding Hospital Centers, the results obtained by estimating latent class models shows that there are 3 groups of such cases, respectively with 52, 40 and 8 percent of cases. In group 1 are mostly individuals belonging to the age group up to39 years without a characteristic type of disease; they moved mostly to the Central Hospital in 2007, 2008 and 2009, in the months of April, May, September, November and December. Group 2 consists of 40% of individuals, mostly from the age group 40 to 44 years old and characterized by all the listed diseases; recorded the highest mean value of PM10 (3,248) and cases were seen mainly in North hospital in 2004, in the months of January, February, March and October, and mostly in the municipality of Amadora. Group 3 is mostly made up of cases with more than 44 years with other respiratory diseases and other respiratory diseases; there were higher values of ozone (average 4,012) and significant values of PM10 (average 1,986) and cases were seen mainly in the Western Hospital in 2005 and 2006, in the months of June, July and August, and mostly the county Sintra.

CONCLUSION:

From the results of referral Hospital, we may conclude that respiratory pathologies classified according to the Disease International Classification (CID-9) can be discriminate by PM10 and Ozone, age, month and year. Moreover we can conclude that CID-9 is correlated with PM10 and age. From the results of Hospital Centers, we may conclude that there is no relationship between ICD9 and PM10 values (p = 0.885) but there is a relationship between ICD9 and ozone values. Meanwhile, the months are related to ICD9 (p = 0.000). The relationship between age and ICD9 is statistically significant (p value =0.000).

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Frailty in the elderly: current identification and alternative markers

Authors: Vanessa Valdiglesias[1], José C. Millán-Calenti[3], María Sánchez-Flores[1,2], Ana Maseda[3], Eduardo Pásaro[1], Laura Lorenzo-López[3], Blanca Laffon[1]

1 *DICOMOSA Group, Department of Psychology, Area of Psychobiology, Universidade da Coruña, Spain*
2 *Department of Cell and Molecular Biology, Universidade da Coruña, Spain*
3 *Gerontology Research Group, Department of Medicine, Universidade da Coruña, Spain*

Presenting Author: Email: vvaldiglesias@udc.es | Tel.: +34 981 167 000 | Fax: + 34 981 167 172

INTRODUCTION:
The current progressive population ageing of the developed societies has conditioned an increase in the age-dependent pathologies and, therefore, a higher possibility of developing disability and/or dependence with the subsequent socioeconomic and sanitary implications. “Frailty”, as a condition opposed to full health or “fitness”, is a multidimensional syndrome with mental and physical connotations which involves an increase in vulnerability, understood as disability and/or dependence, generally imminent. Epidemiologic research to date has led to the identification of a number of risk factors for frailty. Some of them include diseases or physiologic impairments closely related to exposure to environmental factors.

At present, frailty assessment is mostly based on phenotypic features, namely unintentional weight loss, self-reported exhaustion, low physical activity, slow walking speed and grip strength (Fried et al., 2001), or in a cumulative index of health deficits (Rockwood & Mitnitski, 2007).

OBJECTIVES:
On the basis of evidences continuously increasing in the literature in the last years, support for the possible existence of other biomarkers of frailty, mainly at cellular and molecular levels, is being obtained. They could also be considered in order to identify frail elder patients.

MATERIALS AND METHODS:
Cellular and molecular biomarkers of frailty may include indicators of genomic instability, endocrine and immunological status and oxidative stress status.

RESULTS AND DISCUSSION:
Determining and validating these biomarkers would provide a significant advance in old age care since it would allow to anticipate frailty state and detect vulnerable patients before clinical manifestations become evident. Thus, together with the improvement of quality of life in the elderly, it would also let promote personalized healthcare, introduce suitable dependency programs, and reduce socioeconomic and sanitary costs.

CONCLUSION:
Advances about potentially modifiable risk factors for frailty (environmental factors among them) offer now the basis for translational research effort aimed at prevention and treatment of frailty in elder adults. So anticipating frailty state through the use of cellular and molecular biomarkers provides a powerful tool for implementing effective preventive actions by removing/modifying risk factors.

ACKNOWLEDGMENTS:
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Lymphocyte subsets in a population of non-frail elder individuals

Authors: Blanca Laffon[1], Ana Maseda[3], Eduardo Pásaro[1], José C. Millán-Calenti[3], Laura Lorenzo-López[3], María Sánchez-Flores[1,2], Vanessa Valdiglesias[1]

1 *DICOMOSA Group, Department of Psychology, Area of Psychobiology, Universidade da Coruña, Spain*
2 *Department of Cell and Molecular Biology, Universidade da Coruña, Spain*
3 *Gerontology Research Group, Department of Medicine, Universidade da Coruña, Spain*

Presenting Author: Email: blaffon@udc.es | Tel.: +34 981 167 000 | Fax: +34 981 167 172

INTRODUCTION:

Age-related frailty is a growing challenge for societies worldwide. It is generally agreed that frailty is characterised by increased vulnerability to stress due to decline in homeostatic reserve secondary to deregulation in multiple inter-related systems, which results in an increased risk of adverse health outcomes including disability, hospitalisation, institutionalization and death. Among the risk factors identified for frailty, some are associated with environmental chemical exposures, so frailty can be considered as an environmental health negative outcome. Numerous data have been published so far confirming the relationship between frailty and immunological system alterations, including immunosenescence or inflammation markers (reviewed in Collerton et al., 2012). Thus, analysis of immunological changes, such as alterations in lymphocyte subsets, during senescence may provide useful prognostic markers for frailty and mortality. Reference ranges currently used for lymphocyte subpopulations are referred in general to adult individuals, without specifically separating the elderly group.

OBJECTIVES:

To assess the evolution of the different lymphocyte subsets with age in non-frail elder individuals, and to determine other possible physiological or lifestyle factors influencing these immunological variables.

MATERIALS AND METHODS:

The study was performed on 143 elder individuals (aged 65-95) from Galicia (NW of Spain). Frailty status in these individuals, according to five phenotypical features (i.e. weight loss, exhaustion, physical activity, walk time and grip strength) was evaluated following the criteria proposed by Fried et al. (2001). Fresh whole blood samples were collected and used to analyse the percentage of the different lymphocyte subpopulations by means of flow cytometry following García-Lestón et al. (2011): CD3+ T-lymphocytes, CD4+ T-helper lymphocytes, CD8+ T-cytotoxic lymphocytes, CD19+ B-lymphocytes, and CD56+16+ natural killer (NK) cells.

RESULTS AND DISCUSSION:

Although the mean values obtained for all lymphocyte subsets were within the reference ranges previously established for a population of Spanish adults (García-Dabrio et al., 2012), several individuals presented values out of the range limits. A clear influence of age was obtained for CD3+ and for CD4+ cells, especially evident in the group of individuals aged ≥90. Women had higher levels of CD19+ lymphocytes, and no influence of smoking was observed.

CONCLUSION:

Results obtained will serve as a basis to establish comparisons between frail and non-frail elder individuals, in order to determine the usefulness of lymphocyte subsets as prognostic immunological biomarkers for frailty.

ACKNOWLEDGMENTS:
Research funded by a project from Xunta de Galicia (EM 2012/100).

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Cadmium and lead levels in the human brain tissue don't significantly depend on smoking habits

Authors: Patrícia Ramos[1], Nair Pinto[2], Ricardo Mendes[2], Agostinho Santos[2-5], Agostinho Almeida[1]

1 *REQUIMTE, Laboratory of Applied Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal*
2 *National Institute of Legal Medicine and Forensic Sciences, North Branch, Portugal*
3 *Faculty of Medicine, Porto University, Portugal*
4 *School of Health Sciences, University of Minho, Portugal*
5 *CENCIFOR - Forensic Science Center, Portugal*

Presenting Author: Email: patricia-ramos@sapo.pt | Tel.: +351 220 428 667 | Fax: +351 222 003 977

INTRODUCTION:

Worldwide, cigarette smoking causes more than 5 million deaths per year (World Health Organization, 2011). As a result of nicotine addiction, the repeated inhalation of different toxicants in cigarette smoke, including nitrosamines, volatile organic compounds, polycyclic aromatic hydrocarbons and several toxic heavy metals (such as lead and cadmium), leads to smoking-related diseases (Talhout et al., 2011). The scientific literature mainly focuses on the harmful health effects of nicotine, tar, carbon monoxide and other noxious gases emitted in tobacco smoke. Only a small number of studies have been conducted on the role of toxic trace elements in tobacco smoke and their effects on biochemical processes in the human body.

In the general population, the main sources of cadmium (Cd) exposure are cigarette smoke, water and food (e.g. vegetables, cereals and shellfish). Along with deteriorated lead (Pb) painting in older housing, air, soil, food and water (contaminated namely by Pb-containing ceramic dishware, metal plumbing and food cans) are the major sources of exposure to Pb. The contribution of smoking to the total Pb load has become increasingly relevant due to the gradual reduction of Pb addition to gasoline in the last decades(Bernhard, Rossmann, & Wick, 2005).

Prolonged exposure to Cd will cause toxic effects due to its accumulation over time in several tissues including kidneys, liver, central nervous system (CNS) and peripheral neuronal systems. The CNS is the primary target of Pb toxicity but exposure to this metal can also damage hematopoietic, renal and skeletal systems. Under normal conditions, Cd and Pb barely reach the brain in adults due to the brain blood barrier (BBB) protection. Since this structure is not fully mature in early age, children are particularly susceptible to exposure and subsequent brain damage. However, organic Pb compounds penetrate cell membranes and may also cross the BBB in adults; and it was also demonstrated that Cd can increase the permeability of the BBB in adults, leading to brain intracellular accumulation and cellular dysfunction.

Through the inhalation of smoke, either by active or passive smoking, Cd and Pb enter into the body, and it is well known that these (and other) toxic metals exist in higher concentration in smokers' tissues(Bernhard et al., 2005). Due to their long biological half-life, adverse effects on human health observed in old age may result from prolonged intake (even at very low levels) of such toxic elements. There are growing data suggesting that disturbances of metal homeostasis in the human body by cigarette smoking play a keyrole in the pathogenesis of several diseases, but the exact mechanisms remain poorly understood. Increasing evidence has demonstrated that Cd is a possible etiological factor of neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases (Wang & Du, 2013). Lead is also recognized as a risk factor for neurologic and psychiatric disorders, inducing damage in brain regions related to cognitive functions (Verstraeten, Aimo, & Oteiza, 2008).However, most of the current knowledge about the relationship between trace elements and brain functioning is based on animal studies or relies on determinations in cerebrospinal fluid or blood. Studies involving the direct determination of trace elements (both "toxic" and "essential") in

normal and pathological human brain tissue are scarce, limited to a few brain areas and/or involve a reduced number of subjects.

OBJECTIVES:

Based on this background, the main goal of this study was 1) to establish the "normal" range for Cd and Pb in the human brain tissue and evaluate 2) the regional anatomic differences, 3) the changes in relation to age, and 4) the effect of smoking habits on Cd and Pb levels.

MATERIALS AND METHODS:

From neurologically and psychiatrically healthy individuals submitted to autopsy (n=39; 53 -88 years old) the following 14 brain regions were sampled: frontal cortex, superior and middle temporal gyri, caudate nucleus, putamen, globus pallidus, cingulated gyrus, hippocampus, inferior parietal lobule, occipital lobe, midbrain, pons, medulla and cerebellum. After microwave-assisted acid digestion of the samples, Cd and Pb were determined by Inductively Coupled Plasma-Mass Spectrometry.

RESULTS AND DISCUSSION:

Cadmium was present at ca. 2.9-fold higher levels than Pb (mean±sd: 261±121 ng/g versus 91±47 ng/g). Distribution across the different brain regions showed to be quite homogeneous. A tendency for Cd levels to increase with age in some of the brain regions was found (Figure 1), namely in hippocampus and middle temporal gyrus, two regions related to memory functions. On the contrary, Pb seems to remain quite unchanged irrespective of ageing.

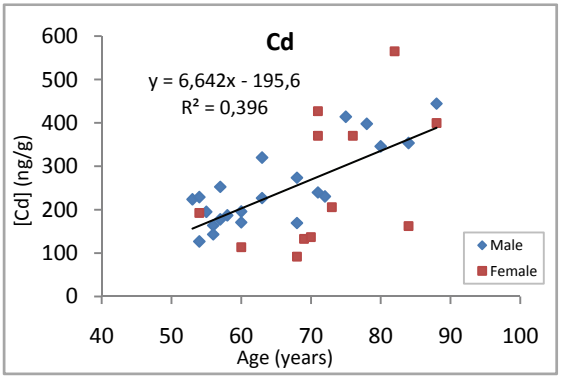


Figure 1 - Relationship between Cd (ng/g) in the human brain tissue and age (n=39).

A gender-related difference was found for Pb (men: 107±40 ng/g versus women 51±29 ng/g; p<0.0001). No significant differences were found between smokers and non-smokers for both Cd and Pb levels (Figure 2). Although higher Cd and Pb levels were found in brain tissue of smoker women, the results did not reach statistical significance.

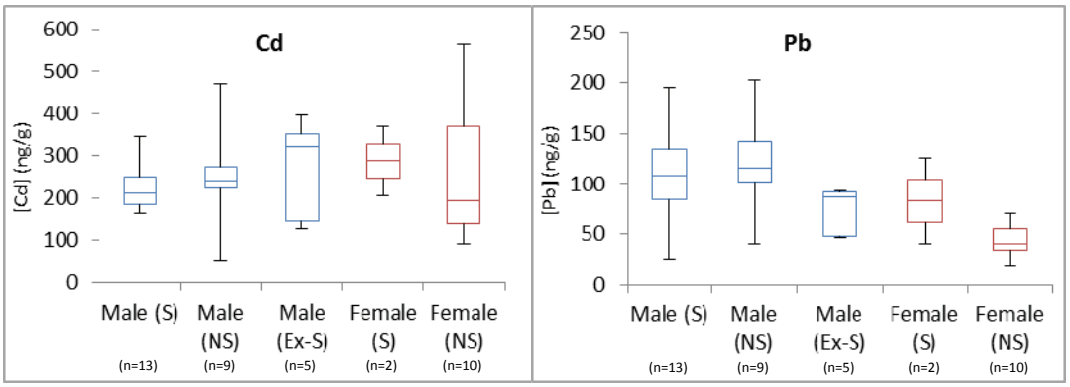


Figure 2 - Cadmium and Pb levels (ng/g) in the human brain tissue in smokers (S), former smokers (Ex-S) and non-smokers (NS) individuals.

CONCLUSION:

Although confounding factors such as occupational exposure or dietary habits were not assessed, this work represents a contribution to clarify the Cd and Pb role in smoking-related diseases process. It provides data on the levels of Cd and Pb in 14 different brain regions of neurologically and psychiatrically healthy individuals ("normal" values), a useful reference for future interpretation of results found in brain tissue affected by neurodegenerative (and other) diseases.

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Elderly at risk during heat waves - what can be done? Results of the climate adaptation study "Cool towns for the elderly"

Authors: Hans-Peter Hutter[1], Arne Arnberger[2], Brigitte Allex[2], Renate Eder[2], Franz Kolland[3], Anna Wanka[3], Beate Blättner[4], Henny Annette Grewe[4], Michael Kundi[1], Peter Wallner[1,5]

1 *Institute of Environmental Health, Center for Public Health, Medical University Vienna, Austria*
2 *Institute of Landscape Development, Recreation and Conservation Planning, University of Natural Resources and Life Sciences Vienna*
3 *Institute of Sociology, University of Vienna*
4 *Department of Nursing and Health Sciences, University of Applied Sciences Fulda*
5 *Medicine and Environmental Protection, Vienna, Austria*

Presenting Author: Email: hans-peter.hutter@meduniwien.ac.at | Tel.: +43 1 40160-34930

INTRODUCTION:

Intensive heat load has negative impacts on human health and results in higher morbidity and mortality during and post heat waves. (e.g. Hajat et al. 2014, Gabriel & Endlicher 2011). Particularly at risk were elderly resp. very elderly and socially isolated persons (WHO 2011). Predicted changes in climate (more frequent heat periods) (Formayer et al. 2008) and demographic changes increase the likelihood of enhanced risk for the elderly during and after heat waves in the future. In the absence of adaptation measures, heat-related deaths would be expected to rise in this risk group (Kravchenko et al. 2013).

OBJECTIVES:

Aim of the study "Cool towns for the elderly - protecting the health of elderly residents against urban heat (STOPHOT)" was, inter alia, to explore how elderly people perceive heat and how they adjust their behavior in response to heat. Targeted adaptation strategies should be developed for this population group at high risk.

MATERIALS AND METHODS:

Using a standard questionnaire (including SF-6, de Jong-Gierveld loneliness scale and assessment of contentment with current living quarters), 400 subjects older than 65 years living in four different urban districts (varying proportion of green spaces, differences in socio-economic structure) were interrogated via computer-assisted telephone interviews in 2011 (first survey). In addition, face-to-face interviews with elderly residents in nursing homes (n=200) were performed. Furthermore, 15 in-depth interviews with stakeholders were carried out (Allex et al. 2013).

The acceptance of adaptation strategies (based on the outcome of the first survey and stakeholder input) was verified by a second survey (2013) of elderly living in heat urban islands (personal interviews, n=200). Main part of the questionnaire was a visual Choice Model. The results were presented and discussed at another workshop. An overview is shown in figure 1.

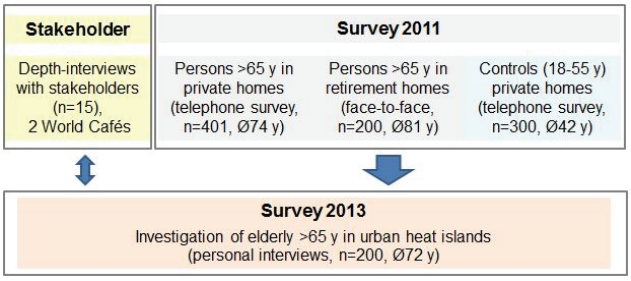


Figure 1 - Study design overview (mixed method design)

RESULTS AND DISCUSSION:

Analysis of the in-depth interviews with stakeholders showed that there was variable perception regarding urban heat and the elderly. Most experts were aware of the issues of climate change and heat waves, but had not considered them with respect to the elderly. The majority of the stakeholders did not feel any direct responsibility regarding these issues, but instead mentioned the need for a multidisciplinary collaboration.

Results of the first survey (2011) showed that participants adjusted their behavior during a heat wave mainly by wearing lighter clothes, increasing their liquid intake, carrying out activities mainly in the morning and the evening, drawing curtains and closing windows during daytime (each >80%). Sixtysix percent of the participants in private homes and 70% in retirement home stay indoors during heat periods (reasons: less heat in indoor environments [75%], health conditions, social isolation, and insecurity [25%]). However, those who are leaving their homes showed significant fewer symptoms during heat events (Arnberger et al. 2013). Half of those with low educational level, but only a quarter of those who had high school or university degrees, perceived great discomfort due to heat stress.

Socially disadvantaged people showed more heat related symptoms. Mental health problems were associated with anxiety during heat periods. This was true for respondents in private as well as in retirement homes. Our results confirm that less or little drinking is associated with more frequent (typical) heat symptoms (e.g. tiredness, dizziness, going to black out, difficulties to breath). Elderly suffering from heat stress were more likely to stay indoors.

Within the **second survey** (2013) participants were asked to rate selected adaptation measures. As most important "more shadow" (e.g. more shadowy seating options in urban areas, more cool areas in parks) was rated. Communication of heat related information (e.g. more information given by physicians) was seen less important.

Although the questionnaire was conducted with participants living in urban heat islands, only half of the respondents had the feeling to live in an especially hot area in Vienna.

Two third of the respondents (68%) were always taking actions as soon as they perceived heat in order to feel more comfortable. No significant differences (age, gender, family status) were seen concerning heat perception at home. With regard to education, 50% of respondents with less than 8 years education and 71% of respondents with more than 8 years education were reacting promptly to heat exposure.

CONCLUSION:

Health protection from heat periods will become increasingly necessary. First analysis showed a positive view of elderly people towards possible heat related measures. Some of our results are confirming existing knowledge, while other results showed the complexity of the research fields "elderly persons and heat" and were difficult to interpret so far.

However, until now only few specific adaptation measures for elderly people are implemented in Vienna. In a next step coping strategies and measures suitable for implementation by the investigated population will be summarized and described in an online manual. This should serve as scientifically sound basis for decision makers to implement measures.

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Daily dose of particulate matter in the elderly respiratory tract

Authors: Marina Almeida-Silva[1,2], Marika Pilou[3], Christos Housiadas[3], Hubert T. Wolterbeek[2], Susana M. Almeida[1]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, EN 10, km 139.7, 2695-066 Bobadela LRS, Portugal.*

2 *Faculty of Applied Sciences, Department of Radiation, Radionuclides and Reactors, Section RIH (Radiation and Isotopes in Health), Technical University of Delft, Delft, The Netherlands.*

3 *Thermal Hydraulics & Multiphase Flow Laboratory, INRASTES, National Centre for Scientific Research "Demokritos", 15310 Agia Paraskevi, Greece.*

Presenting Author: Email: marina@ctn.ist.utl.pt | Tel.: +351 219 946 130

INTRODUCTION:

The concerning about exposure to particulate air pollution and their possible human health effects is not a current problem. Several epidemiological studies have established associations between exposure to Particulate Matter (PM) and adverse human health effects. We are observing an increase of studies developed by the scientific community concerning Indoor Air Quality (IAQ) and its effects upon health, (Canha et al., 2012), since people spend a large part of their life inside the indoor environments (more than 80-90%) which have promoted an increase on exposure to indoor air pollutants (Almeida-Silva et al., 2014). These facts are particularly relevant when we are talking about institutionalized elderly people not only because they are considered a susceptible group but also because they spent the majority of their time indoors (Almeida-Silva et al., 2014). According to the United Nations (2012) the percentage of total population aged 60 years or over in the Europe was 22% for the year 2012 and prospects 34% for 2050 (United Nations, 2012).Portugal is the 8th oldest country in the world and the 6th in Europe, with 23% of population with more than 60 years old. In Portugal, the number of Elderly Care Centers (ECCs) increased 49% between 1998 and 2010 (GEP/MSSS, 2010).

OBJECTIVES:

The main goal of this work was to link the external exposure to the internal dose in the elderly respiratory tract.

MATERIALS AND METHODS:

The current study was carried out in 10 ECCs, located in Lisbon(Figure 1). This region is located in the west of Portugal, on the Atlantic Ocean coast, being the westernmost capital in mainland Europe. The metropolitan area of Lisbon has an area of 2870 km2 and has almost 3 million inhabitants (INE, 2012).

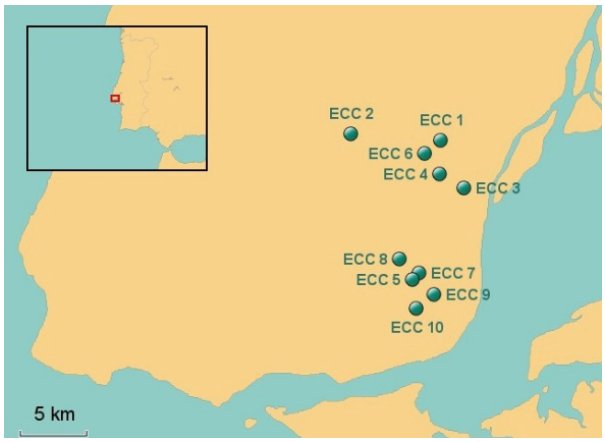
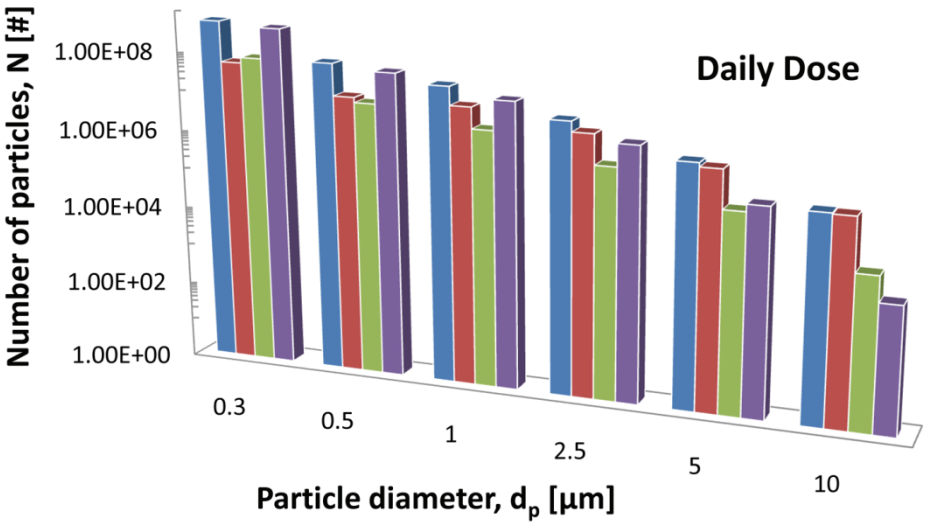


Figure 1 - Geographical distribution of the 10 ECCs selected.

The present work was developed in collaboration with 384 old people living in ECCs which had a range of 7-95 occupants per institution. A time-budget survey (TBS) was applied in order to get information about the time occupancy. In each ECCs particulate matter in different sizes were measured during 7 to 16 hours in two different indoor micro-environments: bedroom and living-room. Then, a mechanistic dosimetry model (Mitsakou et al., 2005, 2007) was used to calculate particle deposition along the human respiratory tract (HRT) during exposure to the aforementioned aerosol sources. The model solves numerically the general dynamic equation (GDE) of the aerosol population in an Eulerian framework. Moreover, the simultaneous action of different mechanisms, such as particle gravitational settling, diffusion, and inertial impaction are taken into account in order to obtain particle deposition.

RESULTS AND DISCUSSION:

The data analysis showed that there are no statistically significant differences between the measurements obtained in the different ECCs. Therefore it was decided to use the average exposure as input in the HRT deposition model. Moreover, the TBS demonstrated that the elderly in average spend 57% of the day in the bedroom and 30% of the day in the living-room (Almeida-Silva et al., 2014).The daily dose in number of particles was calculated assuming that the elderly slept during their stay in the bedroom and were sitting awake during their stay in the living room. In Figure 2, the daily dose per particle diameter is given for the whole HRT, as well as for its different regions. In addition, it was found that the bedroom micro-environment contributed more in the daily dose of sub-micrometer particles (70% for the 0.3μm and 66% for the 0.5μm particles), whereas the contribution of bedroom and living-room are almost equivalent for particles diameters higher than 1μm.



	0.3μm	0.5μm	1μm	2.5μm	5μm	10μm
TOTAL	5.50E+08	7.13E+07	3.03E+07	7.10E+06	1.26E+06	1.52E+05
ET	5.23E+07	1.11E+07	1.04E+07	3.90E+06	9.92E+05	1.44E+05
TB	7.16E+07	8.30E+06	3.03E+06	6.86E+05	1.08E+05	6.70E+03
AI	4.26E+08	5.19E+07	1.69E+07	2.52E+06	1.63E+05	1.54E+03

ET: Extrathoracic, TB: Tracheobronchial, AI: Alveolar Interstitial

Figure 2 - Daily dose (in absolute number of particles) per particle diameter for the total HRT and its regions.

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Cadmium levels in kidney tissue, smoking habits and cardiovascular disease

Authors: Anne Alves[1], Patrícia Ramos[1], Maria Moura[2], Débora Lourenço[2], Ricardo Mendes[2], Agostinho Santos[2-5], Agostinho Almeida[1]

1 *REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal*
2 *National Institute of Legal Medicine and Forensic Sciences, North Branch, Portugal*
3 *Faculty of Medicine, Porto University, Portugal*
4 *School of Health Sciences, University of Minho, Portugal*
5 *CENCIFOR - Forensic Science Center, Portugal*

Presenting Author: Email: alvesannesophie@gmail.com | Tel.: +351 220 428 667 | Fax: +351 222 003 977

INTRODUCTION:

Cadmium (Cd) is a highly toxic trace metal, with an extremely long half-life. Prolonged exposure to Cd can affect a variety of organs, with the kidneys and bones being the most sensitive (ATSDR, 2012).

Exposure to Cd mainly results from contaminated food, cigarettes smoke and occupational exposure (Cd-contaminated workplaces). Accumulation of Cd in tobacco plant can vary widely: average concentrations are 1-2 µg/g of dry weight or 0.5-1.0 µg per cigarette. Approximately 10% of cadmium oxide (CdO) produced during cigarette smoking is absorbed into the blood circulation (Johri, Jacquillet, & Unwin, 2010).

After absorption, Cd is taken up by the hepatocytes, and then from the liver it circulates in the blood bound to metallothioneins (Mt). At the kidney, the Cd-Mt complex, because of its small molecular size, is readily filtered through the glomerular membrane and reabsorbed by proximal tubular cells. Metallothioneins are then catabolized releasing Cd ions in the tubular cells cytoplasm where they induce the synthesis of new Mt molecules (ATSDR 2012). It then remains in the tubular cells and makes up for the major part of the Cd body burden. A number of studies have shown that this accumulation of Cd in kidneys causes tubular dysfunction and renal end stage failure (Bernhard, Rossmann, & Wick, 2005).

Chronic exposure to Cd has been associated with cardiovascular diseases (CVD). Increasing evidence supports that Cd may play a role in the development of a number of traditional CVD risk factors, including hypertension and chronic kidney disease, which could mediate in part the cardiovascular effects of Cd (Tellez-Plaza, Jones, Dominguez-Lucas, Guallar, & Navas-Acien, 2013). However, most existing studies on this topic are simply based on the analysis of body fluids such as blood and urine, since they can be easily obtained.

OBJECTIVES:

Based on this background we have been performing a study aimed to find direct evidence, i.e., in the human body tissues themselves, of the changes in Cd levels with age, the effect of smoking habits and the possible link with CVD. Here we report the results regarding Cd levels in kidney tissue.

MATERIALS AND METHODS:

Kidney samples (cortex and medulla) were collected from individuals submitted to autopsy (n=67; 22-93 years old). Information on gender and cause of dead (namely previously known CVD disease) was also obtained. After microwave-assisted acid digestion of the dried samples, Cd was determined by Inductively Coupled Plasma-Mass Spectrometry. The results were expressed as µg/g on a dry-weight basis.

RESULTS AND DISCUSSION:

Cadmium levels showed to be related with the smoking habits. Higher Cd levels were found in both the kidney cortex and medulla of smokers: 109±49 µg/g versus 45±30 µg/g; p<0.0001 and 55±50 µg/g versus 27±24 µg/g; p= 0.0107, respectively (Figure 1).

Globally (i.e., for the n=67 individuals), a significant difference between Cd levels in the cortex and medulla was found: 70±48 µg/g versus 42±43 µg/g; p=0.0168). The higher levels of Cd in the cortex can be explained by the fact that, as previously mentioned, the renal filtration and reabsorption of Cd-Mt complexes occurs, respectively, at the glomerulus and proximal tubules, which are nephron structures located in renal cortex.

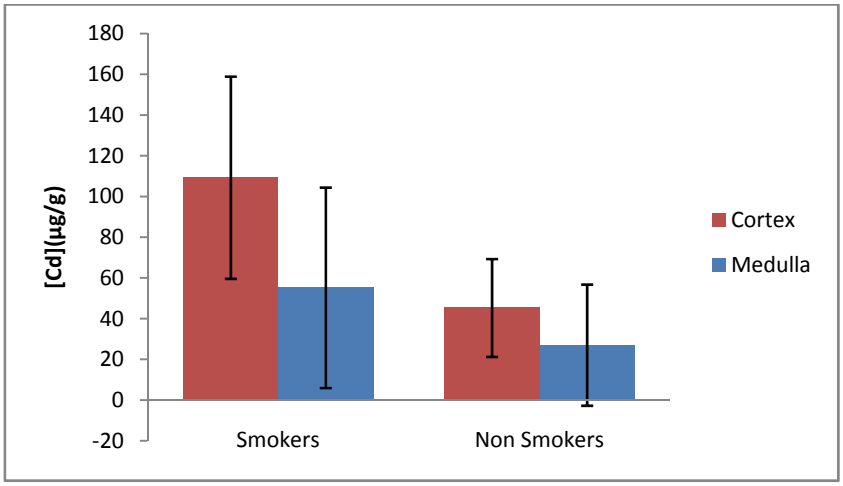


Figure 1 - Cadmium levels (µg/g dry weight) in cortex and medulla of smokers and non-smokers

For Cd levels in the cortex, an age-related increase until 50 years old and then a decrease was found, which is in agreement with previous studies (Figure 2). This decline may be due to an age-related degeneration or Cd-induced damage of the kidney. In the order hand, no significant age-related changes were found for Cd levels in the medulla. No significant difference was also found between male and female individuals.

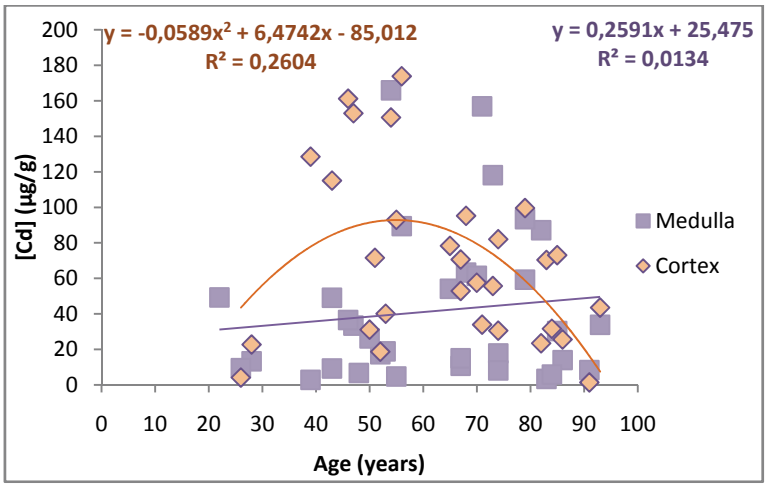


Figure 2 - Relationship between [Cd] (µg/g dry weight) in kidney and age (years)

Higher kidney Cd levels were found in patients with CVD (n=11), but the difference did not reach statistical significance (62±50 µg/g vs. 45±44 µg/g; p=0.3392). Results on this subject have been conflicting. Some authors have even found lower renal levels of Cd in hypertensive than in normotensive patients (Østergaard, 1977).

CONCLUSION:

Smoking habits significantly affect the levels of Cd in renal tissue. Due to the normal mechanism of renal excretion of Cd, significantly higher levels are found in cortex than in medulla. Patients with CVD showed no significantly higher Cd levels. Results on this topic require further studies.

ACKNOWLEDGMENTS:

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Influence of smoking habits on lead, nickel and cadmium levels in lung tissue - evidences from post-mortem analysis

Authors: Mariana Cruz[1], Patrícia Ramos[1], Anne Alves[1], Maria Moura[2], Débora Lourenço[2], Ricardo Mendes[2], Agostinho Santos[2-5], Agostinho Almeida[1]

1 *REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry Faculty of Pharmacy, University of Porto, Portugal*
2 *National Institute of Legal Medicine and Forensic Sciences, North Branch, Portugal*
3 *Faculty of Medicine, Porto University, Portugal*
4 *School of Health Sciences, University of Minho, Portugal*
5 *CENCIFOR - Forensic Science Center, Porto*

Presenting Author: Email: up201206485@up.edu.pt | Tel.: +351 220 428 667 | Fax: +351 222 003 9 77

INTRODUCTION:

Lung cancer is the most common cancer in the world and a major public health problem. According to the International Agency for Research on Cancer (IARC) latest data (GLOBOCAN, 2012), by the year 2012 there were estimated approximately1,824,701 new cases of lung cancer, representing 13.0% of all new cancers. Lung cancer was also the most common cause of death from cancer, with 1,589,800 deaths, representing 19.4% of the total.

The major risk factors for lung cancer (smoking habits, occupational exposure, environmental pollution, diet, age, gender, race, genetic susceptibility, family history and previous respiratory diseases) have been extensively studied. Linked to some of them (e.g., smoking habits, environmental and occupational exposure, diet) is the exposure to trace elements, particularly the so-called heavy metals, such as cadmium (Cd), nickel (Ni) and lead (Pb), whose involvement in the development of lung cancer have become increasingly suspected (Navarro Silvera,& Rohan, 2007).

Most trace elements are found naturally in the environment, and human exposure derives from a variety of sources including air, drinking water and food (Navarro Silvera, & Rohan, 2007). Cd is an environmental pollutant, highly toxic, one of the most important heavy metals when the adverse health effects of smoking are considered (Bernhard, Rossmann, & Wick, 2005). In vivo and in vitro studies suggests that Cd may cause numerous cytotoxic and metabolic effects that have not been sufficiently recognized, such as changes in enzyme activity, changes in proteins with sulphydryl groups (thioneins), induction of oxidative stress and apoptosis, changes in the structure and/or function of cell membranes, changes in DNA structure and altered gene expression, inhibition of ATP production in mitochondria and interaction with zinc (Zn), copper (Cu), calcium (Ca), selenium (Se) and other essential trace elements (Matovic, Buha, Bulat, & Dukic-Cosic, 2011). Ni and Pb are also in the origin of several cellular disorders, like oxidative stress, inhibition of intercellular communication, induction of DNA deletions and aberrations, reasons why they can display tumor promoting activity (Navarro Silvera,& Rohan, 2007).Ni, Cd and Pb are traditionally associated to tobacco smoke, however the link between Ni, Cd and Pb exposure and lung cancer, in particular their effective role in the development of the disease, is not fully understood (Bernhard, Rossmann,& Wick, 2005).

OBJECTIVES:

The present work is part of an enlarged project aimed to study the role of trace elements in lung cancer. The levels of Cd, Ni and Pbin lung tissue of healthy (non-cancer) individuals were determined in order to define the “normal” levels. Gender differences and the effect of smoking habits were also evaluated.

MATERIALS AND METHODS:

Samples (fragments of ca.1 cm3) from the right lung upper lobe were collected during autopsy exams at the National Institute of Legal Medicine and Forensic Sciences (INMLCF), North Branch, Porto. Dried samples (dried to constant weight) were solubilised through a closed-vessel microwave-assisted acid digestion procedure and the solutions obtained were analysed for trace elements concentration by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).Results were expressed in µg/g on a dry-weight basis. Student' s t-test was used for means comparison. Statistical significance was assumed for $P<0.05$.

RESULTS AND DISCUSSION:

Table 1 summarizes the results obtained. Globally Ni and Cd were present at quite similar levels (0.90±0.64 µg/g and 0.80±1.01 µg/g, respectively). Pb was present at much lower values: 0.36±0.27 µg/g.

For Ni and Pb, no significant differences were found between male and female subgroups. Men presented higher mean values than women, especially for Pb (0.41±0.29 vs. 0.22±0.15; P=0.058), but the difference reached no statistical significance. Also, smoking habits didn't show to significantly influence the Ni and Pb content in lung tissue. The mean value was higher in smokers but again the difference reached no statistical significance. The Pb content in cigarettes is around 1.2 g/cigarette, but only about 6% passes to the smoke which is inhaled by smokers. The Ni content in cigarettes varies highly, and may be somewhat higher than the Pb content, reaching 5 g/cigarette (Bernhard, Rossmann, & Wick, 2005). In both case, however, smoking seems to be not the main source of lung exposure to these heavy metals.

Mean Cd content in lung tissue was0.80±1.01µg/g (median = 0.34 µg/g), ranging from 0.056 µg/g to 3.53 µg/g. Men showed significantly higher values than women: 0.96±1.10 µg/g vs.0.20±0.08 µg/g (median: 0.44 and 0.19 µg/g, respectively). Also, smokers showed significantly ($P<0.0001$) higher Cd levels than non-smokers: 1.76±1.10 µg/gvs. 0.27±0.17 µg/g (median: 1.72 µg/g and 0.20 µg/g, respectively).Excluding some particular occupational conditions, smoking is considered to be the main source of Cd exposure in humans. The Cd content in cigarettes ranges between 0.5 and 1.5 µg, but much of it transferred to the smoke (as CdO), and approximately 10% is deposited in the lungs (Bernhard, Rossmann, & Wick, 2005), which explains our results.

It should be noted that the differences found between male and female may simply be due to the different number of smokers in each subgroup: 2 in female subgroup and 9 in the male subgroup.

Table 1 - Results (µg/g dry weight) for Ni and Pb content in lung tissue

Ni	**Total** (n=37)	**Male** (n=27)	**Female** (n=10)	**Smokers** (n=11)	**Non-Smokers** (n=17)
Mean±sd	0.90±0.64	0.88±0.62	0.74±0.42	0.99±0.98	0.88±0.46
P value		*0.547*		*0.678*	
Pb	**Total** (n=36)	**Male** (n=26)	**Female** (n=10)	**Smokers** (n=10)	**Non-Smokers** (n=17)
Mean±sd	0.36±0.27	0.41±0.29	0.22±0.15	0.43±0.38	0.30±0.18
P value		*0.058*		*0.247*	
Cd	**Total** (n=38)	**Male** (n=28)	**Female** (n=10)	**Smokers** (n=11)	**Non-Smokers** (n=18)
Mean±sd	0.80±1.01	0.96±1.10	0.20±0.08	1.76 ±1.10	0.27±0.17
P value		*0.046*		*<0.0001*	

[a]No data available regarding smoking habits for 9 individuals.

CONCLUSION:

This study allowed us to define baseline values for Cd, Ni and Pb in lung tissue. For Cd, significantly higher values (ca. 6 fold) were found in smokers. Such tendency was also found for Ni and Pb, but the difference was not statistically significant, showing that smoking seems to be not an important source of exposure to these

metals. Differences between male and female subgroups were found, reaching statistical significance for Cd, but they can simply be due to different smoking habits.

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Seasonal changes in airborne concentrations of organic solvents and the levels of selected markers of oxidative stress and DNA damage among nail technicians

Authors: Peter Gresner, Radoslaw Swiercz, Maciej Stepnik, Jolanta Gromadzinska, Ewa Twardowska, Wojciech Wasowicz

Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, 8, Sw. Teresy str., 91-348 Lodz, Poland

Presenting Author: Email: pgresner@gmail.com | Tel.: +48 426 314 634 | Fax: +48 426 568 331

INTRODUCTION:

Due to increased popularity of nail salon services among women throughout the western civilizations, the number of nail salons and licensed nail technicians has recently increased. Although regular manicure as well as the making of artificial nails involves a number of toxic chemical substances including organic solvents, related health hazards are still underestimated, and the oversight aimed to evaluate the occupational exposure of nail technicians is insufficient or non-existing at all. Meanwhile, working procedures implying that the chemical products are used close to worker's breathing zones and eyes, can constitute a source of considerable health risks, which may vary substantially throughout the year due to seasonal variations in ventilation behavior directly affecting the rate of air movement through the workplace.

OBJECTIVES:

Aim of this study was to evaluate the differences in concentrations of selected organic solvents in nail salon airborne samples collected during late spring and summer (the so-called "hot") season as well as during late autumn and winter (the so-called "cold") season. We further assessed the levels of biomarkers of oxidative stress and DNA damage among nail technicians and evaluated their correlations with airborne concentrations of organic solvents.

MATERIALS AND METHODS:

One hundred and forty five female nail technicians (median age 34 yrs.; interquartile range 27-42 yrs.) from 129 different nail salons in Lodz district, Poland who regularly perform manicure and/or artificial nail sculpturing during their work-time participated in the study. Airborne samples in the group of "hot" season were collected between May 2012 and July 2012 while those in the group of "cold" season between November 2012 and February 2013 using sampling pump and Anasorb 747 sorbent tubes (SKC Inc., UK).The concentrations of organic solvents were assessed using gas chromatography (Gjolstad, 2006). In each individual the levels of biomarkers of oxidative stress (thiobarbituric acid-reactive species (TBARS), activity of glutathione peroxidase (Gpx) 1, Gpx3, ceruloplasmin, superoxide dismutase) were assessed using spectrophotometric methods (Paglia, 1967; Wasowicz, 1993). The extent of DNA damage was evaluated using the comet assay (McKelvey-Martin, 1993). The Mann-Whitney *U*-test was used in order to assess the significance of differences between groups.

RESULTS AND DISCUSSION:

The airborne concentrations of organic solvents measured in air samples from nail salons collected during "hot" (May - July) and "cold" (November - February) seasons together with respective threshold limit values (TLVs) are presented in Table 1.

Table 1 - Airborne concentrations of organic solvents in nail salons

Organic solvent	TLV (mg/m^3)	Median concentration [range] (mg/m^3)		
		Hot season	Cold season	*p*
Ethanol	1900	1.49 [0.06-16.12]	6.85 [0.35-54.77]	<< 0.0001
Acetone	600	0.93 [0.03-43.23]	0.49 [0.05-61.69]	*NS*
Toluene	100	0.02 [0.004-0.30]	0.01 [0.000-0.62]	< 0.005
2-propanol	900	1.59 [0.11-21.30]	3.45 [0.12-37.70]	< 0.005
2-butanone	200	0.04 [0.007-0.92]	0.05 [0.002-1.63]	0.05
ethyl acetate	200	0.75 [0.07-27.28]	4.75 [0.04-44.54]	< 0.01
isopropyl acetate	600	0.03 [0.010-0.10]	0.01 [0.000-0.22]	<< 0.0001
n-butyl acetate	200	0.29 [0.021-4.87]	0.16 [0.00-18.53]	*NS*

It is obvious that these values did not exceed the TLVs obligatory in Poland, but they differ depending on the season, with ethanol, 2-propanol and ethyl acetate being the most abundant ones. Intriguingly, airborne concentrations of these three solvents were found to be significantly increased during the "cold" season compared to the "hot" one. The levels of selected markers of oxidative stress and DNA damage among nail technicians during "hot" and "cold" seasons are shown in Table 2.

Table 2 - Levels of biomarkers of oxidative stress and DNA damage among nail technicians

Biomarker	Summer	Winter	*p*
TBARS (M)	1.67 [1.03-2.89]	1.71 [1.03-2.81]	*NS*
Gpx3 (U/ml)	0.17 [0.11-0.22]	0.17 [0.12-0.22]	*NS*
Gpx1 (U/gHb)	19.56 [13.9-28.28]	22.75 [13.54-29.59]	< 0.005
Ceruloplasmin (g/l)	0.54 [0.35-1.00]	0.61 [0.32-1.12]	< 0.001
Superoxide dismutase (U/mgHb)	6.81 [5.01-8.74]	6.33 [4.51-7.99]	< 0.0005
DNA single strand breaks (% DNA in tail)	2.64 [0.82-4.31]	3.44[1.84-6.95]	<< 0.0001
DNA single strand breaks (tail moment)	0.21 [0.07-0.40]	0.26 [0.14-0.55]	<< 0.0001
Oxidatively damaged DNA (% DNA in tail)	4.96 [2.35-10.64]	5.98 [4.03-9.52]	< 0.001
Oxidatively damaged DNA (tail moment)	0.45 [0.21-0.91]	0.48 [0.30-0.89]	*NS*

Data suggest that the "cold" season of the year is associated with significantly increased levels of biomarkers of DNA fragmentation and oxidatively caused DNA damage as compared to the "hot" season. Nevertheless, out of the five biomarkers of oxidative stress, only activity of cytosolic Gpx1 and the concentration of ceruloplasmin were found to be significantly increased in the "cold" season. The airborne concentrations of ethanol were found to significantly correlate with the levels of DNA single strand breaks (R_S = 0.32 for tail % DNA, R_S = 0.31 for tail moment; $p<0.05$) as well as with the activity of red blood cells Gpx1 (R_S=0.18; $p<0.05$).

CONCLUSION:

Presented data suggest, that during the "cold" season, i.e. the late autumn and winter time, there is an increased concentration of airborne organic solvents in the air of nail salons compared to the "hot" season. Although direct evidence was presented only for ethanol, the airborne concentration of which was found to be positively associated with the extent of DNA damage and glutathione peroxidase activity, such increased concentrations of airborne solvents may supposedly be linked with increased oxidative stress and DNA damage among nail technicians.

ACKNOWLEDGMENTS:

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Cytotoxity induced by extracts of *Pisolithustinctorius* spores on human cancer and normal cell lines - evaluation of the anticancer potential

Authors: Ricardo Alves[1], Marco Preto[2], Rui S. Oliveira[1,3,4], Rosário Martins[2,3]

1 *Department of Environmental Health, School of Allied Health Sciences of Polytechnic Institute of Porto, Portugal*
2 *CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal*
3 *Research Centre on Health and Environment, School of Allied Health Sciences, Polytechnic Institute of Porto, Portugal*
4 *Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Portugal*

Presenting Author: Email: ricardoalvest@gmail.com | Tel.: +351 912 370 077

INTRODUCTION:

Cancer is one of the most important causes of death and morbidity worldwide. According to the World Health Organization 8.2 million people have died from cancer in 2012. The treatment of this fast growing disease is regarded as a major challenge in health for the XXI century. Conventional anti-cancer therapy is usually aggressive to the patients due to systemic toxicity caused by lack of specificity of the chemotherapy drugs (Kidd, 2000). This has triggered the interest of the scientific community to search for new and more effective therapies, less aggressive than conventional treatments. Used since antiquity in oriental civilizations, fungi, in particular mushrooms have been shown to limit or reverse tumor growth (Kidd, 2000). *Pisolithustinctorius* (Basidiomycota) is a soil fungus with world-wide distribution that it known to form ectomycorrhizal symbioses in forest ecosystems (Kope & Fortin, 1990). The sporocarps of this fungus contain Pisosterol, a triterpene that has been shown to have antitumor activity against some cancer cell lines (Montenegro et al., 2004). Nevertheless, no studies have focused on the anticancer potential of other structures such as spores and thus the anticancer potential of *P. tinctorius*, remains largely unknown.

OBJECTIVES:

Considering that spores can be a good source of new pharmacological compounds, the main objective of this study was to evaluate the potential of *P.tinctorius*spores as a source of anticancer compounds.

MATERIALS AND METHODS:

Extractions: *P.tinctorius*samples were collected from a forest ecosystem in Northern Portugal. The spores were separated from the sporocarps and freeze dried. A crude extract was prepared with 20.43g of spores and 75ml of a mixture of dichloromethane/methanol (2:1). The mixture was sonicated, centrifuged and the pellet re-extracted three times, rendering a total volume of 240 ml, which was then evaporated to dryness under reduced pressure. The residue was chromatographed over silica gel with hexane/ethyl acetate and methanol (with progressively increasing polarity) giving eleven fractions (A with lowest polarity to K highest polarity).

MTT assay: The cytotoxicity of the crude extract and fractions was tested against the osteosarcoma cell line MG63, colon adenocarcinoma cell line RKO, breast carcinoma cell line T47D and brain microvascular endothelial cell line hCMEC/D3. Cells were grown in DMEMGlutamaxmedium supplemented with 10% fetal bovine serum (FBS), 2.5 µg/ml fungizone and penicillin-streptomycin (100 IU/ml-1 and100 µg/ml, respectively).For the MTT assay cells were seeded in triplicate in 96 wells plates at a cell density of $3.3x10^4$cel/ml. After 24h adhesion, the medium was removed and cells were exposed to new medium with the crude extract at a concentration of 1mg/ml and the fractions at0.1mg/ml during 24, 48 and 72 h in a CO_2 incubator at 37ºC. Cell viability was determined by the ability of living cells to reduce 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-*2H*-tetrazolium bromide (MTT). At the end of each period of incubation, MTT was added at 0.05mg/ml. After3 h of incubation formazancrystals were dissolved in 100µlDMSO and the absorbance was measured at 550 nm.

RESULTS AND DISCUSSION:

The results concerning the cytotoxicity of the crude extract showed that this extract was able to inhibit cell viability in all cancer cell lines (Fig. 1). This result is supported by the results obtained with the fractions, since the crude extract represents a mixture of the compounds that are then separated in the fractions. Concerning the cytotoxicity of the fractions, fractions, D, F, G and H, were the ones where the lowest rate of viable cells was registered, suggesting the presence of compounds with strong cytotoxic activity. However, we found that the different cancer cell lines were not affected to the same extent when exposed to the same fraction. As example, in fraction H, the results showed 5% of viable cells in RKO and T74D after 48h incubation, while in MG63 the rate of viable cells was 90%. This could mean that the cytotoxic compounds that are present in fraction H are selective towards RKO and T74D showing different modes of action. Concerning the hCMEC/D3 cell line when exposed to D, F, G, and H fractions, the number of viable cells was much higher than that found in cancer cell lines, which indicates that the fractions are much more cytotoxic to cancer than to normal cells, reinforcing the idea of the presence of anticancer compounds in these fractions.

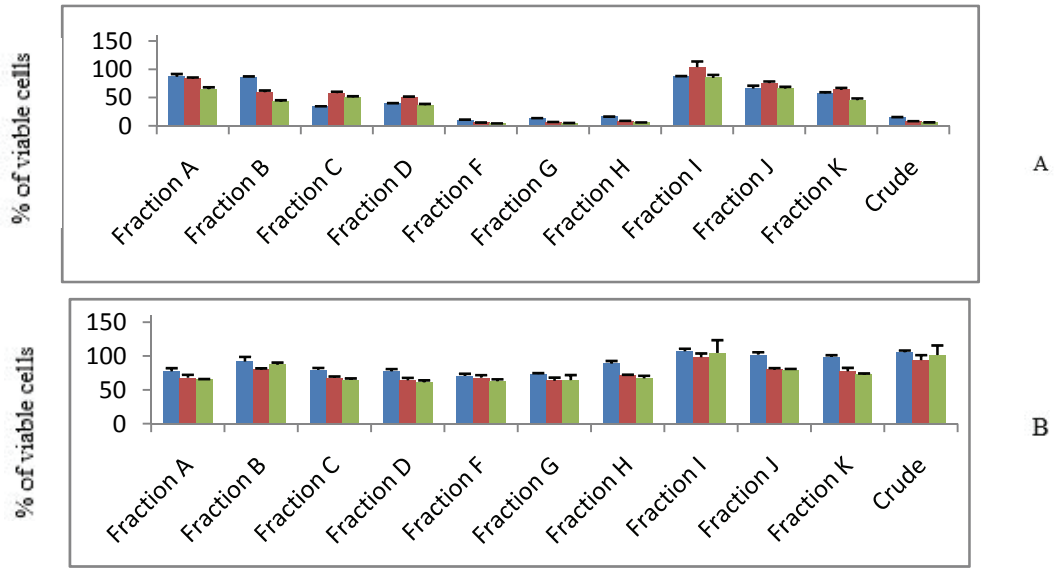


Figure 1 - Effect of crude extract and fractions of *Pisolithustinctorius* spores on the viability of T47D cells **(A)** and hCMEC/D3 cells **(B)**.The cells were exposed for 24, 48 and 72h at a concentration of 0.1mg/ml for the fractions and 1mg/ml for the crude extract. Cell viability was determined by the MTT dye reduction assay. Values are present as means ±SD of triplicate analyses when compared with control group (n = 3).

CONCLUSION:

To our knowledge this is the first study concerning the cytotoxic potential of *P. tinctorius* spores on cancer cell lines. The study demonstrates that the spores of *P. tinctorius* are an interesting source of compounds with anticancer potential and in this sense it represents the initial step in the discovery of new sources of anticancer compounds. It was possible to identify several fractions with selective cytotoxicity toward the cancer lines and normal cells, supporting the possibility of therapeutic potential for the treatment of human cancer forms. Further studies are on-going such as the evaluation of the effects of different concentrations of the fractions in the cancer cell lines and the identifications of the compounds with anticancer activity that are present in the obtained *P.tinctorius* fractions, more specifically, in the fractions D, F, G, and H.

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Microbiological Characterization of Thermal Waters

Authors: Adriana Machado[1], Manuela Amorim[1,2], Stephanie Ferreira[1,2], Jorge Condeço[1,2,3],Sandra Mota[1,2], Anabela Moreira[1,2], António Augusto[1,2,4]

1 *Department of Biomedical and Public Health Laboratory Sciences, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

2 *Research Centre of Health and Environment, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

3 *Portuguese Blood Bank and Transplantation Institute, Portugal*

4 *Biogerm, SA, Maia, Portugal*

Presenting Author: Email: mas@estsp.ipp.pt | Tel.: +351 222 092 128

INTRODUCTION:

Thermal waters are used for disease prevention or treatment, for rehabilitation or welfare. The health risks associated with the use of these waters depends not only on the nature of the waters, but also on the manner of its use and the immune status of users. Exposure to microorganism scan occur through inhalation, ingestion or contact. The related severity and diversity of diseases leads to the verification of bacteriological standards established by law in order to ensure its quality and safety.

OBJECTIVES:

To characterize microbiologically thermal waters of the northern region of Portugal.

MATERIALS AND METHODS:

We conducted a study on water microbiological analysis results of northern Portugal, obtained between January 3, 2013 and December 12, 2013. The microbiological parameters studied to characterize the thermal waters included total microorganisms count at 37°C , 24 hours; microorganisms count at 22 °C, 72 hours; and *Escherichiacoli* and other coliforms, *Enterococcus* spp, sulphite-reducing anaerobes spores, *Pseudomonasaeruginosa* and *Legionella* spp detection. These parameters were analyzed according to sampling location (Vichy shower, water jet, bath tub, water captation, and water filter) and time (December to April and May to November). We classified the results according tomicrobiological standards established by Portuguese law (Ministerial Order no. 1220/2000 of December 29).

RESULTS AND DISCUSSION:

We studied 158 water samples' records. Results are presented in Tables 1 and 2. Of all records, 54,1% of samples were in compliance regarding microorganisms count at 37°C, and 91,08% regarding microorganisms at 22°C. All samples were considered satisfactory for *E. coli*, total coliforms and sulphite-reducing anaerobes spores counts, regardless the sampling location. *Enterococcus* spp counts were in compliance with standard values in 99,4% of total samples, while 98,7% presented satisfactory *Pseudomonasaeruginosa* count. Satisfactory samples' frequency seems to be evenly distributed along the year (table 2).

Table 1 - Absolute and relative frequency of samples (n (%)) in compliance with microbiological standards, according to sampling location.

Microbiological parameter	Standard values	Sampling location					
		Vichy shower (n=27)	Water jet (n=27)	Bath tub (n=27)	Water filter (n=50)	water captation (n=27)	TOTAL
Microorganisms at 37 °C (n=158)	≤5CFU/ml	16 (59,3)	9 (33,3)	16 (59,3)	17 (34,0)	27 (100,0)	85 (53,8)
Microorganisms at 22 °C (n=158)	≤20 CFU/ml	26 (96,3)	18 (66,7)	27 (100,0)	46 (92,0)	27 (100,0)	144 (91,1)
Coliforms (n=158)	≤ 0/ 250 ml	27 (100,0)	27 (100,0)	27 (100,0)	50 (100,0)	27 (100,0)	158 (100,0)
Escherichia coli (n=158)	≤ 0/ 250 ml	27 (100,0)	27 (100,0)	27 (100,0)	50 (100,0)	27 (100,0)	158 (100,0)
Enterococosspp (n=158)	≤ 0/ 250 ml	27 (100,0)	27 (100,0)	26 (96,3)	50 (100,0)	27 (100,0)	157 (99,4)
Pseudomonas aeruginosa (n=158)	≤ 0/ 250 ml	27 (100,0)	27 (100,0)	26 (96,3)	49 (98,0)	27 (100,0)	156 (98,7)
sulphite-reducing anaerobes spores (n=158)	≤ 0/ 50 ml	27 (100,0)	27 (100,0)	27 (100,0)	50 (100,0)	27 (100,0)	158 (100,0)
Legionella spp (n=7)	≤ 0/ 1000 ml	0 (0,00)	0 (0,00)	0 (0,00)	7 (100,00)	0 (0,00)	7 (100,00)
TOTAL N (%)		16 (59,26)	9 (33,33)	16 (59,26)	17 (34,00)	27(100,00)	85 (53,80)

Table 2 - Absolute and relative frequency of samples (n, (%)) in compliance with microbiological standards, according to sampling time.

Microbiological parameter	Sampling location and time									
	Vichy shower (n=27)		Water jet (n=27)		Bath tub (n=27)		Water filter (n=50)		Water pump (n=27)	
	Dec - Apr	May-Nov	Dec - Apr	May-Nov	Dec - Apr	May-Nov	Dec - Apr	May-Nov	Dec - Apr	May-Nov
Microorganisms at 37 °C	9 (75,0)	7 (46,7)	3 (25,0)	6 (40,0)	6 (50,0)	10 (66,7)	10 (50,0)	7 (23,3)	12 (100,0)	15 (100,0)
Microorganisms at 22 °C (n=158)	12 (100,0)	13 (86,7)	7 (58,3)	11 (73,3)	12 (100,0)	15 (100,0)	18 (90,0)	28 (93,3)	12 (100,0)	15 (100,0)
Coliforms	12 (100,0)	15 (100,0)	12 (100,0)	15 (100,0)	12 (100,0)	15 (100,0)	20 (100,0)	30 (100,0)	12 (100,0)	15 (100,0)
Escherichia coli	12 (100,0)	15 (100,0)	12 (100,0)	15 (100,0)	12 (100,0)	15 (100,0)	20 (100,0)	30 (100,0)	12 (100,0)	15 (100,0)
Enterococosspp	12 (100,0)	15 (100,0)	12 (100,0)	15 (100,0)	11 (91,7)	15 (100,0)	20 (100,0)	30 (100,0)	12 (100,0)	15 (100,0)
Pseudomonas aeruginosa	12 (100,0)	15 (100,0)	12 (100,0)	15 (100,0)	11 (91,7)	15 (100,0)	19 (95,0)	30 (100,0)	12 (100,0)	15 (100,0)
Sulphite-reducing anaerobes spores	12 (100,0)	15 (100,0)	12 (100,0)	15 (100,0)	12 (100,0)	15 (100,0)	20 (100,0)	30 (100,0)	12 (100,0)	15 (100,0)
Legionella spp			0 (0,0)	0 (0,0)			3 (100,0)	4 (100,0)		

CONCLUSION:

Despite of the reduced number of non-conform results, this study provides indicators for the development of improvement and corrective measures, to ensure microbiological quality and safety of the use of thermal waters.

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Anatomic region differences and age-related changes on Na^+ and K^+ levels in human brain

Authors: Edgar Pinto[1,2], P. Ramos[3], A. Carvalhido[3], N. Pinto[4], R. Mendes[4], A. Santos[4], A. Almeida[3]

1 *REQUIMTE, Department of Chemical Sciences, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal*

2 *CISA -Research Center in Environment and Health, ESTSP-IPP*

3 *REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal.*

4 National Institute of Legal Medicine and Forensic Sciences, North Branch, Portugal

Presenting Author: Email: ecp@estsp.ipp.pt | Tel.: +351 916 941 708

INTRODUCTION:

Neurodegenerative diseases (ND) have been the subject of intensive research in last years in an attempt to understand the mechanisms underlying the neurodegeneration process. The etiology of these diseases is multifactorial, involving a complex interaction between the aging, genetic predisposition and environmental factors. In particular, the role of metals as risk factors for neurodegenerative disorders is currently being studied. As regards to this topic, the knowledge of metal distribution in the brain is of major importance [2].Evidence suggests that Alzheimer's disease (AD) is associated with impaired depressed Na^+/K^+ ATPase levels in AD brain that might lead to a cellular ion imbalance [1]. Recent studies show that a pronounced variability exists in the distribution of several elements (Ca, Fe, Mg) in brain tissues according to brain area and the age of individuals [3,4].

OBJECTIVES:

The goals of this work were to study: (1) the regional anatomic differences of Na+ and K+ levels within the brain; (2) the changes on Na^+ and K^+ levels in relation to age; and 3) the differences in Na+ and K+ levels between individuals with and without evidence of ND.

MATERIALS AND METHODS:

From each neurologically and psychiatrically healthy individual submitted to autopsy (n=44) the following 14 areas were sampled: (1) frontal cortex; (2A) superior and (2B) middle temporal; (3A) caudate nucleus, (3B) putamen, (3C) globuspallidus; (4) cingulated gyrus; (5) hippocampus; (6) inferior parietal lobule; (7) occipital lobe; (8) midbrain; (9) pons; (10) medulla; and (11) cerebellum. Samples from individuals with previous diagnosis of Alzheimer's (n=2) and Parkinson's disease (n=1) were also collected. After microwave-assisted acid digestion of the samples Na^+ and K^+ levels were determined by atomic absorption spectrometry (AAS).

RESULTS AND DISCUSSION:

Considering the mean value for the 14 areas studied, K (12.5 ± 1.3 mg/g) was present at higher levels than Na^+ (7.6 ± 1.3 mg/g). The distribution of Na^+ and K^+ within the brain tissue showed to be not homogeneous. Highest levels of K^+ were found in the middle temporal gyri (15.9 ± 3.5 mg/g) and putamen (15.5 ± 2.4 mg/g), whereas highest levels of Na^+ were found in the frontal cortex (11.5 ± 2.4 mg/g). Medulla and cerebellum were the regions with the lowest levels of K^+ and Na^+, respectively. In specific brain areas, Na^+ and K^+ levels were age-related. Positive correlation between Na^+ levels and age was found in several brain areas (e.g., middle temporal gyrus, caudate, cingulated gyrus, hippocampus, inferior parietal lobule). For K^+, a negative correlation with age was observed in the frontal cortex. When compared with age-matched controls, Na^+ levels from individuals affected by Alzheimer's disease were found significantly increased in some brain regions. In both Alzheimer's disease patients, increased Na^+ levels were found in the superior temporal gyri (20.8% and 12.8%) and globuspallidus (52.5% and 56.8%, respectively). For K^+, no significant differences

were observed. In the Parkinson's disease patient, Na^+ levels were decreased in most of the brain areas except frontal cortex, hippocampus and cerebellum. The K^+ content was increased in 9 of the brain regions and decreased in the remaining 5 regions.

CONCLUSION:

In conclusion, K^+ is present at higher levels than Na^+. The elements levels at the different brain regions vary significantly, although the K^+ and Na^+ levels are not correlated with each other in the same brain regions. Therefore, the K^+/Na^+ ratio also varies significantly from region to region. Generally, Na^+ increase seems to be age-related, however this tendency was not constant in the different brain regions. Patients with ND presented altered Na^+ and K^+ levels in some brain regions but results are inconclusive. This study provides a comprehensive picture of Na^+ and K^+ distribution within the brain tissue. This data may be a relevant contribution for defining "normal" human brain levels of Na^+ and K^+, allowing future interpretation of the levels found in ND, in an attempt to clarify their role in the disease process.

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Assessment of Variation in Cancer risk due to exposure to ambient air at major intersections in Kanpur city, India

Authors: Manish Agrawal[1], Anubha Goel[1]

1 *Department of Civil Engineering, Indian Institute of Technology Kanpur, Kanpur 208016, India*

Presenting Author: Email: amanish@iitk.ac.in | Tel.: +91-876 577 6535

INTRODUCTION:

Air quality research in India has focused on monitoring of ambient levels of Particulate Matter (mainly PM10, & PM2.5) and their health effects. Increase in vehicle density, introduction of newer fuels, and implementation of stricter emission norms highlights the need for research on amount and characteristics of PM emitted from vehicles under varying load and operating conditions. Rise in vehicle density is linked with increase in reported cases of asthma and other related health problems. Petrol and Diesel fuelled vehicles are primarily used in India and their emissions have been identified as prominent sources of fine PM (Siegmann et al., 2008). Study on fine PM (dp< 1µm), which are major health concern since they carry a higher fraction of toxic pollutants such as PAHs are of major interest since due to their small size they can penetrate deeper into human lungs. Thus estimation of potential health effects like cancer risk (Yadav & Satsangi, 2013) need to be performed. Simultaneously links between variation in RSPM load and cancer risk due to inhalation of ambient air at locations with high traffic volume need to be identified.

OBJECTIVES:

This study examines variation in traffic density and RSPM load at different locations, mainly major traffic intersections, in Kanpur city. Estimation of cancer risk at some locations for 2007 to 2010 was performed to assess health risk due to inhalation of these particles.

MATERIALS AND METHODS:

a. **Collection of RSPM (Respirable Suspended Particulate Matter) data at major traffic intersections in Kanpur city:**
   Data from 2007 to 2013 was used and was collected from Environmental Information System website of Central Pollution control Board.

b. **Incremental lifetime cancer risks:**
   This has been calculated by multiplying the cancer potency factor of a given carcinogen with the chronic daily intake (CDI).

$$\mathrm{CDI} = \frac{\mathrm{C(mg/m^3)} \times \mathrm{Intake\ rate(m^3/day)} \times \mathrm{Exposure(days/life)}}{\mathrm{Body\ weight(kg)} \times 70\mathrm{(years/life)} \times 365\mathrm{(days/year)}} \qquad \text{(Eq.1)}$$

Where: C is concentration level of RSPM at major intersections of Kanpur (Table 1) and intake rate is the amount of air that a person inhales each day (Yadav & Satsangi, 2013).

RESULTS AND DISCUSSION:

Figure 1 describes trends in traffic observed at a major traffic intersection in the city. The data clearly shows that traffic volume and occurrence of traffic peak hour has changed over time. Vehicle density for this particular location has risen sharply - up by 250% from 2007 to 2013.

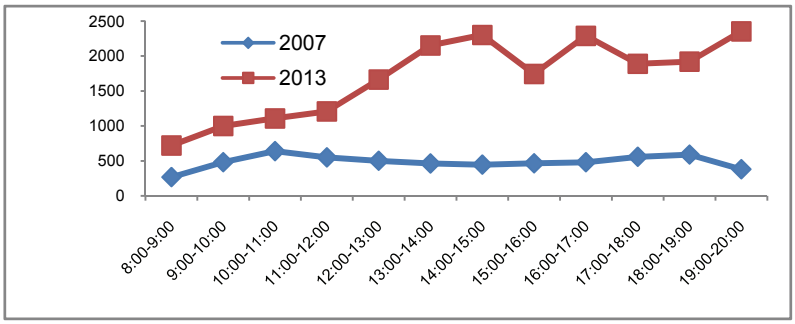


Figure 1 - 4W passenger vehicle density comparison at major traffic intersection ('*Parade*')

Table 1 presents RSPM Load at monitoring stations close to major intersections in Kanpur city from 2007 to 2010.

Table 1 - RSPM load at monitoring stations close to major intersections

(*Source*: Environmental Information System, CPCB)

RSPM Level (Annual Average Concentration in µg/m3)				
Location	**2007**	**2008**	**2009**	**2010**
Fazalganj	205	225	232	221
Jajmau	196	210	201	NA
Deputy ka Padao	198	215	216	NA
Kidwai Nagar	197	201	204	NA
Sharda Nagar	185	210	NA	NA
Vikas Nagar	160	NA	NA	194
Dabauli	NA	NA	208	196

Table 2 describes the cancer risk from 2007 to 2010 at traffic locations listed in Table 1 on the basis of Equation 1.

Table 2 - Cancer Risk with respect to inhalation of RSPM at major intersections

Cancer Risk (mg/kg/day)				
Location	**2007**	**2008**	**2009**	**2010**
Fazalganj	0.47	0.52	0.53	0.51
Jajmau	0.45	0.48	0.46	NA
Deputy ka Padao	0.45	0.49	0.5	NA
Kidwai Nagar	0.45	0.46	0.47	NA
Sharda Nagar	0.42	0.48	NA	NA
Vikas Nagar	0.37	NA	NA	0.44
Dabauli	NA	NA	0.48	0.45

CONCLUSION:

Data clearly shows that trends for nature and amount of traffic have changed significantly over the past six years. Peak day traffic now (2013) occurs in the afternoon & in late evening (2 to 3 PM; 7 to 8 PM) while in 2010, peak traffic was observed in morning & in the evening (10 to 11 AM; 5 to 7 PM). As seen in figure 1, vehicle density has increased sharply by 250% at one location. An expected increase in RSPM levels is evident by data in Table 1. As expected, cancer risk calculated by assuming only 20 minutes of exposure every day for 40 years, results in very high values at major traffic intersections (Table 2). The risks vary and show an increasing trend with time. The sharp increase in cancer risk at Sharda Nagar and Vikas Nagar can be linked to rise in RSPM load (>10% and > 20% at both locations respectively). Investigation of particle size

distribution on roads combined with toxicity of PM-bound metals and their possible risks to human health is warranted. In addition investigation of vehicle types leading to high PM load is important for air pollution control and development of effective risk management strategies.

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The effect of cigarette smoking on leptin and IGF-I levels in pregnant women: relation to birth weight and length of newborn

Authors: Magdalena Chelchowska[1], Jadwiga Ambroszkiewicz[1], Leszek Lewandowski[2], Joanna Gajewska[1], Joanna Mazur[3], Tomasz M. Maciejewski[2]

1 *Screening Test Department, Poland,*
2 *Department of Obstetrics and Gynecology,*
3 *Department of Child and Adolescent Health, Institute of Mother and Child, Warsaw, Poland*

Presenting Author: Email: magdalena.chelchowska@imid.med.pl | Tel.: +48 223 277 260 | Fax: +48 223 277 260

INTRODUCTION:

Despite the fact that in the last years a decrease in the number of active smokers has been observed, cigarette smoking still plays a significant role among hazardous health-related behaviors. This is particularly disturbing for pregnant women. Epidemiology studies conducted at the Institute of Mother and Child indicated that in Poland between 25 to 30% of pregnant women were active smokers and close to 60% were passive smokers (home and/or occupational exposure). It is widely accepted that maternal smoking during pregnancy is associated with a low birth weight and reduced length but the mechanisms by which this occurs has not been adequately clarified. Toxic compounds of tobacco smoke alter the blood flow to the placenta and change the balance between proliferation and differentiation of the cytotrophoblast. A consequence of the placental dysfunction can be reduced active transport of essential nutrients to the fetus. In addition, observed in smoking pregnant women decreased transfer of glucose to the fetus may disorder insulin-like growth factors and leptin axis which are considered to be involved in fetal growth regulation.

OBJECTIVES:

We examined the effect of tobacco smoking on concentrations of leptin and insulin-like growth factor I (IGF-I) in serum of maternal-cord pairs. We also studied the correlations between these biochemical parameters and birth weight and length of newborn.

MATERIALS AND METHODS:

Samples were drawn from a prospective observational study for adverse effect of tobacco smoking pregnant women engaged in regular clinic visits in the Department of Obstetrics and Gynecology Institute of Mother and Child in Warsaw. Seventy five healthy pregnant women were asked to cooperate in this study. All pregnant volunteers signed a written informed consent form, approved by the Institute's Ethical Committee. Only singleton pregnancies were included. Exclusion criteria were: toxemia, hypertension, diabetes mellitus, active hepatitis, renal and cardiovascular disease, inflammatory conditions and preeclampsia. Socio-economic status of all subjects was similar. All of them were living in urban area. At the first visit, a history of smoking was obtained by directly questioning the pregnant women. Smokers were defined as those women who reported their smoking habit as maintained at a rate of minimum five cigarettes per day and minimum two years before conception, and continued smoking during pregnancy. Non-smokers were defined as those women who had never smoked and were not exposed to environmental tobacco smoke during their pregnancy (smoking spouse or co-workers). The classification was confirmed by measurement of serum cotinine concentration in pregnant women - the major metabolite of nicotine. The concentration of cotinine on the level of 15 ug/L was accepted as the limit value between non-smoking and smoking group.

Venous blood samples were collected in III trimester (median: 39 wk; range: 38-42 wk). Cord blood was collected immediately post partum from the umbilical vein after clamping of the cord.
Serum concentrations of leptin, IGF-I and cotinine were determined by immunoenzymatic methods using a commercial available kits (leptin and IGF-I - DRG, Germany; cotinine Calbiotech Inc., USA). Statistical

analysis was done using the STATISTICA 10.0 (StatSoft, Poland) and the significance level was set at $p<0.05$.

RESULTS AND DISCUSSION:

We observed from Table 1 that there were no significant differences between the studied groups with respect to ethnic origin, age, mean gestational age at delivery, and neonatal gender. This is important for the analysis, because if these factors were different, they could alter newborn anthropometric parameters. Maternal characteristics were comparable in both groups except for cigarette smoking habits. In the group of smoking mothers mean serum cotinine concentration was 78.7µg/L and correlated positively with the daily number of cigarettes consumed (r=0.70; p=0.009). The mean number of cigarettes per day for smokers was 8.9, and the mean duration of smoking before conception was 8.8 years. All newborn infants were born in full term (gestational age >37 wk) and there were no negative pregnancy outcomes or complications during delivery in the non-smoking as well as smoking women. It is known that smoking during pregnancy promoted lower birth weight of neonates. In our study, the mean birth weight of the smoking mothers' infants was lower by about 400g ($p<0.001$) and their birth body length by 1,5cm ($p<0.01$) and negatively correlated with number of cigarettes smoked per day ($r=-0.65$; $p<0.001$).

Table 1 - Characteristics of the study population

Characteristics	Non-smoking	Smoking
Age (years)	29.5 ± 0.6	30.6 ± 0.5
Ethnic origin: Caucasian (%)	100	100
Gestational age of birth (weeks)	39 (38-41)	39 (38-42)
Delivery (n)		
Cesarean	1/35	2/40
Normal vaginal	34/35	38/40
Neonatal gender (F/M) (n)	15/20	20/20
Birth weight (g)	3548 ± 465	3128 ± 423**
Birth body length (cm)	56.1 ± 2.6	54.4 ± 2.8*
Apgar score (5th min)	10 (10 - 10	10 (10 - 10)
Number of cigarettes/day	0	8.9 ± 4.5
Time of smoking before conception (year)	0	8.8 ± 3.9
Concentration of cotinine in serum (µg/L)	0	78.7 (48.3 - 133.6)

Values are means ± standard deviation (SD);Values are median and interquartile range (10th-90th percentiles);*p=0.0074; **p=0.0001

There is an increasing number of evidence that exposure to tobacco smoking during pregnancy alter blood flow to the placenta, change the balance between cytotrophoblast proliferation and differentiation, and could lead to functional interferences of this organ. Therefore, it is logical to reason that many placental products, including IGF-I and leptin are low in placental insufficiency. Our data confirm that maternal smoking during pregnancy was significantly associated with decreased serum levels of IGF-I in mothers and newborns (Table 2).

Table 2 - Serum concentrations of selected biochemical parameters in blood of studied pregnant women and in umbilical cord blood

	Non-smoking		Smoking	
Concentration (µg/L)	Maternal	Cord Blood	Maternal	Cord Blood
Leptin	20,3 ± 12,7	10,3 ±7,1	21,8 ± 13,7	6,5 ± 5,8
IGF-I	381,9 ± 111,8	84,4 ± 3,9	316,6 ± 100,0****	61,7 ± 36,5***

Values are means ± standard deviation (SD); ***p=0.0191; ****p=0.0099

Additionally, a recent analysis showed a significant inverse association between cigarette dose and IGF-I concentrations in mothers as well as in the fetus (r=-0.49; r=-0.-40, $p<0.05$ respectively). Compatible with previous reports, we found that maternal and fetal leptin concentrations were similar in smoking and tobacco

abstinent groups. Despite of this fact, the significant negative relationship between the reported number of cigarettes smoked daily during pregnancy and cord blood leptin was observed ($r=-0.31$, $p<0.05$). We observed the individual, large differences in serum leptin levels result in large standard deviation. Therefore it is possible that larger number of observations might have resulted in a statistical difference between the groups. In agreement with others study we found a positive correlation between birth weight and concentrations of IGF-I in blood of mothers ($r=0.5$; $p<0.01$) and cord blood ($r=0.40$; $p<0.05$) as well as between birth weight and leptin level of newborn ($r=0.42$; $p<0.05$). There was no significant association between newborn birth length and studied biochemical parameters.

CONCLUSION:

Our findings suggest that smoking during pregnancy decreases maternal and fetal serum IGF-I levels but does not have a significant effect on maternal blood leptin concentrations. It seems to be that maternal and cord blood levels of insulin-like growth factor I and cord blood leptin levels could play an important role in the regulation on fetal growth. The association between lower birth weight of neonates among smoking mothers and level of these parameters concerns the negative effect of tobacco smoking on newborn growth and development. Thus, the educational care plane for women to quit smoking tobacco during pregnancy should be elaborated and included in primary medical servis.

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Trends in tobacco smoking among Polish adolescents in 2006-2014 in relation to the selected socio-demographic factors

Authors: Joanna Mazur[1], Anna Dzielska[1], Magdalena Chelchowska[2], Anna Kowalewska[3]

1 *Department of Child and Adolescent*
2 *Screening Test Department, Institute of Mother and Child*
3 *Faculty of Pedagogy Warsaw University, Warsaw, Poland*

Presenting Author: Email: magdalena.chelchowska@imid.med.pl | Tel.: +48 223 277 260 | Fax: +48 223 277 260

INTRODUCTION:

For years, the countries of Eastern and Central Europe were characterized by different life style patterns than in the countries of Western Europe. As a rule, boys were more often than girls involved in risky health behaviors. Also, non-linear (U-shaped) relationship between socio-economic status and risky behaviors was observed with high prevalence of risk behaviors in rich families.

International HBSC (*Health Behavior in School-aged Children*) studies provide an unique opportunity to monitor trends in health behaviors and provide periodically repeated international comparisons based on data from over 40 countries. Gender and cultural convergence in adolescent drunkenness was well described; however similar studies on smoking are still lacking. In Poland, the trends in health behavior were well described in the national monograph, but these analyzes still require a follow-up. It is also recommended to draw separate trends for boys and girls, and for groups differing in family socio-economic status and in selected neighborhood characteristics.

OBJECTIVES:

The purpose of the present study is to describe trends in daily smoking among Polish adolescents between 2006 and 2014, using preliminary results of the new HBSC study. As a second goal we try to explore socio-economic factors related to increasing trends in regular smoking among adolescent girls and students living in deprived areas.

MATERIALS AND METHODS:

Analyses were based on data from the samples of 15-year-olds who participated in Poland the HBSC study in 2005/2006, 2009/2010 and 2013/2014 (total N = 4965). In each round of the study, a nationally representative sample from the same regions was used with a school class as a main sampling unit. Only preliminary results of the new 2013/2014 survey were available, representing about 75% of the final sample.

Among many questions about health behaviors, children had to answer "*How often do you smoke tobacco at present?*" (1-*every day*; 4-*I do not smoke*). A dichotomous variable was created, representing daily smoking. Family socio-economic status (SES) was described by a subjective indicator describing „how well off" each respondent perceived their family to be (*PFW - perceived family well-off*). The marginal categories were combined, yielding three groups of families: poor, average, wealthy. Local area socio-economic (LASES) status was described by three items coming from the National Longitudinal Survey of Children in Canada and recommended in the HBSC protocol since 2002 as optional package. A summative index ranged 0-6 points was created and then divided into three categories: serious problems in the local area; moderate problems and problem free area.

Changes in daily smoking were analyzed with Chi squared test in the total group and separately for boys and girls. At first, logistic regression was estimated with four independent variables: gender, PFW, LASES and

year of the study. In the next stage, three similar models were estimated on the basis of data from three consecutive surveys. Analyses were performed using SPSS v.17 software.

RESULTS AND DISCUSSION:

Daily smoking rates in these samples underwent some fluctuations in 2006-2014, with no clear trend. An upward significant trend was observed in the last 4 years in girls only. The opposite trend was suggested in boys, by close to significant decrease especially in 2010-2014 (Table 1). In the 2013/14 survey, for the first time, there was no significant difference in the smoking prevalence among boys and girls; but taking into account the absolute values, girls smoked more often.

Table 1 - Tobacco smoking (%) in 15-year-old Polish adolescents in relation to gender and year of survey

Group	2006	2010	2014	p (surveys)
Total	12.3	11.0	11.7	0.4599
Boys	14.8	13.8	10.7	0.0944
Girls	10.0	8.2	12.4	0.0287
p (boys vs. girls)	0.0005	0.0004	0.3696	

In the same period, the level of family wealth measured by the PFW remained at a similar level. Respectively at average, 5.9%; 69.0% and 25.1% of young respondents perceived their families as poor, average and well-off. Perception of the neighborhood SES began to change only in recent years. In 2010-2014, the percentage of children living in deprived areas decreased from 19.7% to 14.0%.

All the time, tobacco smoking is strongly determined by socio-economic factors (Table 2). Based on the data from the combined sample, daily smoking was more prevalent in poor families and in regions with more local problems, which applied both to boys and girls. Taking into account the most recent data (2013/14), the strong influence of socio-economic factors was well seen only in girls.

Logistic regression analysis estimated on the total sample of 4965 adolescents, showed that all three socio-demographic predictors were significant but not the year of the study. One of the most interesting findings refers to the changing set of predictors. In 2006 both gender, PFW and LASES were significant; in 2010 only two independent variables remained in the final model (without PFW); and in 2013/14 only one (LASES).

Table 2 - Tobacco smoking (%) in 15-year-old Polish adolescents in relation to socio-economic factors (combined sample 2006-2014)

Socio-economic	Family level (PFW)		Neighborhood level (LASES)	
status	Boys	Girls	Boys	Girls
poor	20.2	20.1	18.1	17.5
average	12.4	9.2	13.0	8.9
well-off	15.3	9.9	10.2	7.3
p	0.0173	0.0000	0.0027	0.0000

In further, studies should analyze the current trends in the use of other psychoactive substances in relation to socio-economic and demographic factors. More objective measures of family affluence should also be included.

CONCLUSION:

Preliminary results of the new HBSC study conducted in Poland suggest unfavorable trends in tobacco smoking only in adolescent girls. It is worth to pay attention to the environmental aspects of smoking prevalence, since the neighborhood SES remained the only significant factor in all subsequent rounds of the HBSC study, including the most recent one. Reducing disparities between more and less deprived areas will require comprehensive efforts and equity-based policies that address the social determinants of smoking in adolescence.

Changes in patterns of the use of selected psychoactive substances observed in Poland can be regarded as part of the epidemiological transition.

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The effect of different type of diets on fat tissue and bone metabolism parameters in prepubertal children

Authors: Jadwiga Ambroszkiewicz[1], Witold Klemarczyk[2], Magdalena Chelchowska[1], Grazyna Rowicka[2], Malgorzata Strucinska[2], Joanna Gajewska[1]

1 *Screening Department*

2 *Department of Nutrition, Institute of Mother and Child, Kasprzaka 17a, 01-211 Warsaw, Poland*

Presenting Author: Email: jagoda.ambroszkiewicz@imid.med.pl | Tel.: +48 223 277 260 | Fax: +48 223 277 260

INTRODUCTION:

Vegetarian diets are seen as an alternative to the traditional model of nutrition and are growing in popularity in developed countries, including Poland. The official position of the American Dietetic Association points out that well-planned vegetarian diets are healthy, nutritionally adequate and appropriate for individuals at all stages of life, including children, adolescents, adults and elderly. Researchers suggest that vegetarianism can be a healthful dietary option which offers a number of nutritional benefits and is associated with reduced risk of certain chronic diseases, including diabetes, coronary heart disease, and some cancers. Moreover, vegetarians consume a diet high in fruit and vegetables and low in energy density, which put them at decreased risk for obesity. On the other hand, individuals choosing to follow vegetarian restrictive diets might experience mineral and vitamins deficiencies and notice that using fortified foods or supplements can be helpful in meeting dietary recommendations. This is especially important in childhood and adolescence, when growth and development are most intensive.

OBJECTIVES:

The aim of our study was to investigate associations between fat tissue and bone metabolism markers and their relations with anthropometric parameters in vegetarian and omnivorous prepubertal children.

MATERIALS AND METHODS:

We examined 50 healthy prepubertal children aged 5-10 years who had been referred to the Department of Nutrition at the Institute of Mother and Child in Warsaw for dietary consultation. Children were divided into subgroups: vegetarians (n=25) and normal-weight omnivores (n=25). Anthropometric measurement (weight, height) was performed in all children and body mass index (BMI) was calculated. To determine bone mineral density, fat mass and the percentage of body fat, dual-energy X-ray absorptiometry scan was performed using a Lunar Prodigy (GE, USA). Dietary constituents were assessed by questionnaire and calculated using the nutritional computer program Food4. Serum levels of calcium, phosphate, vitamin D, were assessed by standard laboratory methods. Concentrations of markers of bone metabolism (osteocalcin-OC, carboxyterminal telopeptide of collagen type I-CTX) and leptin were determined by immunoenzymatic assays. For statistical calculation we used Statistica 10.0 PL computer software.

RESULTS AND DISCUSSION:

Our children on vegetarian diet was in good health with no noticeable problems and were under regular medical and nutritional care. Generally, all studied subjects grew normally and had total energy and macronutrient intakes within the recommended daily intake for children (Table1). However, vegetarian diet contained lower intake of fat and higher carbohydrates in comparison to omnivorous diet. Our vegetarian children had similar intake of phosphorus, but significantly ($p<0.05$) lower intake of calcium and vitamin D.

Table 1 - Average daily energy and nutrients intake of examined children compared to recommended daily intake

	Children on vegetarian diet	Children on omnivorous diet
Energy (kcal/day)	1478±420	1612±397
% energy from protein	13.8±2.6	14.1±2.7
% energy from carbohydrates	57.1±5.6	54.3±6.1
% energy from fat	30.3±5.3	32.9±6.1
Dietary calcium (mg)	565±326	811±311*
Dietary phosphorus (mg)	916±346	937±312
Dietary vitamin D (µg)	2.38±1.12	3.34±1.39*

Data were presented as mean value ± SD; *p<0.05

There were no significant differences in body weight, height and BMI between vegetarians and children on traditional mixed diet. Children on vegetarian diet had slightly lower fat mass and bone mineral density (total body BMD and lumbar spine BMD) than omnivores (Table 2). Serum concentration of leptin was significantly lower in vegetarian children compared with omnivores (p<0.01). We observed also decreased by about 10% concentration of osteocalcin (bone formation marker) and slightly increased levels of CTX (bone resorption marker). Hence, the ratio of OC to CTX was lower in vegetarians than in omnivorous children. We found positive correlation between serum leptin level and BMI as well as fat mass. No statistically significant correlations between leptin and bone markers were observed.

Table 2 - Anthropometric and biochemical parameters of children on vegetarian and omnivorous diet

	Children on vegetarian diet	Children on omnivorous diet
Anthropometric parameters:		
Age (years)	7.2±2.0	7.0±1.8
Body weight (kg)	23.8±6.8	25.8±6.7
Body height (cm)	126.6±12.9	129.2±10.6
BMI	15.5±1.8	15.7±1.9
Percentage of fat (%)	17.2±5.7	19.1±7.9
Fat mass (kg)	4.28±2.20	4.76±3.2
Total BMC (g)	810±250	826±242
Total BMD (g/cm^2)	0.811±0.057	0.891±0.065
Z-score total BMD	-0.45±0.72	-0.32±0.91
Lumbar spine BMD L1-L4 (g/cm^2)	0.617±0.106	0.633±0.100
Z-score lumbar spine BMD L1-L4	-0.51±0.96	-0.41±0.80
Biochemical markers:		
Leptin (ng/ml)	2.9±2.1	5.7±4.1**
Calcium (mmol/l)	2.34±0.11	2.40±0.12
Phosphate (mmol/l)	1.65±0.13	1.72±0.15
25-hydroxyvitamin D (ng/ml)	22.2±9.5	28.8±4.4*
OC (ng/ml)	65.0±25.6	71.1±24.7
CTX (ng/ml)	1.815±0.448	1.650±0.516
Ratio of OC/CTX	0.36	0.42

Data were presented as mean value ± SD; *p<0.05; **p<0.01); BMI - body mass index, BMC - bone mineral content, BMD - bone mineral density, OC-osteocalcin, CTX- carboxyterminal telopeptide of collagen type I

CONCLUSION:

We observed that different kind of diet may influence on biochemical bone and fat tissue markers in prepubertal children. Determination of leptin and markers of bone formation and bone resorption can be clinically useful in the medical and nutritional care of vegetarian children in order to prevent bone abnormalities.

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Changes in concentrations of adipokines in obese children after a 3-month weight reduction programme

Authors: Joanna Gajewska[1], Jadwiga Ambroszkiewicz[1], Witold Klemarczyk[2], Magdalena Chelchowska[1], Anna Zielinska[3], Halina Weker

1 *Screening Department, Institute of Mother and Child, Warsaw, Poland*
2 *Department of Nutrition, Institute of Mother and Child, Warsaw, Poland*
3 *Department of Anthropology, Institute of Mother and Child, Warsaw, Poland*

Presenting Author: Email: jagoda.ambroszkiewicz@imid.med.pl | Tel.: +48 223 277 260 | Fax: +48 223 277 260

INTRODUCTION:
Excessive weight in children and adolescents causes metabolic disorders, orthopaedic problems and in the future can also influence the health of adult population. It is widely recognized that lifestyle intervention, including modification of nutritional habits and physical activity, is the most important therapy to reduce weight excess in childhood and adolescence. However, anthropometric parameters may not identify all changes associated with lifestyle modification. It seems, that adipokines can be clinically useful markers for the monitoring of therapy efficacy but it is not completely understood.

OBJECTIVES:
The aim of this study was to assess the level of leptin, leptin receptor and adiponectin in prepubertal obese children after the 3-month weight reduction therapy.

MATERIALS AND METHODS:
Changes in nutrition, anthropometric and biochemical parameters were determined in 55 obese children (z-score BMI≥2SD) aged 4-10 years, before and after a 3-months lifestyle intervention programme. The exclusion criteria were: (a) the presence of endocrine disorders or genetic syndromes, including syndromic obesity; (b) chronic medical conditions; (c) taking medications that could affect growth, pubertal development, nutritional or dietary status. Pubertal stage was determined according to the Tanner scale, and subjects, who showed pubertal development before and at the end of intervention were excluded. The weight loss programme consisted of dietary (1200-1400 kcal/day) and physical activity modifications and behaviour therapy, including individual psychological care of the child and its family (2). The reference group consisted of 35 healthy normal-weight children (z-score BMI <-1+1>). All children and their parents were asked to record the type and amount of food and beverages consumed for two consecutive weekdays and one weekend day. Average daily food rations and their nutritional value were calculated using the nutritional analysis software Food2. Serum levels of leptin, leptin receptor and adiponectin were determined using immunoenzymatic kits. Parameters were determined twice in obese children (before and after 3-month therapy) and once in the control subjects.

RESULTS AND DISCUSSION:
All obese children were divided after therapy into two subgroups: responsive subjects (RS) and non-responsive subjects (NRS) (Table 1). In subgroup RS with decreased BMI by 10% ($p<0.01$) after the 3-months therapy we found also decreased concentration of leptin (about 3-fold, $p<0.0001$) and increased concentrations of leptin receptor (about 20%, $p<0.0001$) and adiponectin (about 10%, $p<0.05$). In NRS subgroup without changes in BMI during therapy, no significant differences in most biochemical parameters were observed. After therapy, only the level of leptin (about 30%, $p<0.05$) was decreased. Total energy intake in RS subgroup after therapy was lower than before ($p<0.00001$). Moreover diet of this subgroup was characterized with a lower intake of fat, carbohydrate ($p<0.0001$) and protein ($p<0.05$) (Table 2). In NRS subgroup we found also lower intake of total energy after therapy than before ($p<0.001$), but the differences in this group were not so significant as compared with differences in RS subgroup. Moreover, the diet of NRS subgroup was characterized with a lower intake of carbohydrate ($p<0.0001$) and fat ($p<0.05$), but the intake

of cholesterol was unchanged. It suggests, that this diet during therapy was not exactly compatible with recommendations, therefore children in NRS subgroup did not change of their BMI values after 3 months of intervention. The lack changes in biochemical parameters as well as BMI values were observed by many authors. However, we found that leptin may decrease during therapy without BMI changes. It is not excluded, that leptin may reflect the changes in fat tissue metabolism as a result of dietary modification, more sensitive than leptin receptor and adiponectin.

Table 1 - Anthropometric and biochemical measurements in obese children and controls

Parameters	Responsive subjects (RS) Before	After	Non-responsive subjects (NRS) Before	After	Controls
N	30	30	25	25	30
Age (years)	7.7±1.1	7.9±1.0	8.2±1.8	8.4±1.8	7.8±1.9
BMI (kg/m^2)	25.3±3.0	22.7±2.8**	25.4±3.4	24.8±3.2	16.1±2.1♦♦
Fat mass (%)	41.0±5.5	36.8±6.0*	40.5±7.4	40.7±8.3	20.2±5.6♦♦
Leptin (ng/ml)	36.1±18.1	13.1±11.0***	39.6±19.3	26.6±17.2*	5.86±3.50♦♦
Leptin receptor (ng/ml)	21.8±5.5	26.8±8.9***	25.6±9.7	25.4±7.4	40.8±13.9♦♦
Adiponectin (µg/ml)	7.09±2.71	7.95±2.72*	6.63±3.25	7.39±2.83	9.17±3.69♦

*p<0.05 in obese children after in comparison to before
**p<0.01 in obese children after in comparison to before
***p< 0.0001 in obese children after in comparison to before
♦p<0.05 in control group in comparison to RS and NRS groups
♦♦p<0.0001 in control group in comparison to RS and NRS groups

Table 2 - Dietary intake of obese children and controls

Parameters	Responsive subjects (RS) Before	After	Non-responsive subjects (NRS) Before	After	Controls
Energy intake (kcal/day)	1640.1±492	1210±350****	1764±83	1290±380**	1680±479♦ ,,
Protein (g)	59.4± 18.1	48.6±12.2*	57.7 ±16.0	49.6±13.0	55.6±18.3
Fat (g)	63.2±22.8	43.2±16.8***	69.8±24.7	46.1±18.2*	63.0±19.5♦ ,,
Cholesterol (mg)	229.1 ±111.7	164.3±52.0*	214.0±72.7	193.8±57.4	210.7±72.6,,
Carbohydrate (g)	221.5±73.0	167.2±47.5***	241.2±70.8	178.9±55.0***	236.5±77.0♦ ,,
Sucrose (g)	68.3±32.1	36.8± 20.2****	64.3±28.9	43.1±21.8*	70.4±32.7♦ ,,

*p<0.01 in obese children after in comparison to before
**p<0.001 in obese children after in comparison to before
***p<0.0001 in obese children after in comparison to before
****p<0.00001 in obese children after in comparison to before
♦p<0.01 in control group in comparison to after of NRS group
♦♦p<0.001 in control group in comparison to after of RS group

CONCLUSION:

Our findings support the beneficial effects of lifestyle intervention if it is associated with weight loss and with tendency to normalization of leptin, leptin receptor and adiponectin concentrations. In addition, leptin level may also show the positive changes in fat tissue metabolism without BMI changes in obese children after therapy. We suggest, that all studied adipokines may be useful in monitoring of therapy effectiveness in responsive as well as in non-responsive obese subjects.

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Respiratory Virus on Flu Syndrome

Authors: Céu leitão[1], Ana Almeida[1], Fernando Bellém[1], Isabel Dias[2], Isabel Fernandes[2], Margarida Carvalho[2], Paula Santos[2], Renato Abreu[1]

1 *Sciences Health School of Lisbon Polytechnic, Portugal*
2 *Hospital Curry Cabral*

Presenting Author: Email: mcleitao@estesl.ipl.pt | Tel.: +351 218 980 400 | Fax: +351 218 980 460

INTRODUCTION:

Flu (or influenza)is an extremely contagious respiratory illness, spread person to person, by coughs and sneezes. The timing of flu is unpredictable, but it is especially common in winter and early spring, which is why it is also known as "seasonal flu" (Hayward AC et al, 2014). The most Flu syndromes are caused by different groups of virus and not by Influenza virus only (Creer DD et al, 2006). High-risk groups, such as people with asthma and diabetes, heart disease, cancer, with a compromised immune system, chronic pulmonary disease, congenital/acquired cardiac disease, pregnant women, adults 65 and older and children younger than five and especially younger than 2 years old, are more likely to have a more severe disease process, with the highest risk of dying from seasonal flu complications (Esposito S et al, 2010).

OBJECTIVES:

It is important to identify clinically relevant respiratory illnesses (Paba P et al, 2014), so, this investigation intended to determine which respiratory viruses are responsible for “Flu syndrome”, in a sample of 402 patient adults, admitted at a central hospital, in Lisbon, all symptomatic cases, from April to December 2009.

MATERIALS AND METHODS:

The molecular diagnosis, by multiplex real time RT-PCR, was performed for the simultaneous detection of 13 respiratory viruses. Respiratory virus researched were: Influenza A (IfA), Influenza B (IfB), Parainfluenza 1-3 (PIV 1-3), Parainfluenza 2-4 (PIV 2-4), Respiratory syncytial virus (RSV), Adenovirus (AdV), Enterovirus (EV), Rhinovirus (RV), Metapneumovirus (MPV), Coronavirus GP1 (GP1), Coronavirus GP2 (GP2), and SARS. Viral nucleic acid extraction was performed in a 3 level bio-security laboratory, using NucliSenseasyMAG (Bio-Merieux) and BioROBOTEZ (Qiagen).

RESULTS AND DISCUSSION:

Out of 402 specimens examined, 185 (46%) had PCR-confirmed disease for: Rhinovirus 114 (28,3%), Coronavirus e Parainfluenza 1 e 3, 17 (4,2%), Parainfluenza 2 e 4, 12 (3%), Influenza B, 8 (2%), seasonal A flu H3, 5 (1,2%), Enterovirus, 4 (1%), Adenovirus e Respiratory syncytial virus, 3 (0.7%), seasonal A flu H1, 2 (0,5%). Attending that this study was performed during the 2009 H1N1 outbreak, it is important to settle that a large proportion of influenza syndromes are caused by non-influenza virus (Table 1). As in Tsukagoshi.H. et al (2013), acute respiratory illness due to various virus is the most common cause of upper respiratory infection in Humans and a major cause of morbidity and mortality, leading to diseases such as bronchiolitis and pneumonia

Table 1- Prevalence respiratory virus detected by RT-PCR in real time.

Vírus	(%)
Adenovírus	0,7%
Enterovírus	1%
Coronavírus	4,2%
SARS	0%
Gripe Sazonal A H1	0,5%
Gripe A H3	1,2%
Gripe A H5	0%
Influenza B	2%
Parainfluenza 1 e 3	4,2%
Parainfluenza 2 e 4	3%
Rinovírus	**28,4%**
RSV	0,7%
Negative	**54%**

CONCLUSION:

The promptness of the results for the diagnosis of respiratory virus by molecular biology techniques, in contrast with other (serological and cultural),as well as a higher sensitivity and specificity, is extremely important, especially during periods of epidemic outbreaks, when hospitals have an excessive number of emergency incidents, in services overcrowded. This situation increase negligence errors, like delayed and wrong therapies, which translates into very serious consequences, including death (Hayward AC et al, 2014), particularly among the High-risk groups of patients.

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Developing risk of non-alcoholic steatohepatitis disease in individuals without liver disease, a comparative study

Authors: Renato Abreu[1], Ana Almeida[1], Carla Pires[1], Céu Leitão[1], Fernando Bellém[1], Ricardo Paulo[1]

1 *Sciences Health School of Lisbon Polytechnic, Portugal*

Presenting Author: Email: renato.abreu@estesl.ipl.pt | Tel.: +351 218 980 400 | Fax: +351 218 980 460

INTRODUCTION:

Non-alcoholic Steatohepatitis (NASH) has increased a considerable interest in recent years, among scientific community, since its prevalence is higher (20% -30%) in developed countries (Silverman, J. F., 1990; Ângulo P., 2002; Ong J.P. & Younossi, Z. M., 2007).Some studies report that after hepatitis C, NASH is the most common diagnosis in patients presenting persistent abnormalities of liver function(Day C.P. 2002).

OBJECTIVES:

This investigation designed the study of biochemical parameters among two different populations: with disease of NASH and without NASH.

MATERIALS AND METHODS:

The studied groups were: group 1- Control - included 47 subjects without NASH (25 females and 13 males) and group 2 - NASH - 11 subjects (2 females and 9 males) diagnosed with NASH by liver biopsy or ultrasound. After blood collection, the biochemical parameters were assessed on the equipment *TARGA3000®*.

RESULTS AND DISCUSSION:

Significant differences (p<0, 5) were found among the two groups (Table 1), namely on Triglycerides, AST and ALT. Aminotransferases are the variables that showed a more marked difference. The observed values of AST and ALT were elevated in 100% and 72, 7%, correspondingly, on patients with NASH. Only one subject from group 1 had higher values of Aminotransferases then the average observed for group 2.

Table 1 - Global results and statistical data

	Group 1 - Control				Group 2 - NASH				
	média	sd	min.	max.	Average	sd	min.	max.	P value
Glicose (mg/dL)	102,42	19,92	77,50	156,00	108,75	12,82	94,00	133,00	0,131#
HDL (mg/dL)	60,07	10,18	38,60	84,60	49,68	8,25	36,30	60,70	**0,003***
LDL (mg/dL)	140,65	43,20	66,10	256,10	113,20	23,20	74,10	143,80	**0,009***
Colesterol (mg/dL)	225,29	56,83	148,00	367,00	192,82	27,80	137,00	228,00	0,265#
Triglicerides (mg/dL)	122,83	89,61	42,50	446,00	149,70	69,00	98,70	329,00	0,045#
AST (UI/L)	16,97	6,49	2,80	35,20	29,87	10,10	19,30	57,80	**0,000#**
ALT (UI/L)	7,78	5,98	2,20	33,10	24,86	8,74	14,80	39,00	**0,000#**

sd - standard deviation; min-minimum value; max-maximum value; #p value for Mann-Whitney test;
* p value for T test (independent samples)

CONCLUSION:

These results enlighten the need for surveying and monitoring apparently healthy population in order to be effective in primary prevention of NASH and other metabolic disorders. According to Day C.P. (2002) 15% to 50% of patients with NASH develop progressive liver disease or even cirrhosis.

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Evaluation of the influence of obesity on lipid profile in Type II diabetics

Authors: Ana Almeida[1], Céu leitão[1], Fernando Bellém[1], Joana Apolónio[1,] Renato Abreu[1], Roberta Medeiros[1]

1 *High School of Health Sciences of Polytechnic of Lisbon, Portugal*

Presenting Author: Email: ana.almeida@estesl.ipl.pt | Tel.: +351 218 980 400 | Fax: +351 218 980 460

INTRODUCTION:

Nowadays there is a growing number of obese people with type II diabetes mellitus (DM II), and that fact, associated with an increased risk of dyslipidemia, causes cardiovascular problems(Cheung B., 2008; SPA, 2008).

OBJECTIVES:

The aim of the study was to assess the influence of obesity, by calculating body mass index (BMI), on lipid profile of individuals with DM II.

MATERIALS AND METHODS:

Seventy individuals with DM II ≥ 18 years were selected, of which 35 were obese (O) and 35nonobese (NO). The sample was withdrawn from medical records of patients of the service of Diabetology of a south Hospital, in Portugal. The parameters of interest analyzed were: total cholesterol (TC), LDL, HDL, triglycerides(TG), fasting glycemia and glycated haemoglobin(HbA1c), inits statistical analysis, the independent student t test, correlation coefficient of *Pearson* and chi-square were used.

RESULTS AND DISCUSSION:

Regarding the lipid profile, the values obtained showed that their average CT and TG are slightly higher in the group of O, in relation to NO (O: CT = 181.43 mg/dL and TG = 143.6 mg/dL; NO: CT = 180.60 mg/dL and TG = 126.3 mg/dL) but however, there were not. As for LDL, HDL, fasting glucose and HbA1c, the averages presented for each variable showed higher values in the NO group (O: LDL = 100.9 mg/dL, HDL = 44.89 mg/dL, glucose = 175.4 mg/dL, HbA1c = 7.9%; NO: LDL=103.9 mg/dL, HDL = 46.63 mg/dL, glucose = 188.6 mg/dL, HbA1c = 8.2%) and there weren't significant differences between those groups.

CONCLUSION:

It was concluded that, in the sample under study, obesity was not a crucial factor for the modification on lipid profile, because there were no significant differences between the lipid profile of DM II, O and NO.

Key Words: Type II Diabetes Mellitus, Obesity, Lipid Profile, BMI, Glycemia, HbA1c.

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POLLUTION AND ENVIRONMENTAL MANAGEMENT ABSTRACTS

Individual and population exposure to volatile organic compounds in a urban industrialized area

Authors: Joana Ferreira[1], Joana Valente[1], Teresa Nunes[1], Margarita Evtyugina[1], Carlos Borrego[1], Myriam Lopes[1]

1 *CESAM & Department of Environment and Planning. University of Aveiro, Portugal*

Presenting Author: Email: jferreira@ua.pt.pt | Tel.: +351 234 370 200 | Fax: +351 234 370 309

INTRODUCTION:

The intense industrial development from the second half of the 20th century, coupled with population growth and population concentration in urban areas, has accentuated the concern on the evaluation of the potential effects and impacts from air pollutant emissions on environment and human health. The current work methodology was applied to the Estarreja region, an urban area that has one of the largest chemical complexes in Portugal, which has been recently under expansion. During the 80's of the last century, air quality started to be monitored in this region, but only the classics pollutants were addressed. Volatile organic compounds (VOC) include several compounds with negative human health effects, like aromatic compounds. Due to a complexity of sources, industrial, traffic, agriculture and natural, that can drive air quality in the region, field campaigns were planned, involving VOC and nitrogen dioxide (NO_2) measurements with passive tubes, to obtain a spatial screening of their concentrations.

Health effects of air pollution are the result of a sequence of events, which include release of pollutants, their atmospheric transport, dispersion and transformation, and the contact and uptake of pollution before the health effects take place. The conditions for these events vary considerably and have to be accounted for, in order to ensure a proper assessment. Exposure studies can be carried out to obtain estimates of the exposure of an individual (personal exposure) or for a larger population group (population exposure). The general approach for exposure estimation can be expressed by:

$$Exp_i = \sum_{j=1}^{n} C_j t_{i,j} \qquad \text{(Equation 1)}$$

where Exp_i is the total exposure for person/group i over the specified period of time; C_j is the pollutant concentration in each microenvironment j and $t_{i,j}$ is the time spent by the person i in microenvironment j.

OBJECTIVES:

This paper explores two different approaches (individual and population exposure) to analyze the human exposure to VOC in a Portuguese urban industrialized area, Estarreja.

MATERIALS AND METHODS:

To model human exposure over a selected region by a deterministic approach, three types of input data are needed: the population characterization (number of people and daily time-activity pattern), the spatial distribution of the microenvironments visited by the population and the temporal variation of pollutants concentrations in each microenvironment. In this study the outdoor pollutants concentrations were obtained in four sampling campaigns with a weekly duration performed between March to June 2012.A set of passive tubes were distributed for 32 sampling locations in an area with ˜100 km2 (Figure 1). Radiello passive tubes and Palmes type tubes were used to VOC and NO_2 concentration measurements respectively. For more details see Nunes et al., 2013. Indoor concentrations were calculated using indoor-outdoor relationships from the literature.

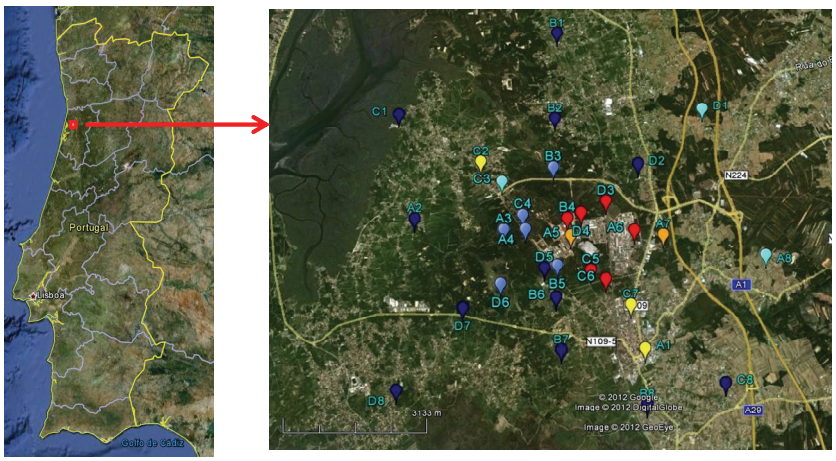


Figure 1 - Sampling locations in the Estarreja region.

For the individual exposure study, the individual time-activity profiles were built considering the weekly personal schedule obtained for each individual by personal interview. The individuals recruited for the study were volunteers that worked and lived within the study area. In the case of population exposure, population characterization and daily activity patterns are given by the Portuguese National Statistics Institute (INE), based on inquiries to the Portuguese resident population.

RESULTS AND DISCUSSION:

Among the seventeen VOC quantified regularly at each measurement point toluene is always the most abundant compound. Pollutant concentrations varied with place and time, and are much influenced by meteorological conditions.

The calculation of the population exposure has permitted the estimation of the spatial distribution of the VOC exposure of the population subgroups considered, and the identification of the microenvironments where a larger number of people are exposed to VOC. On the other hand, the individual exposure methodology allowed the identification of the inter-individual differences from person to person.

The results of the present study show that the air quality of Estarreja region is significantly influenced by Estarreja Chemical Complex; nevertheless other sources contributed as well for the levels of VOC and NO_2 in the studied area. Traffic, and related sources like fuelling stations, are responsible for some concentrations hotspots within the study domain. The industrial complex and residential urban areas are the spots where VOC outdoor concentrations are higher. The concentrations of BTEX (benzene, toluene, ethylbenzene and xylene) observed are characteristic of urban areas bigger than Estarreja.

CONCLUSION:

The individual and population exposure study shows similar results, indicating that the population of Estarreja has exposure levels comparable with other urban areas. Preliminary results show that, although the highest VOC concentrations were found in the urban and industrial complex areas, the population exposure spatial pattern does not evidence these areas with higher exposure. People spend most of their time indoors where VOC and NO_2 concentrations are lower than outdoors. The analysis shows that the studied individuals', that in a traditional study would be considered to have the same exposure, since they work/live in a restricted geographical area, show high VOC and NO_2 exposure variability.

ACKNOWLEDGMENTS:

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New sensing technologies for air pollution control and exposure assessment: field evaluation of ozone micro-sensors

Authors: João Ginja[1], Nicolas Moser[2], Ana Margarida Costa[1], Miguel Coutinho[1], Carlos Borrego[1,3]

1 *IDAD - Institute of Environment and Development, Aveiro, Portugal*
2 *SGX Sensortech SA, Corcelles-Cormondreche, Switzerland*
3 *CESAM and Department of Environment and Planning, University of Aveiro, Portugal*

Presenting Author: Email: joao.ginja@ua.pt | Tel.: +351 234 400 800 | Fax: +351 234 400 819

INTRODUCTION:

The current air quality monitoring strategy is manly based on measurements from fixed monitoring stations, not always reflecting the exposure and effects on humans. Advanced air pollution control systems based on low-cost sensing technologies opened a new vision for air quality control and exposure assessment. Their performances allow for a new strategy, resulting in fast responses, low operating costs and high efficiencies that cannot be achieved with conventional approaches. Nevertheless, much research remains to be done to integrate these new technologies, particularly on the quality check of the sensors performance against conventional methods in field exercises (Aleixandre & Gerboles, 2012; Mead et al., 2013).

OBJECTIVES:

This work presents a performance evaluation of ozone micro-sensors against standard method during field campaigns performed from November 2013 to January 2014. The application of new sensors side by side with standardised equipment in field studies allows assessing the reliability and uncertainty of these low-cost sensors, especially regarding an accurate detection of pollutant concentration peaks.

MATERIALS AND METHODS:

The measurement campaigns were conducted in two major Portuguese airports, Lisbon and Oporto, in 6 monitoring sites, from October 2013 to January 2014.The micro-sensors were installed in a shelter at the top of roof of IDAD's mobile laboratory. IDAD's Laboratory measured several atmospheric and meteorological parameters: NO, NOx, NO_2, CO, O_3, SO_2, BTEX, temperature, relative humidity, radiation, precipitation, wind velocity and wind direction. The comparison exercise was performed with the reference equipment for O_3 (ultraviolet photometry). For MEMS (Micro Electro Mechanical Systems) sensors, gas detection is based on the physical principle involving the modulation of conductance within a layer of semiconductor material. The impedance characteristics of the semiconductor are altered through reactions with oxidizing gases present in the air. The pollutant concentration is calculated as the measured resistance (R_S) adjusted by the calibration and temperature compensation parameters.

RESULTS AND DISCUSSION:

A statistical analysis of field results was conducted allowing a performance evaluation of these low-cost sensors. The results were treated for each measurement following bibliographic references and information from the manufacturer, namely temperature correction and methodology for calculation of the function for each sensor (Gerboles & Buzica, 2009; Gerboles et al., 2011;Spinelle et al., 2013). Figure 1 presents an example of correlations between the data from micro-sensors and ozone reference analyser. For ozone was possible to identify measurements with a strong correlation between micro-sensor and UV analyser. Preliminary correlations with R^2 between 0,68 and 0,88 were achieved for a significant number of measurements.

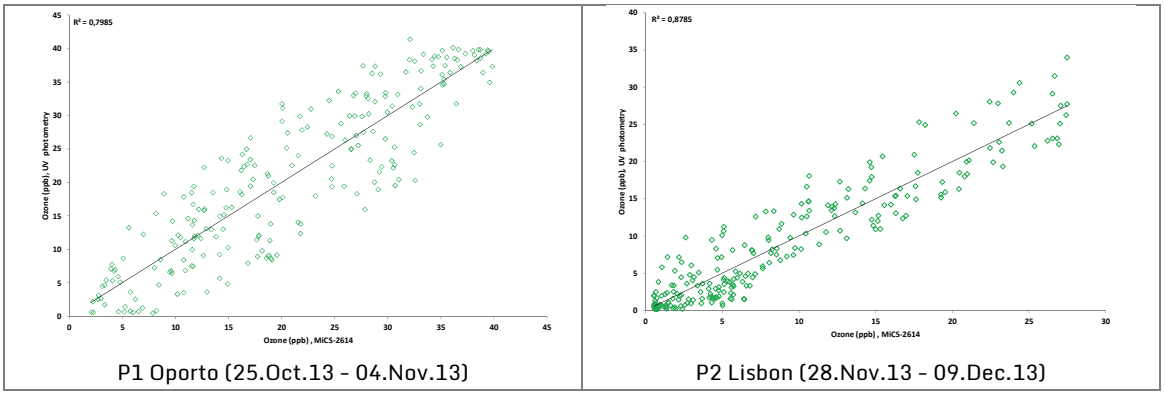


Figure 1 - Example of correlation between micro-sensor and UV analyser

The present work showed promising results for O_3 micro-sensors. Examples of reference measurements and calculated concentration from micro-sensor signal are presented in Figure 2. Several of the measurements presented strong correlations and equivalence between daily concentration profiles.

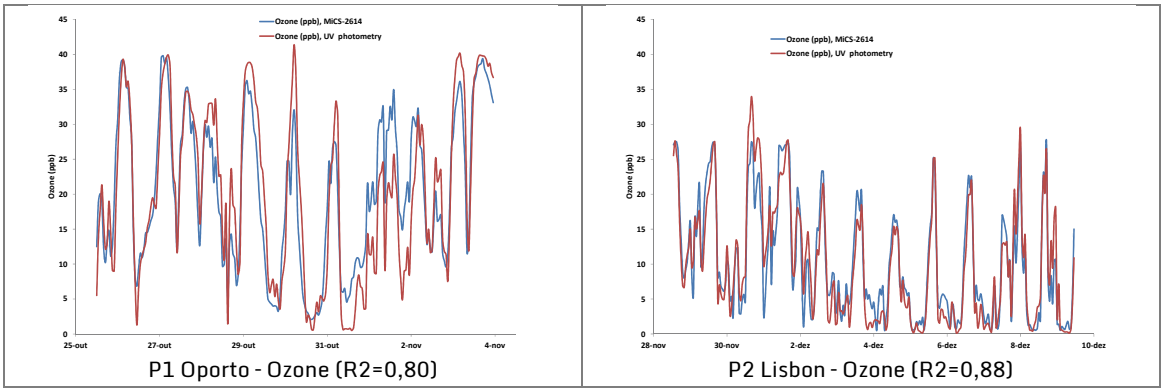


Figure 2 - Example of correspondence between reference measurements and micro-sensor results for O3

CONCLUSION:

The utilisation of AQ micro-sensors is still not mentioned for regulatory purposes in European legislation, nevertheless their use can be particularly valuable to have highly spatially and temporally resolved air quality data and to improve exposure assessment. Although there is a significance research and development of low cost sensors for pollutant monitoring, data treatment of sensors signals from field campaigns remains limited and challenging. The preliminary evaluation performed confirms that O_3micro-sensors could be a promising technique for air quality monitoring. Their performances can lead tonew strategies for air quality control, rapid mapping of air pollution over small areas, validation of atmospheric dispersion models or evaluation of population exposure.

ACKNOWLEDGMENTS:

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Air pollution by PM10 in Portugal between 2001 and 2011

Authors: Tiago Faria[1,2], Susana Marta Almeida[1]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, EN 10, km 139.7, 2695-066 Bobadela LRS, Portugal.*

2 *Environmental Health RG, Lisbon School of Health Technology, Polytechnique Institute of Lisbon, Lisbon , Portugal*

Presenting Author: Email: tiagofaria@ctn.ist.utl.pt | Tel.: +351 219 946 130

INTRODUCTION:

Portugal is located in the southwest of Europe, whose territory is situated in the western part of the Iberian Peninsula and Europe. It is a country with 10,487,289 inhabitants and 92,212,02 km^2, being bordered on the north and east by Spain and the south and west by the Atlantic Ocean. During last three decades special efforts have been made in Europe aiming to reduce air pollution and more importantly, to reduce the adverse impacts of atmospheric pollutants. Although these efforts led to a reduction of risks and effects, air pollution in Europe is still a matter of concern (Slezakova et al, 2011). Several directives have been introduced to control levels of certain pollutants and to monitor their concentrations in the air. The Directive 2008/50/EC on ambient air quality assessment and management is the latest and covers the revision of previously existing legislation and introduces new air quality standards. In this Directive, 50µg/m^3 of PM_{10} mustn't be exceeded in more than 35 days an year and 40µg/m^3 is the annual limit value. Several works have been showed that the EU directive targets for air quality in Portugal are rarely achieved. (EEA, 2012). Moreover, WHO has recently identified that Portugal is one of the 80 countries that exceed the reference values for particulate matter (WHO, 2012).

OBJECTIVES:

The aim of this study was to study the air pollution by PM_{10} in Portugal between 2001 and 2011.

MATERIALS AND METHODS:

Data were collected from 60 stations of air quality monitoring throughout mainland Portugal, divided by 5 regions - 1) North, 2) Centre, 3) Lisbon and Tejo Valley, 4) Alentejo and 5) Algarve - covering over 40 counties. Each station was divided into 5 different types, namely: a) Traffic, b) Industrial, c) Background - Urban, d) Background - Sub-Urban and e) Background - Rural. The measurements at the stations were sent to a central database (Base Air Quality) in order to be available to the public. These data were provided by the Portuguese Environment Agency, with contribution from the Committee for Coordination and Regional Development (CCDR). Different approaches were used to interpret the data including temporal and spatial analysis using Geographical Information Systems.

RESULTS AND DISCUSSION:

In Figure 1, it's possible to observe that the average concentration of PM_{10} in Portugal decreased over the last 10 years, with annual averages since 2004 below the current limit value of 40µg/m^3. This data are in agreement with the European Environment Agency who showed, in 2012, that Portugal was the country with main reported reduction, with a 29%, between 2000 and 2010. In average, the North of the country presented the highest values over the years, followed by Lisbon and Tejo Valley. Furthermore, since 2008, the values in both stations have been quite similar or even higher in Lisbon and Tejo Valley. Between 2001 and 2011, it was record a PM_{10} reduction of 60% and 35% in North and Lisbon and Tejo Valley, respectively. These data are corroborated by Borrego (2009), where the major urban areas, North (Porto) and Lisbon were also the areas with higher values in 2007 compared with the rest, and Lisbon presents lower values than Porto.

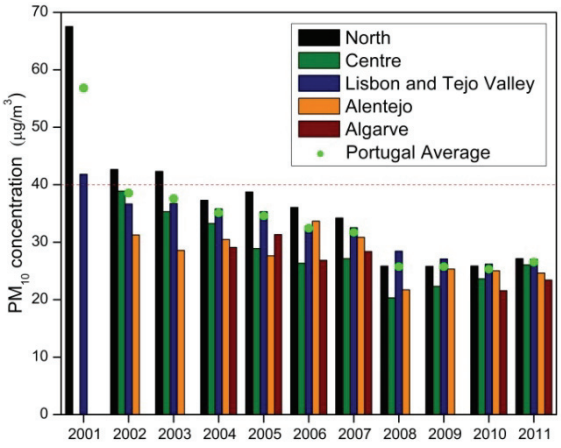


Figure 1 - Concentration of PM_{10} in Portugal by region. Results are presented in µg/m^3.

In Figure 2 it's possible to observe that monitoring stations located along the high traffic areas have higher concentrations of PM_{10}. The EU directive target for PM_{10} of 40µg/m^3 was only achieved in 2007. The stations near of industries and sub-urban areas are the second type of station that presented higher particles concentration. The sub-urban areas are characterized by being near of urban centers, having cheaper houses and having the possibility of industry nearby. These data are similar to those presented by Figueiredo, 2013 which in Estarreja municipality (Centre Region of Portugal) found results that demonstrated industrial activity and road traffic, are the major contributors to the air quality degradation over the study region.

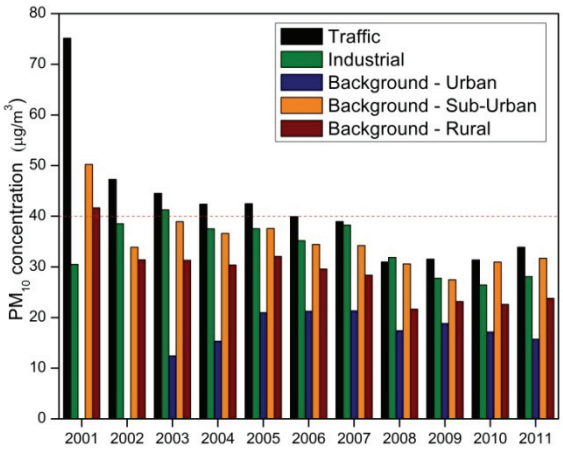


Figure 2 - Concentration of PM10 by type of monitoring stations. Results are presented in µg/m3.

CONCLUSION:

Between 2001 and 2011 the annual levels of PM_{10} decreased in Portugal. The EU directive target for PM_{10} of 40µg/m^3 was only achieved in 2004. Despite this improvement, it is fundamental to continue with efforts reducing emissions of these pollutants, in order to protect public health, since it is well known that particles may promote serious diseases upon human health.

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Assessment of metal concentrations in airborne PM10 from multifarious sources in a heavy industrial area

Authors: J. Lage[1,2], S.M. Almeida[1], M.A. Reis[1], P.C. Chaves[1], S. Garcia[2], B.G. Fernández[3], H.Th. Wolterbeek[2]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, 139.7 km, 2695-066 Bobadela LRS, Portugal*

2 *Faculty of Applied Sciences, Department of Radiation, Radionuclides and Reactors, Section RIH, Technical University of Delft, Delft, The Netherlands*

3 *Instituto de Soldadura e Qualidade, Av. Prof. Dr. Cavaco Silva, 33, 2740-120 Porto Salvo, Portugal*

4 Global R&D, ArcelorMittal, Avilés, Spain

Presenting Author: Email: joanalage@ctn.ist.utl.pt | Tel.: +351 967 648 123

INTRODUCTION:

Air pollution is one of the most pressing environmental concerns due its potential impact on health and environmental quality. Amongst air pollutants, air particulate matter (APM) is of particular concern with fine particles receiving worldwide attention due to their ability to cause adverse health effects, as increased respiratory and cardiovascular diseases, and mortality. Moreover, the most recent data indicate that in 2010, 223000 deaths from lung cancer worldwide resulted from air pollution (IARC, 2013). The greatest anthropogenic threats to air quality come from industrial activities, vehicle emissions and residential wood burning. Due the continuous increase of industrial activity and their impact on the air quality the development of air quality monitoring studies are even more important. Furthermore, studies of air quality assessment can contribute to the improvement of the local air monitoring control strategies.

OBJECTIVES:

The objective of this work was 1) to assess the air quality of an industrial area composed by a steelwork, a cement industry, a power plant and a harbor, and 2) to assess the impact of these industries to the local air.

MATERIALS AND METHODS:

The applied methodology consisted on the collection of PM samples industrial area in two sampling campaigns, performed in the winter and summer seasons, and in four sampling periods in order to identify specific sources. For that one low volume sampler and one high volume sampler, operating side by side were used to collect coarse and fine particles. Afterwards filters were analyzed by Instrumental Neutron Activation Analysis (INAA) (Bowen and Gibbons, 1963) with the $k_0$methodology (De Corte, 1987) and by Particle Induced X-Ray Emission (PIXE) (Johansson and Campbell, 1988) for the elemental concentration and by ion chromatography, indophenol-blue spectrophotometry and atomic absorption spectroscopy for the determination of water-soluble inorganic ions.

RESULTS AND DISCUSSION:

Source apportionment with Positive Matrix Factorization (PMF) was applied and seven emission sources were identified. The first source reflected the sea-spray composition, having high shares of Cl and Na, and contributed on average to 22% of the PM_{10} mass. The second source, which contributed on average to 11% of the total PM_{10} mass, was mainly composed by As, Cr, Cu, Ni, Pb, Sb and Zn and was attributed to mixed combustion processes and traffic. The third source contained high percentages of NH_4^+ that derives from gas to particle conversion processes. The contribution of this factor to PM_{10} mass was on average 12%. The fourth factor was made of Br and contributed for 1.8% of the total PM_{10} mass. The fifth source was dominated by NO_3^- and SO_4^{2-} and contributed on average for 19% of the PM_{10} mass concentration. The sixth source carried high percentages of Al, Ca, La, Si, Ti and V and accounted for 14% of the PM_{10}. These are the major constituents of soil and point out the fingerprint of mineral dust. The seventh factor was associated with steel production as it is defined by Fe and Mn. This source accounted for 21% of PM_{10} mass concentration.

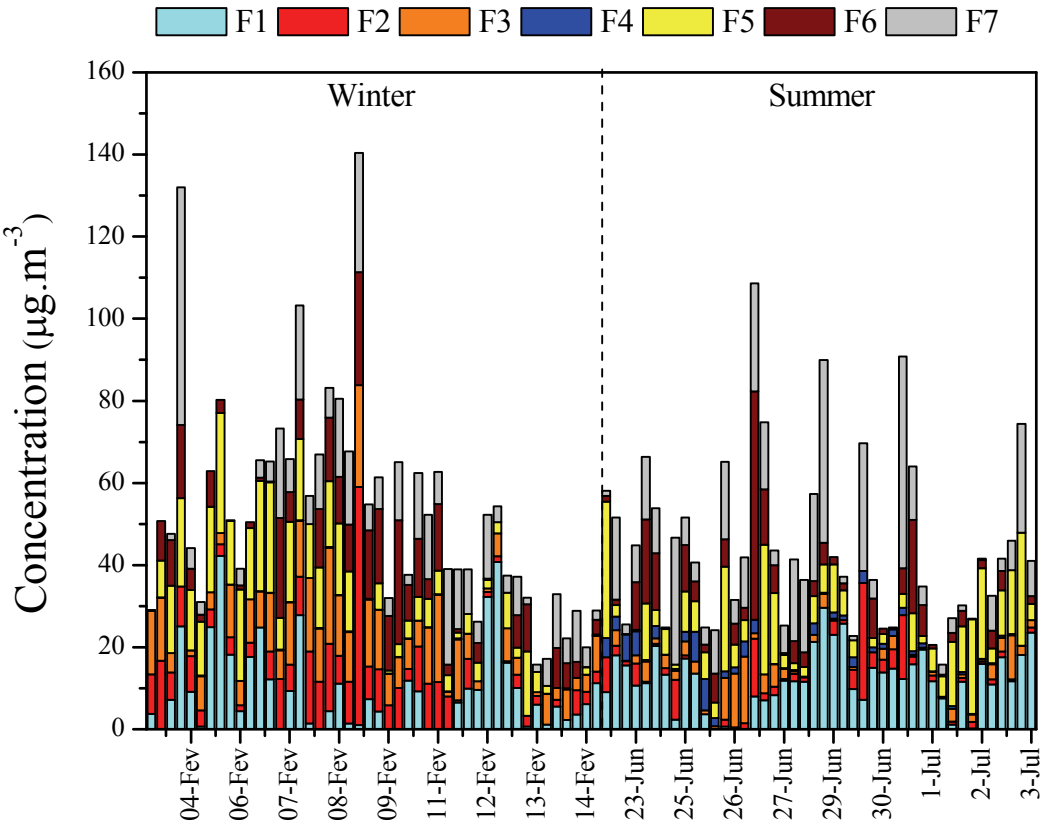


Figure 1 - Source contribution for PM10 total mass concentration time series (µg.m3).

CONCLUSION:

This work contributes to the identification of the main emission sources that influence the local air quality, which are they the marine aerosol, mixed combustion processes and traffic, secondary aerosols, soil and mineral dust and steel production. It was also identified that the steelwork as a major contributor to local PM_{10} concentrations and is the major emission source of heavy metals as Fe and Mn. The contribution of this kind of studies is relevant once, the performance of a good source apportionment to identify specific air pollution sources is essential for the development of cost-effective strategies of pollution control in target areas.

ACKNOWLEDGMENTS:

This work was supported by Portuguese “Fundação para a Ciência e Tecnologia” under J. Lage PhD fellowship SFRH/BD/79084/2011, and by European Community's Research Fund for Coal and Steel (RFCS) under grant agreement no RFSR-CT-2009-00029.

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Airborne exposure related health effects in urban environments are socio-spatially distributed

Authors: Ulrich Franck[1], Heinz-Josef Klimeczek[2], Annegret Kindler[3]

1 *Core Facility Studies, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany*
2 *Senate Department for Urban Development and the Environment, Berlin, Germany*
3 *Department Urban and Environmental Sociology, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany*

Presenting Author: Email: ulrich.franck@ufz.de | Tel.: +49 341 235 1540 | Fax: +49 341 235 451540

INTRODUCTION:
Human health and well-being are strongly affected by exposure to airborne pollutants. Urban exposures play an important role because of the high number of people affected and due to traffic and industrial sources of air pollutants. Inner-urban differences of human exposure are often higher than mean differences between cities in a country. Additionally, the majority of cities is characterized by socio-spatial segregation. Therefore, public health in urban environments is strongly related to the location of the urban district in the city and the question of environmental justice.

OBJECTIVES:
Because of the related health risks, it is necessary to investigate the inner-urban spatial distributions of human exposures. Additionally the question arises if the social situation of population is also related to airborne outdoor exposures. Special attention is turned on emissions of urban traffic.

Many socio-economic indicators can characterize the social situation in the urban areas. This study also compared the suitability of various socio-economic indicators.

The different human exposures in the areas of the city were related to health risk differences.

MATERIALS AND METHODS:
The capital of Germany, Berlin, covers an area of 892 km^2. With a population of 3.43 million people, Berlin is Germany' s largest city and is the second in the European Union. In 2006, 1.416 million motor vehicles were registered in the city. In 2006, a new spatial basis was introduced for monitoring and planning of different urban development processes including demographic and social developments in the city (Welsch et al., 2011). These planning area units (PLAs) were defined by uniform urban structure types and local environment, large roads and traffic arteries, as well as natural barriers (e.g. rivers and lakes). The classification generated 447 areas which each rather homogenous with respect to social parameters, traffic, and building structure.

Particulate matter (PM10, PM2.5) and nitrogen oxides (NO_X, NO_2) are two of the most important airborne pollutants in German cities. The PM10 and NO_X concentrations, additional reference data for selected characteristic monitoring stations and for street sections, were provided by the Berlin Senate Department for Health, Environment and Consumer Protection. This study aimed on long-term exposures characterizing the PLAs. Hence, yearly mean values were used. These data were given on the basis of 1 km^2 grid cells. Because this study is based on PLAs as spatial units the area weighted annual average values were calculated from this data for all PLAs. NO_2 and PM2.5 concentrations have been calculated using the data on NO_X and PM10. Additional contributions of these pollutants by urban traffic have been taken into account based on the area of main streets in the PLAs.
The social indicators include information about unemployment (percentages of unemployed, unemployed in the age between 15 and 24, people unemployed longer than one year, beneficiaries of welfare

aid/unemployment pay. A dynamic indicator also includes changes of parameters and migration balances of different groups.

The mortality risks in areas of Berlin have been calculated for the example of cardiovascular diseases based on data on health risks of Lepeule et al. (Lepeule et al., 2012).

RESULTS AND DISCUSSION:

The socio-spatial differentiation in Berlin resulted in a concentration of disadvantaged PLAs in the city center and within the low emission zone. In this area, PLAs with high status index tended to develop negatively.

Exposure by both pollutants, PM2.5 and NO_2, was highest in the Berlin city center which includes the low emission zone. People in the periphery of the city suffered from lowest exposure levels in Berlin. This fact underlines the reasonable localization of the low emission zone at least with respect to reduction of the exposure of the inhabitants of this area and the related adverse health effects.

NO_2 and PM2.5 exposures were correlated (Pearson's correlation coefficient 0.92, $p<0.001$). This indicates that both pollutants were emitted by the same source types.

There was a strong tendency to lower exposures at increasing social indices. High and very high values of all social indicators were associated to significantly lower exposures whereas for all indices the lowest values were associated to the highest human exposures in the PLAs. The percentage of population exposed to different levels of air pollution strongly depended on the social indicator. The lower the social index the more inhabitants were exposed to high and very high levels of pollutant concentrations. Conversely, there existed no PLAs with high index values which suffered from the highest exposure concentrations.

CONCLUSION:

In Berlin, mean exposure concentrations of NO_2 and PM2.5 were moderate in comparison to recommendations of the EU and the WHO. But, the concentrations significantly differ between the 447 planning areas. Exposure to airborne pollutants is higher in socially disadvantaged areas and contributes to environmental injustice. All city dwellers of Berlin, but especially people living in socially discriminated planning areas would significantly benefit from a reduction of NO_2 and PM2.5 exposure concentrations.

ACKNOWLEDGMENTS:

The authors wish to thank the Berlin Senate Department for Health, Environment and Consumer Protection for the possibility to use their excellent data base.

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Evolution of air quality in the municipalities of Aveiro, Lisbon, Portimão and Oporto (2005-2011)

Authors: Vasconcelos Pinto, M.[1]; Ribeiro, I.[3]; Figueiredo, J.P.[2]; Ferreira, A.[1]

1 *Department of Environmental Health from Coimbra Health School, Polytechnic Institute of Coimbra*
2 *Department of Complementary Sciences from Coimbra Health School, Polytechnic Institute of Coimbra*
1 *Degree in Environmental Health, Coimbra Health School, Polytechnic Institute of Coimbra*

Presenting Author: Email: martavasconcelos@estescoimbra.pt | Tel.: +351 239 802 430 | Fax: +351 239 813 395

INTRODUCTION:

Over the past decades, air quality has been the subject of study and legislative action against the growth of industrial activity and population, which has contributed to its degradation. As the consequences of this degradation cause several effects on human health and ecosystems it is necessary to implement measures to the air quality control. A national network of air quality monitoring applied to the entire Portuguese territory allows us to analyze the concentrations of air pollutants emitted by various emission sources in Portugal.

OBJECTIVES:

This study aims to analyze the evolution of the air quality in the cities of Aveiro, Lisbon, Portimão and Oporto in the period2005-2011.

MATERIALS AND METHODS:

Type of observational and retrospective cohort study. The analytical collection was supported by the web QualAR (Portuguese Environment Agency). The pollutants studied were PM_{10}, NO_2, CO, $O_3$and C_6H_6. The selected air quality stations for this study of air quality evolution were based on the same type of environment and the same focus of pollution. All the evaluated stations are characterized by an urban environment and areas of intense car traffic. For the description of the parameters it was applied simple descriptive measures. The statistical strategies applied were: Student t-test for the population mean, General Linear Model: Repeated Measures ANOVA and I to factor.

The interpretation of statistical tests was based on a confidence level of 95% to a maximum error up to 5%. The statistical processing of the acquired data was performed using the SPSS Statistic software version 21.0 and MedCalc Statistical Software. The annual limits of air pollutants assessed were analyzed regarding D.L.n.º 102/2010 of 23 of September (Table 1).

Table 1 - Legal parameters required by D.L. 102/2010 for air pollutants

Pollutant	Parameter	Considered Period	Limit Value	Margin of Tolerance
PM_{10}	Annual limit value for the Protection of Human Health	1 calendar year	40 µg/m^3	---
NO_2	Annual limit value for the Protection of Human Health	1 calendar year	40 µg/m^3	2005 - 10; 2006 - 8; 2007 - 6; 2008 - 4; 2009 - 2; 2010 - 0
CO	Limit value for the Protection of Human Health	Daily maximum of 8h average	10 000 µg/m^3	---
C_6H_6	Annual limit value for the Protection of Human Health	1 calendar year	5 µg/m^3	2005 - 5; 2006 - 4; 2007 - 3; 2008 - 2; 2009 - 1; 2010 - 0
O_3	Target value for the Protection of Human Health	Daily maximum of 8h average	120 µg/m^3	---

RESULTS AND DISCUSSION:

The Figure 1 summarizes the average of time-based estimation of some parameters under study. Regarding the distribution of the concentration of CO in four stations studied between 2005 and 2011 in a period of 8 h, the average of the concentration of this pollutant did not exceeded the limit value for protection of human health (10 000 μg/m^3) significantly ($p < 0.0001$).

Analyzing the average concentration of $PM_{10}$from 2005 to 2011it was found that, in 2007and 2011the Aveiro station concentration exceeded the annual limit value for protection of human health (40 μg/m^3). The Lisbon station showed the highest number of exceedances in all years of study. Portimão's station showed exceedances in the years2005, 2007and 2011, being the years 2007and 2011 the ones that showed higher concentrations. At Oporto station, the year 2005 was the one who showed exceedance of the limit value, being very close to the reference value ($p < 0.001$).

With respect to NO_2, Lisbon station recorded a concentration above the limit value ($p < 0.001$) in all the years with very high concentrations compared with the reference value (40 μg/m^3). The Aveiro station had no exceedances being its average concentration relatively lower compared to the limit value. Portimão haven't shown an annual average concentration of this pollutant above the limit value / risk to human health. The station located in the city of Oporto presented exceedances to the limit value throughout the analyzed years, slightly above the reference value. It was also found that the large urban centers, Lisbon and Oporto were those with concentrations well above the annual limit value for NO_2 concentration.

In relation to pollutant O_3 (limit value: 120 μg/m^3), it was necessary to analyze the data for periods of 8 hours. The monitoring station of Oporto was the only one to evaluate the concentration of this pollutant. Considering all the daily periods of 8H, the year 2010 was the most critical in terms of mean concentration, since registered the highest concentrations (despite being less than the value of risk to human health). It was also found that for the 8 hours period, the 8H01 to 16H period showed the higher concentrations of this pollutant.

The pollutant $C_6H_6$was only monitored in Aveiro and Portimão stations. The annual concentrations registered were below the annual limit value for protection of human health. This pollutant was not monitored in the years of 2008, 2009 and 2011in Portimão. 2007 was the year that recorded the higher values of concentration of this pollutant in Portimão. Aveiro's Station showed the highest concentrations, compared with the Portimão station, reaching its maximum in 2005. Yet, is note worthy that the concentration of this pollutant decreased, in both stations, in the recent years.

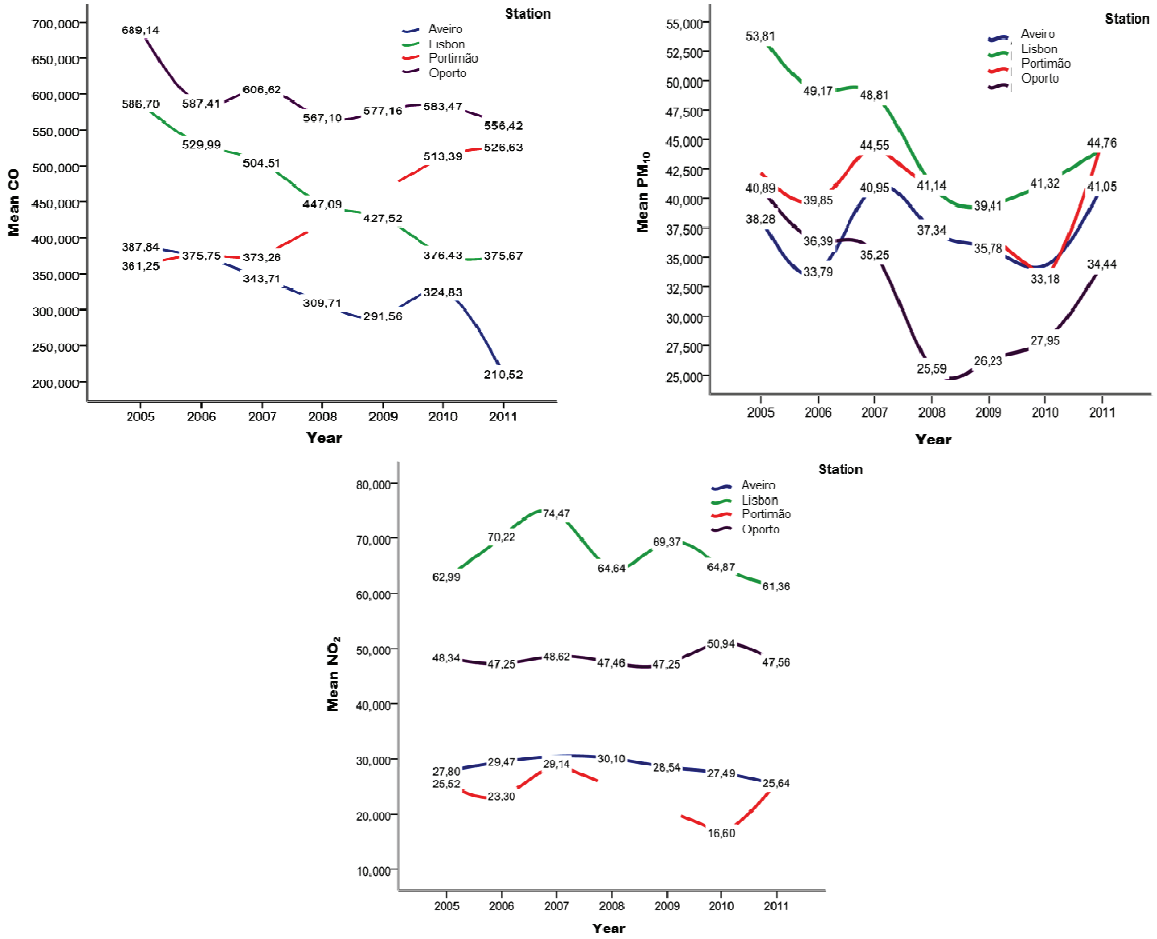


Figure 2 - Evolution of the concentration of CO, $PM_{10}$and NO_2inAveiro, Lisbon, Faro and Oporto stations from 2005 to 2011

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Characterization of ambient suspended particles in an urban area affected by industrialized activities loading at Setubal, Portugal

Authors: Alexandra Silva[1,2], Susana Marta Almeida[1], Cristina Oliveira[3]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, EN 10, km 139.7, 2695-066 Bobadela LRS, Portugal.*

2 *Universidade de Aveiro, Departamento de Ambiente e Ordenamento, 3 810-193 Aveiro, Portugal*

3 *CCMM-Department of Chemistry and Biochemistry, Faculty of Science, Lisbon, Portugal*

Presenting Author: Email: alexandra.silva@ctn.ist.utl.pt | Tel.: +351 219 946 130

INTRODUCTION:

Urban areas are affected not only by road traffic but also by industrial activities that are frequently located close the cities. The industrial activities in cities along the accompanying urban expansion increase contribute for the highest levels of pollution and the largest targets of impact.

In the Iberian Peninsula, PM pollution assumes a special importance, including Setúbal area, due different sun favorable climatologic factors [1-2], the geographical proximity to the coast and northern [3] and PM health effects [4].

Depending on the size, shape and chemical composition of the PM significant health effects can occur. According to Directive 2008/50/CE,PM_{10} are routinely measured in Europe. $PM_{2.5}$ are also receiving worldwide attention, as they have a potential high impact on the human health, principally on that part of the population living in urban areas affected by high traffic density or industry. However, PM information is incomplete to perform an exhaustive study of the nature of several sources of contamination and the processes which affect the air quality.

OBJECTIVES:

The present work was developed within the project PMfugitive and intends to perform a chemical characterization of particulate matter ($PM_{2.5}$ and $PM_{2.5-10}$) sampled in Setúbal - Portugal, to analyse seasonal trends and to identify sources and formation mechanisms of particles.

MATERIALS AND METHODS:

With about 125 000 inhabitants, Setúbal is located in the west of Portugal, on the Atlantic Ocean coast at the point where the river Sado flows into the Atlantic.

During the winter and summer of 2011, $PM_{2.5}$ and $PM_{2.5-10}$samples were collected simultaneously in two monitoring stations, classified as urban traffic, and in an Industrial area in Setúbal. Sampling was performed on a 12h basis using a Gent sampler. Filter mass before and after sampling was obtained as the average of three measurements, when observed variations were less than 5%.

The exposed nuclepore filters were cut into two parts, one of them was analysed by Instrumental Neutron Activation Analysis using the k0 methodology (k0-INAA) for the determination of chemical elements and another part was used by Ion-exchange Chromatography for the determination of water soluble ions.

RESULTS AND DISCUSSION:

Source apportionment was performed by integrating time variability, night and day comparisons, enrichment factors, fine to coarse ratios and principal component analysis.

$PM_{2.5}$ and $PM_{2.5-10}$ total mass concentrations measured during the sampling campaigns are presented in Figure 1. Values are discriminated by season and daily period.

For the urban traffic station (Quebedo) fine particulate mass concentration varied between 2.0 and 35 $\mu g.m^{-3}$ in winter and between 4.0 and 21 $\mu g.m^{-3}$ in summer. Mann Whitney U-test showed that $PM_{2.5}$ total mass concentration did not significantly differed between seasons (p=0.26). For coarse fraction, considerably high concentrations were obtained during the summer (16 $\mu g.m^{-3}$) comparing with the winter (12 $\mu g.m^{-3}$), probably due to higher re-suspension conditions that occur during the dry season. Significant differences were not found between day and night periods both for fine (p=0.98) and coarse (p=0.94) fractions.

For the Industrial station fine particulate mass concentration varied between 1.6 and 34 $\mu g.m^{-3}$ in winter and between 4.0 and 21 $\mu g.m^{-3}$ in summer. For coarse fraction, the average concentration was 15 $\mu g.m^{-3}$ in winter and 16$\mu g.m^{-3}$ in summer. Significant differences were not found for these season periods for the both fractions.

PMF results show that in Quebedo the major $PM_{2.5}$ aerosol mass contributors included vehicle exhaust (36%) and secondary aerosol (24%). In the coarse fraction, NO^{3-}(46%) and marine (16%) and mineral (16%) aerosol contributions were predominant (Figure 2).

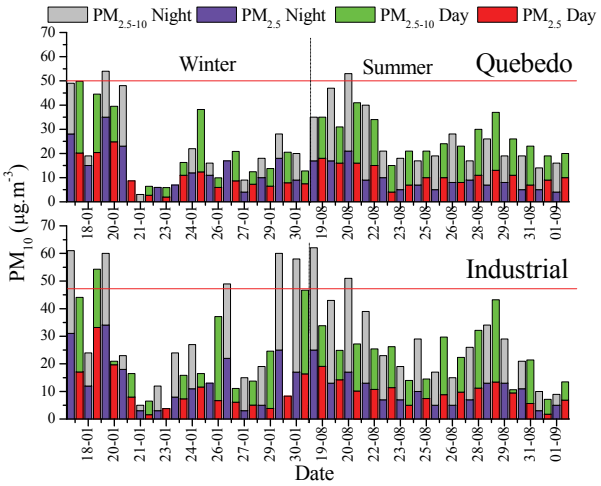


Figure 1 - Day and night $PM_{2.5}$, $PM_{2.5-10}$ and PM_{10} concentrations measured in the urban traffic and industrial station (Quebedo and Industrial) during the winter and summer periods. Values are in $\mu g.m^{-3}$.

Fine fraction:
F1- Vehicle exhaust,
F2-Soil,
F3- Cement Industry,
F4-Secondary Aerosol,
F5- Sea.
Coarse fraction:
G1-Secondary Aerosol,
G2-Sea,
G3-Nitrate,
G4-Vehicleexhaust,
G5- Soil.

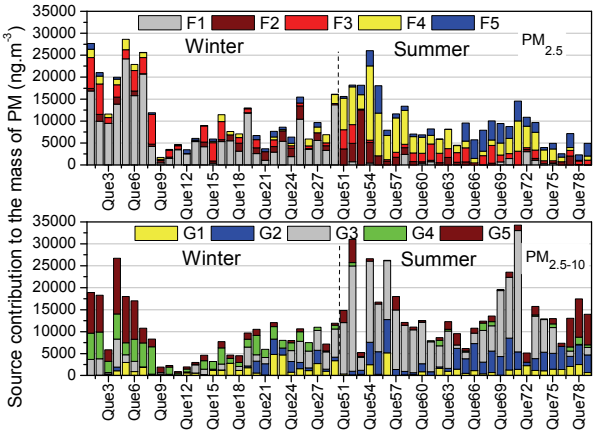


Figure 2 - Source contribution to $PM_{2.5}$ and $PM_{2.5-10}$ concentrations ($ng.m^{-3}$) in Urban Traffic Station (Quebedo).

CONCLUSION:

These approaches identified the emission sources: sea, soil, secondary aerosol, combustion and traffic and the results identified vehicle exhaust as the principal source contribution.

ACKNOWLEDGMENTS:

We gratefully acknowledge FCT for funding the project PMfugitive (PTDC/AAC-AMB/098825/2008) and the PhD fellowship SFRH/BD/78698/2011.

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A rising risk to human health: assessment of light pollution on two dark sky regions of Portugal

Authors: Raul Lima[1,2], José Pinto da Cunha[3], Nuno Peixinho[2,4]

1 *Physics, School of Allied Health Sciences of Polytechnic of Porto, Portugal*
2 *Center for Geophysics of the University of Coimbra/Astronomical Observatory of the University of Coimbra*
3 *Physics Department, University of Coimbra*
4 *Astronomy Unit, University of Antofagasta*

Presenting Author: Email: raul.s.lima@gmail.com | Tel.: +351 960 225 091

INTRODUCTION:

Bad public and private artificial light planning is the main cause for light pollution (LP). Night illumination is part of modern societies, reaching increasing levels with consequences on various fields that only recently began to be studied. Residents of large cities are almost totally deprived from a starry night. UNESCO, UNTWO and IAU's Declaration in Defence of the Night Sky and the Right to Starlight state that "an unpolluted night sky that allows the enjoyment and contemplation of the firmament should be considered an inalienable right equivalent to all other socio-cultural and environmental rights. Hence the progressive degradation of the night sky must be regarded as a fundamental loss" and that "the intelligent use of artificial lighting that minimizes sky glow and avoids obtrusive visual impact on both humans and wildlife should be promoted. This strategy would involve a more efficient use of energy so as to meet the wider commitments made on climate change, and for the protection of the environment"(Marín & Jafari, 2007).The observation and study of the night sky both at professional and casual or amateur levels is the first to be affected by light pollution thus being the primary reason to study LP until recently. However, other LP effects are already known to affect the human being such as modifications of the circadian rhythm(Stevens, 2006). More recent studies suggest LP as a risk factor for human breast and prostate cancer(Haim & Portnov, 2013). Other direct and indirect effects on the environment include natural resources consumption and effects on biodiversity, namely nocturnal fauna(Rich & Longcore, 2005).The creation of dark-skies protected areas is one form to preserve the night sky of some regions that still have a good dark sky quality. It is also a way to draw the attention of public and stakeholders to this growing problem. More than a local effect caused mainly by large urban centres, light pollution propagates to regions that are tensor more kilometres away from those populated regions, affecting places once with good night sky quality even a few years ago. Two examples of regions in Portugal that offer good dark skies are, at the north of the country, the Peneda-Gerês National Park and, at the south, the Alentejo's region of Alqueva, both commonly known to have good dark skies but that have not been previously measured. The latter got its classification as «dark sky reserve» based on the values of night sky brightness obtained for this work.

OBJECTIVES:

Light pollution increases as a consequence of bad lighting planning and of the growth of cities(Walker, 1977). The creation of «dark sky preserves» is not sufficient unless other accompanying measures are taken to prevent the propagation of light pollution within those areas. The assessment of the places of study regarding sky brightness is then of the utmost importance in order to act both on the prevention side and on the improvement of the dark sky quality. Being recognized as a source of pollution with effects beyond astronomy, namely on human health, a permanent record of the night sky darkness becomes a necessity to trace the evolution of LP. This work is part of an ongoing PhD thesis and quantifies the sky darkness of two large areas of Portugal (Fig. 1), aiming to be a starting point to a permanent record of light pollution levels at those places. In Galiza (Spain) a similar work has effectively started where several stations register on a permanent basis the brightness of local night sky (Salvador Bará, personal communication, March 6, 2014. Also URL: http://webspersoais.usc.es/persoais/salva.bara/GalicianNSBnetwork.html).

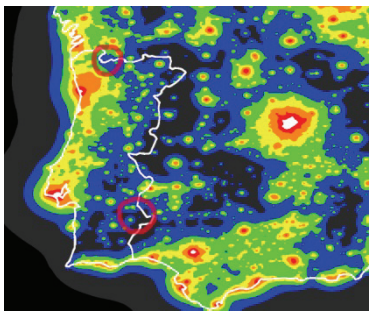


Fig. 1 - Areas of study superimposed on night sky brightness map (PNPG, red circle above, Alqueva, red circle below). Adapted from Cinzano P, Falchi F, Elvidge CD (2001) The first World Atlas of the artificial night sky brightness. Mon Not R AstronSoc 328:689-707

MATERIALS AND METHODS:

The measurements were obtained at the zenith on moonless nights, between dusk and dawn astronomical crepuscules to minimize natural light contribution and to guarantee that only artificial light contribution was being measured against natural dark sky brightness. We used a portable Unihedron® Sky Quality Meter-L and an iPhone unit with a GPS application (MotionX-GPS v21.1) for georeferentiation mostly along the roads that cross both regions. The campaigns started in 2010 and the next campaign is due to the next summer (2014).

RESULTS AND DISCUSSION:

The Dark Sky® Alqueva Reserve has a large area (around 3000 sq. km) of almost continuous good quality night sky darkness, negatively affected only at regions close to its main populated areas, namely Reguengos de Monsaraz (pop. 7261 inh.; source: INE 2011) (Fig. 2).

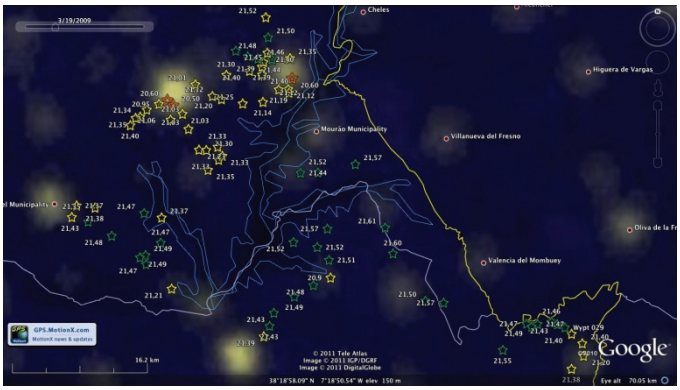


Fig. 2 - Sample of results for Alqueva from one of the campaigns. All values in mag/arcsec^2.

The smaller (c. 710 sq. km) and loosely populated Peneda-Gerês' National Park (PNPG) is mostly affected by LP at its south and south western most parts, where many small but highly concentrated populated areas prevail. Light pollution contribution here therefore is mainly from those sources. We have had readings above 21,8 mag/arcsec^2 on some places on both regions, consistent with excellent dark sky quality. These are the first results obtained from the ground for both regions. The results for all the region of Alqueva were used to apply for classification as Starlight Tourism Destination from the Starlight Foundation (UNESCO/United Nations World Tourism Organization/Instituto de Astrofisica de Canarias). The Dark Sky® Reserve was the first site in the world to achieve this certification (December 2011). The first results for Peneda-Gerês National Park from this work were also used for the application for regulation regarding light pollution within the Park, which became effective with the Park's latest Management Plan.

We found that the main contribution to light pollution in the PNPG comes from regions outside the Park extending for kilometers away (Comunidades Intermunicipais of Alto Minho, Cávado, Alto Tâmega, Ave and Grande Porto), whereas the main source to light pollution in the Reserva Dark Sky® Alqueva comes from small towns within and at the borders of the Reserva (namely Reguengos de Monsaraz). On both areas the

small towns contribution is almost negligible except from within those towns, where glare from bad lighting blocks the vision of all but the brightest stars. A few hundred meters away from those localities the sky darkness is again of very good quality.

CONCLUSION:

We have analysed the sky brightness on two regions in Portugal known to be of good quality regarding darkness, in order to quantify and assess the major sources of light pollution to that areas. We present the first results of the night sky brightness for those two regions. The night sky on both Alqueva and Peneda-Gerês, albeit being regions with different characteristics, has exceptional quality that urges to be preserved. Being a growing menace to human health, we emphasise the necessity of continuous monitoring of light pollution to reduce it where possible, namely on populated cities and avoid propagation to well preserved places.

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Tolerance of *Venerupis decussata* and *Venerupis philippinarum* clams to lead

Authors: Cátia Velez[1,2], Rosa Freitas[1,2], Paulo Cardoso[1], Vanessa Carreqosa[1], Sara Antunes[2,3], Roberto Martins[1,2], Amadeu M.V.M Soares[1,2], Etelvina Figueira[1,2]

1 *Departamento de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal*

2 *CESAM - Centro de Estudos do Ambiente e do Mar, Universidade de Aveiro, 3810-193 Aveiro, Portugal*

3 *Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal.*

Presenting Author: Email: catiavelez@ua-pt | Tel.: +351 234 370 350 | Fax: +351 234 372 587

INTRODUCTION:

The aquatic ecosystems receive a large amount of contaminants from industrial activities, agriculture, urbanization and domestic effluents, being the water, sediment and organisms exposed to a variety of metals, essential and non-essential, such as lead (Pb), one of the most toxic to marine environment (Company et al. 2011).

Lead, occurring in a soluble form in seawater, can be directly absorbed by aquatic organisms, accumulated by biogenic particles (Fisher et al. 1996), adsorbed to non-living particles or precipitated with carbonate or sulphide (Eisler, 1981). Thus, filter feeders like bivalves are particularly exposed to this contaminant (Figueira et al., 2012).

The clams *Venerupisdecussata* (European clam) and *V. philippinarum* (endemic species from the Indo-Pacific region) are species with a significant role in the ecosystem and have high socioeconomic value. *V. philippinarum* is faster-growing and more resistant towards environmental physical stress than the native species *V. decussata*, competing not only on aquaculture farms but also in their natural habitat.

In Ria de Aveiro, *V. philippinarum* has been rapidly spreading throughout the ecosystem, leading to a decrease in abundance of *V. decussata*. Recent studies conducted with both clam species, collected in the same area, showed differences regarding bioaccumulation and tolerance to cadmium (Cd) (Figueira et al. 2012).

OBJECTIVES:

Study and compare the bioaccumulation, bioconcentration and tolerance of the two sympatric clam species, *V. decussata (native)* and *V. Philippinarum* (invasive), when exposed to Pb. The biochemical responses of both species after exposition to Pb were also evaluated.

MATERIALS AND METHODS:

Clams species *V. decussate* and *V. philippinarum* living in sympatry were collected in the Ria de Aveiro.

In the laboratory, organisms of both species were initially exposed to the same Pb concentrations (0, 3.6, 6, 10, 17 and 28 mg L^{-1}). However, 100% of the *V. decussata* specimens died after 48h of exposure and all *V. philippinarum* individuals survived up to the end of the experiment (96 h). Therefore, after several different sets of concentrations tested, both species were submitted to different Pb ranges inducing similar mortality levels (0, 0.1, 0.23, 0.45, 0.9, 1.8 and 3.6 mgL^{-1} for *V. decussata* and 0, 28, 47, 78, 136, 218 and 365 mgL^{-1} for *V. philippinarum*) during 96h at 18±1ºC (salinity 28.0±1.5 and photoperiod 12h). After exposure, mortality, $LC_{50}$and Cl_{95}values were determined for both species.

Pb accumulations in the soluble and insoluble fractions of soft tissues were determined by ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) in both clam species under environmental and laboratory conditions. The Bioaccumulation (BAF) and Bioconcentration Factor (BCF) were determined. BAF corresponded to the accumulation by organisms under natural environmental, influenced by water and dietary sources, while BCF is defined as the accumulation in organisms through water contamination measured under laboratory conditions (McGeer et al., 2003).
Indicators of cellular damage (total soluble protein, Lipid Peroxidation (LPO)), antioxidant enzyme activity (Catalase, CAT; Superoxide dismutase, SOD) and metallothioneins (MTs) were quantified, according to Figueira et al. (2012) using clamssoft tissues.

RESULTS AND DISCUSSION:

The invasive species (*V. philippinarum*) presented lower mortality at Pb concentrations 100 times higher than the native (*V. decussata*). The LC_{50} confirmed that the invasive species, *V. philippinarum*, present higher tolerance (about 160 times higher) towards Pb concentration than the native species, *V. decussate* (*V. philippinarum*: LC_{50} = 103.8 mg L^{-1}; CI_{95}: 79.4 - 177.9 mg L^{-1}; *V. decussata*: LC_{50} = 0.65 mg L^{-1}; CI_{95}: 0.38 - 1.09 mg L^{-1}). BAF values were similar for both species in natural conditions. However, BCF values are more 25 to141 times higher in *V.decussata* than in *V. philippinarum,* supporting the higher mortality rates, at lower Pb concentrations, in *V. decussata.*

The biochemical responses induced by Pb, such as alteration in total protein content and lipid peroxidation, the activity of antioxidant enzymes and the induction of MTs revealed differences between both species (Figure 1).

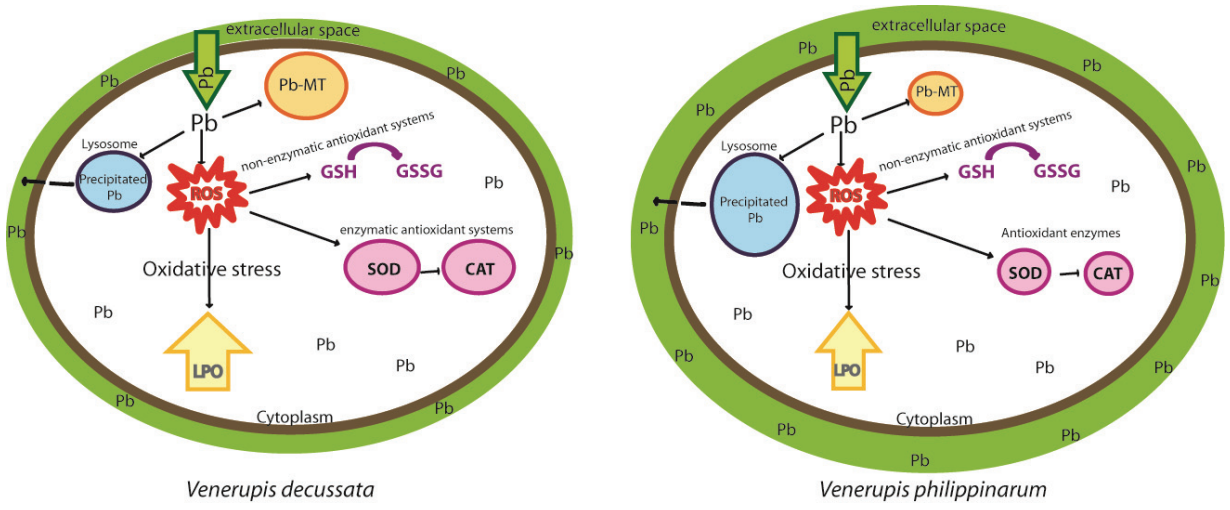


Figure 1 - Tolerance mechanism triggered by Pb exposure in *V. decussata* and *V. philippinarum*. *V. decussate* accumulates lessPb levels (green arrows) than *V. philippinarum* when exposed to 100 times lower concentrations of Pb - *V. philippinarum* restricts more efficiently Pb entry in the cell.In *V. decussata*, Pb enters into the cell, inducing MTsand Pb precipitation in lysosomes. Precipitated Pb can be stored intracellularly or excreted out of the cell. The free Pb in the cytoplasm increases reactive oxygen species (ROS). To prevent cellular damages, the activity of the antioxidant enzymes (catalase (CAT) and superoxide dismutase (SOD)) increases. The elimination of ROS can also be caused by non-enzymatic processes such as the oxidation (GSSG) of reduced (GSH) glutathione. When these mechanisms fail, oxidative stress overcomes and lipid peroxidation increases. In *V. philippinarum* a higher proportion of Pb accumulated is precipitated and less Pb ions remain free in the cytosol, resulting in lower ROS increase, lower activity of antioxidant enzymes and lower LPO.

Differences in the tolerance towards Pb may be explained by differences in accumulation and partitioning. Both clams allocated most of the metal to the insoluble fraction, being this mechanism especially evident for *V. philippinarum*. Previous studies (Figueira et al. 2012) also revealed, for both species, higher percentage (84.5-98.2%) of Cd in the insoluble fraction than in soluble fraction, when submitted to the same concentration.

CONCLUSION:

The native species (*V. decussata*) presented lower tolerance to Pb than the exotic species (*V. philippinarum*) due to the higher accumulation (100 times)of this metal. Similar free Pb concentrations in the cytosol and identical biochemical responses between both species are observed when exposed to completely different (100 times) Pb ranges.

ACKNOWLEDGMENTS:

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Exploration of the spatial Composite Risk Index (CRI) for the characterization of toxicokinetics in petrochemical active areas.

Authors: Richard Olawoyin[1,2], Charles W. McGlothlin[1]

1 *Prevention Research Center, School of Health Sciences, Oakland University, Rochester, Michigan, 48309 USA*
2 *Environmental Health and Safety Engineering, Pennsylvania State University, University Park, Pennsylvania, USA*

Presenting Author: Email: olawoyin@oakland.edu | Tel.: +1 248-364-8653 | Fax: +1 248-364-8657

INTRODUCTION:

The spatial modeling of the petrochemical active regions in the Niger Delta (ND), Nigeria was carried out through the analysis, exploration and extraction of geospatial data and resultant risk maps were generated. The pollutants assessed include; heavy metals, polychlorinated aromatic hydrocarbons (PAHs), benzene-toluene-ethylene-xylene (BTEX), and total petroleum hydrocarbons (TPHs) and properties of the pollutants such as bioaccumulation, persistence and toxicity were used to calculate the Hazard Index (HI) and thus created a ranking system. The Composite Risk Index (CRI) was developed successively considering the concentrations of all pollutants and the computed HI using the samples collected in ND areas of Nigeria. The carcinogenic PAHs showed spatial abundance in the areas sampled and elevated levels of soil heavy metals were also observed. In this study, mathematical tool such as the artificial neural network (ANN) self-organizing map (SOM) and geostatistical analysis such as kriging were applied to develop the risk map of the areas which represent the spatial spread of the CRI. The results show that the application of spatially developed integral risk map for pollutant assessment is effective and facilitates decision making with regards to the environment and the humans exposed in the region.

OBJECTIVES:

The use of mathematical tool such as the artificial neural network (ANN) self-organizing map (SOM) and geostatistical analysis such as kriging to develop the risk map of polluted areas which represent the spatial spread of the integral risk to human health from petrochemical pollution.

MATERIALS AND METHODS:

Hazard assessment (HA) The identification, classification and assessment (ICA) of hazards are important steps in evaluating the quality of environment materials. ICA is primarily a function of the PBT of the chemicals of concern (CoC). The application of PBT criteria for the minimization of CoCs and identification of these substances have been successfully used in various studies. The proxy for hydrolysis and biodegradation can be considered as the biota is exposed to a variety of chemicals which can be classified as the measure of persistence. The importance of HA include the following; hazard classifications of CoC; priority substances ranking; further investigations into contaminated sites; derivation of quality criteria for environmental materials and the use of exposure data for the assessment of ecological risk. The calculation of the Composite Risk Index (RIc) was carried out using information from the concentration of each pollutant measured from the sample and the hazard index of the chemicals. The mathematical equation for this expression is as shown in equation 1.

$$\boldsymbol{RI_c} = \left\{\frac{\sum(\boldsymbol{HI_{PBT}} * \ \boldsymbol{P_{ci}})}{\boldsymbol{P_n}}\right\} \ldots\ldots \quad \ldots\ldots \quad \ldots \quad \ldots\ldots \quad \ldots \quad eq.1$$

Where;RI_c= composite risk index; HI_{PBT}= hazard index; P_{ci}= pollutant concentration; P_n= number of pollutants. ***Hazard Index*** **(HI_{PBT}):** This is the measure of the hazard effect of a substance relative to other compounds in the same series. The PBT is used to calculate the HI_{PBT} . PBT can be defined as P - Persistence: This is the measure of the half-lives of substances in environmental materials (sediments, soil, water and

air); B - Bioaccumulation: The **EPI Suite**™ KOWWIN™ can be used to compute the logarithm of the bioconcentration factor (log BCF) which estimates the bioaccumulation of substances in environmental materials using the octonol-water constant (K_{ow}) using an atom/fragment contribution method; T - Toxicity: The degree to which irreversible damages are induced in organisms due to exposures to harmful substances, this could cause lethality to organs, tissues or the entire body. It is also dose dependent and it can be measured by assessing the effects on the toxicant recipient. The HI_{PBT} is computed by adding up variables according to their PBT (i.e. P + B + T =HI_{PBT}).

Environmental samples were collected in the Niger Delta area (NDA) at five locations in Bonny (BN), Eriemu (ER), Odidi (OD) and Ughelli (OG). The concentrations of all pollutants in the samples (soil, sediment, and water) were determined. Analyses of the heavy metals were done using extraction methods following the US EPA method 3050B. Polycyclic aromatic hydrocarbons (PAHs) were treated using mass spectrometry detection (MSD). Details on sample collection and analysis are presented in Olawoyin *et al.,* (2012, 2013). About 103 total samples were collected for soil, sediment and water from the five different locations all around the study areas (BN, ER, OD, OG and WR).

RESULTS AND DISCUSSION:

Spatial modeling of the petrochemical regions of the NDA was carried out using eq. 1. The RI_c conforming to each sample point was computed and the results obtained were consistent with the other indices calculated. The areas of heavy petrochemical operations showed very high RI_c with the highest value of 2.7 at ER-SS16A. The risk map and contour map of the different sampled locations in the ND area which represent the geospatial distribution of the $RI_c$are presented. As shown on Fig. 1, at the BN location, the highest risk areas were observed towards the crude oil export terminals and facilities, which decrease into residential areas.

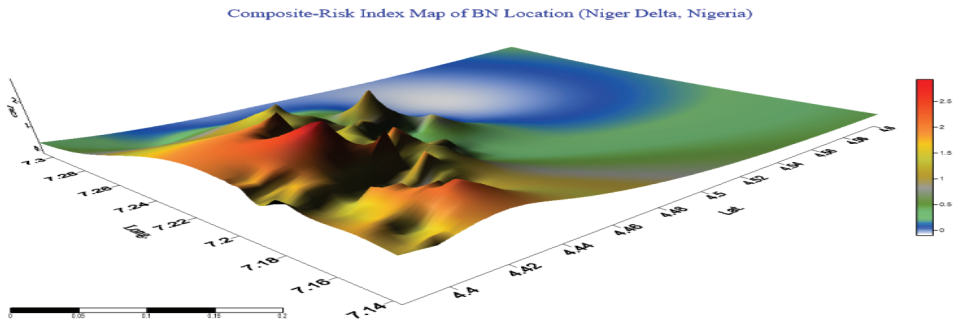


Fig. 1 - Composite risk map of the BN Location, Niger Delta, Nigeria

The risk level of concern is defined by any area having RI_c of 0.5 or more, based on baseline values. The mean RI_c for the residential areas was determined to be 1.8; this indicates elevated risk levels to the residents of the BN area. Hotspots were identified at three areas with exceedingly higher RI_c than the mean RI_c in the region. The most impacted zones were in the NW and W corners and some zones in the central areas with risk values of up to 2.4 and there is a high percentage of human population in these zones. The RI_cmap for the BN location was overlaid on the geographic maps as shown in Fig. 2, applied to all locations.

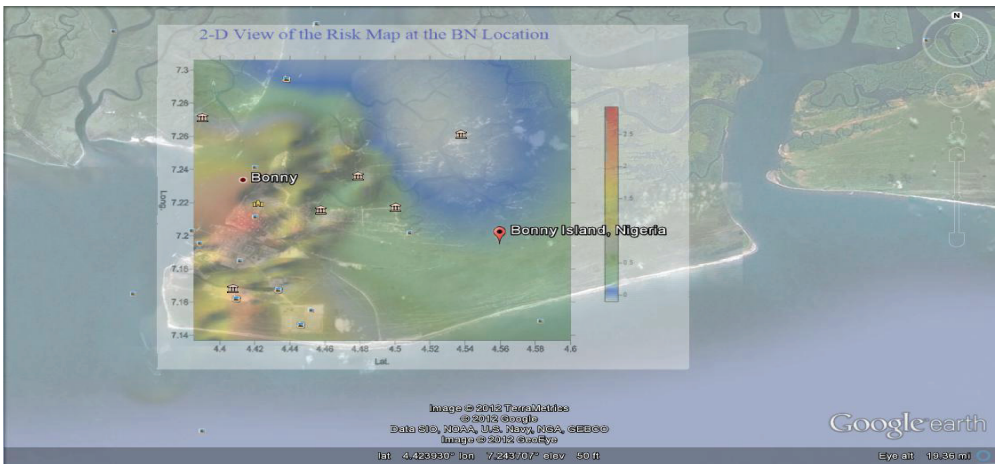


Fig. 2 - Composite-Risk Map of BN Location Overlaying the Physical Map of the BN Location using Google Earth

CONCLUSION:

Spatial modeling was used to characterize the composite risk which illustrates the global and local risk of the areas of interest. The obtainable chemical concentrations of each pollutant were observed and used as the bases for the calculation of the risk indices. The methodology applied in this study involved the assessment of human health risk base on chemicals levels in environmental materials by identifying the hazards, analyzing the dose-response of the chemicals, assessing the exposure rate and finally characterizing the risk (Goldstein, 2005). These procedures are all inclusive which incorporates the interest of all stakeholders such as the petrochemical operators, the residents of these communities, local and state governments, environmental managers and the general public.

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Association between atmospheric pollutants and hospital admissions in Lisbon

Authors: Ana Cruz[1,3*], Marta Almeida[2], Célia Alves[4], Maria Freitas[2], Hubert Wolterbeek[3]

1 *Escola Superior de Tecnologia e Gestão de Oliveira do Hospital, Instituto Politécnico de Coimbra, Oliveira do Hospital, Portugal*

2 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Loures, Portugal*

3 *College of Applied Sciences, Department of Radiation, Radionuclides and Reactors, Section RIH, Technical University of Delft, Delft, The Netherlands*

4 *CESAM, Universidade de Aveiro, Aveiro, Portugal*

Presenting Author: Email: ana.cruz@estgoh.ipc.pt | Tel.: +351 963 476 700 | Fax: 238 605 179

INTRODUCTION:

Ambient air pollution is recognised as one of the potential environmental risk factors causing many health hazards to the exposed population. This has been demonstrated in numerous studies conducted across the Globe. Numerous longitudinal, ecological and epidemiological studies have been shown a relation between outdoor levels of atmospheric pollutants and adverse health effects, especially associated with respiratory and cardiovascular hospital admissions.

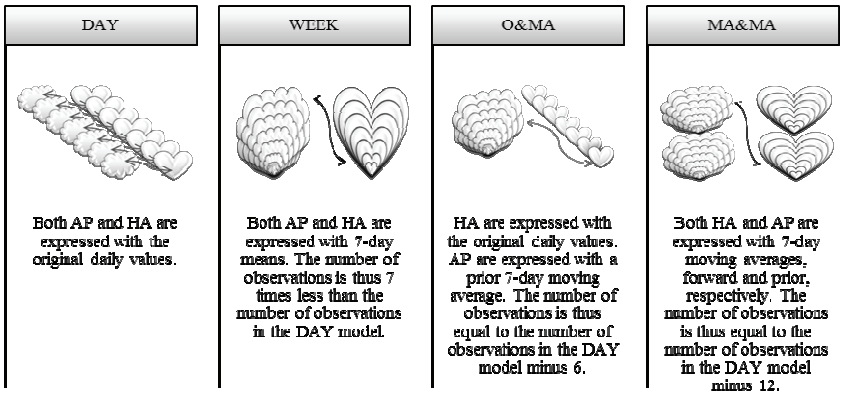
OBJECTIVES:

The aim of this work is to assess the influence of atmospheric pollution over the hospital admissions in Lisbon, by ordinary least squares linear regression (ols).

Materials and methods: the air pollutant data (CO, NO, NO_2, SO_2, O_3, PM_{10} AND $PM_{2.5}$) were obtained from 13 monitoring stations of the portuguese environmental agency, which provide hourly observations. Hospital admission data were collected from the central administration of the health system (ACSS) and were compiled by age: <15, 15-64; >64 years old. the study period was 2005-2008.

In an attempt to better understand the effect of the pollutants on hospital admissions for the different diseases, four different ways of aggregating atmospheric pollutants and hospital admission variables, were considered over time. Studies on human health effects usually use daily data; although, recent epidemiological studies have been using exposures in the form of moving averages, distributed lags or data aggregated over several days or even weeks (Schwartz 2000; Sarmento et al. 2009). In this study, we started by using a daily database (DAY model). From this daily database we have created other three with different aggregations that were then used in WEEK, O&MA and MA&MA models, according to Table 1, as applied in Almeida et al. (2014).

Table 1 - Model specifications for exposure versus response variables considering different ways of aggregation over time (AP - atmospheric pollutants; HA - hospital admissions).



Ordinary Least Squares Linear Regression (OLS) was performed with Excel 2007 and SPSS 17.0 software packages. In each model, the dependent and independent variables were, respectively, the hospital admissions and the atmospheric pollutants.

Control of seasonal confounding was performed by including 12 dummy variables for each month. The dummy variables took on either the value zero or the average value of all daily observations falling in that month (considering all the years). For instance, the dummy variable for January assumed the value of zero for all observations, except for January, where the average daily observations for hospital admissions were considered. These dummy variables were used in all models.

Weekend effects were controlled, in models DAY and O&MA, by including a weekend dummy, zero on weekends and 1 on weekdays. In the models WEEK and MA&MA, there was no weekend effect due to the use of weekly averages. No considerations were taken for holidays.

RESULTS AND DISCUSSION:

In this study 37 relationships between air pollutants and health data were found to have a positive significant linear correlation for at least one of the four models (DAY, WEEK, O&MA; MA&MA). All atmospheric pollutants, except O_3, showed significant and positive correlations with all typologies of hospital admissions, except asthma.

The highest associations were obtained with the MA&MA model (p=0.000), which predicted that an increase of 10 $\mu g.m^{-3}$ in NO_2 and PM_{10} concentrations were associated with an increase in hospital admissions of about 1.9% for childhood respiratory diseases.

CONCLUSION:

This study strongly suggests that there is a significant causal relationship between atmospheric pollutants and health problems.

Further studies will be developed to evaluate and improve the robustness of the model through the inclusion of meteorological data (which can be confounding factors). The adequate treatment of outliers will be done, their impact on the model will be evaluated and trend/seasonal control will be assessed as well.

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Assessing a health risk thanks to regional mappings based on local perceptions: A comparative study of three different hazards in three sites: Laos, Tunisia and Ecuador.

Authors: Nicolas Maestripieri[1], Mehdi Saqalli[1], Bénédicte Veyrac-Ben Ahmed[1], Sylvia Becerra[2], Marguerite Munoz[2], Ribolzi Olivier[2]

1 *GEODE Laboratory (Geography of Environment), 5, Allées A. Machado, 31058 Toulouse, CEDEX 9, France*
2 *GET Laboratory (Geosciences Environment Toulouse), 14, avenue Édouard Belin, 31400 Toulouse, France*

Presenting Author: Email: maestripieri@univ-tlse2.fr | Tel.: +33 663 244 869

INTRODUCTION:

While human societies remained exposed to natural hazards, anthropogenic dynamics have dramatically increased the exposure to human-originated factors: hazards and risks are more and more coming from human-originated sources. Meanwhile, thanks to economic development, vulnerabilities to such dangers can be managed, controlled or reduced thanks to public policies, through environmental control, sanitation and medical infrastructures and practices (Becerra, 2012). However, assessing such vulnerabilities faces difficulties. Along the evaluation of such risks and hazards themselves through environmental and epidemiological measurements (Ghorbel et al. 2010), evaluations and modelisations, evaluating human vulnerabilities and capacities to face such hazards requires to understand the perceptions of people who can or are already affected, which conditions the local acknowledgement of such dangers but also the differential evaluation among the concerned public stakeholders and population.

OBJECTIVES:

The goal of these researches is to better understand how a human-originated contamination by some pollutants is perceived by local stakeholders. The most efficient and most practicable way to such an evaluation goes through spatialising the corresponding aleas, along other constraints people may consider. We have assessed three mapped evaluations based on local perception at the regional level in three different sites in three different countries, each one corresponding to three different contaminations (tropical diseases, heavy metals and petrol). This communication tends to present these results and compare them in order to establish the efficiency of such a method for different contaminations and different social and environmental conditions.

MATERIALS AND METHODS:

The Perception-Based Regional Mapping (PBRM) method is based on interviews of pairs of local people, supported by a map of the concerned area on which a tracing paper is set (Figure 1, Saqalli et al., 2009). A series of maps is therefore obtained, combined and analyzed through a Geographical Information System. The advantage of such a method is to obtain the local hierarchy of factors that described the territory according to the sampled population, from which the hazard can be evaluated. Such maps provide the inherent multidisciplinary environment of the concerned population, including local economy, sociology and environmental constraints that may condition the capacity to face environmental hazards. Moreover, because the map is already positioned, it can, unless a mental map, be compared and confronted to other spatial evaluations of the same hazard for validation. Finally, it is a low-cost and rapid method. The PBRM may be exact in terms of variable collecting and precise topologically, it cannot be precise spatially. Plus, a census of a perceived hazard does not prove the existence and/or extent of this hazard. Despite this, the census tests the adequacy between the existence and/or extent of the real hazard and the perceived hazard.

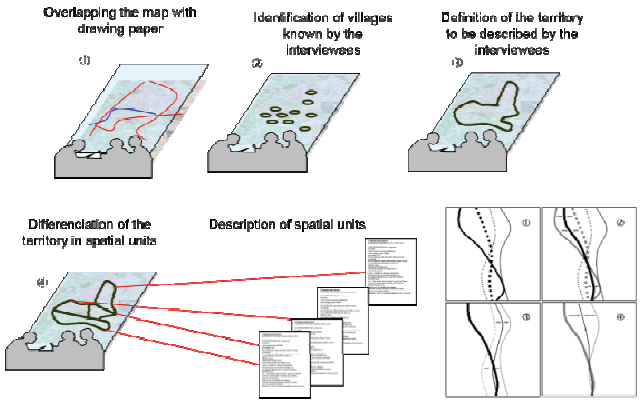


Figure 1 - The different steps of the PBRM process

RESULTS AND DISCUSSION:

Laos, Tunisia and Ecuador have all in common their exposure to contaminants coming from the exploitation of natural resources, which actually drove the interest of environmental scientists on such places: in the region of Luang Phabang (Laos PDR) (Ribolzi et al., 2011), it is the exploitation and paradoxically the spatial restriction of farming and the concentration of the population along rivers that may condition the exposure to bacterial contaminants. Around the former mines of plumb and cadmium of the Jebel Ressas Mount (Tunisia), the signal of the exposure to the heavy metals of the terrils is de facto mixed with other and more visible exposures (nitrates from intensive farming, dusts from a cementary plant nearby). In the Ecuadorian Oriente, the petrol contamination may be paradoxically so well-known as a global risk but not as a spatially precise one that it can be considered now as part of the social landscape, along other social vulnerabilities such as unemployment and insecurity.

For each case study, the PBRM highlight common features such as Figure 2, a result of the investigation in the surroundings of Luang Phabang (Laos PDR) (figure 2):

The environmental hazards we investigate were rarely pointed out by the interviewees as the most important issues:

1. As chronic problems, they are “covered” by individually more vital issues (infrastructures, employment). As problems seen as complex and non-solvable at the individual scale, they are perceived as “beyond the scope” of one person and thereby often neglected.
2. As human-originated and human-affecting issues, there is an obvious correspondence between population density, sanitary risks related to the investigated hazards and a relative weakness of the State per capita: for instance, in Lao PDR, there may be more hospitals in high populated areas but actually less infrastructures per capita.

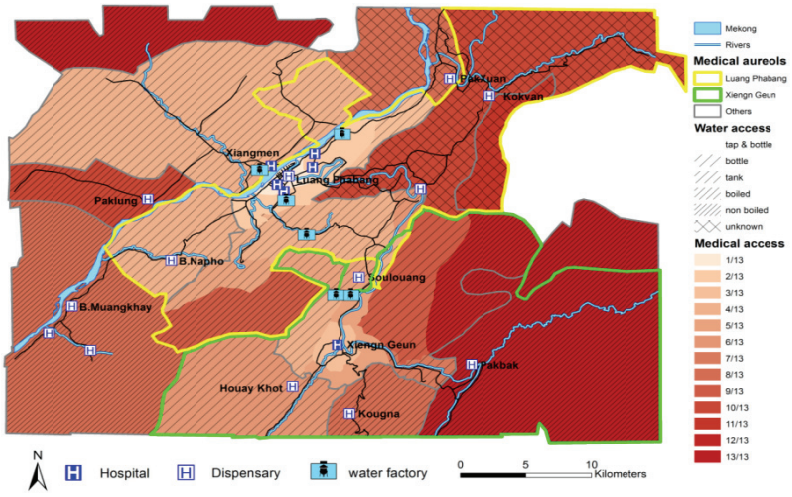


Figure 2 - Spatialisation of medical aureoles and medical/water access according to PBRM health interviewees

CONCLUSION:

Such a method can be useful for apprehending rapidly the gap between perceived and "real" risks but also the inherent mixture of feelings and perceptions public stakeholders have to deal with. Finally, visualizing spatial differentiations may allow going beyond the mean sanitary cover until the median sanitary cover.

ACKNOWLEDGMENTS:

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Health impact assessment of traffic noise in Madrid

Authors: Cristina Linares[1], Alberto Recio[2], Aurelio Tobías[3], Julio Díaz[1]

1 *National School of Public Health, Carlos III Institute of Health, Madrid, Spain.*
2 *Department of Preventive Medicine and Public Health, Autonomous University of Madrid, Madrid, Spain.*
3 *Institute of Environmental Assessment and Water Research, Spanish Council for Scientific Research, Barcelona, Spain.*

Presenting Author: Email: aurelio.tobias@idaea.csic.es | Tel.: +34 934 006 100

INTRODUCTION:
The relationship between occupational noise and health outcomes has been widely investigated. Regarding environmental noise levels, the attention is focused on road traffic noise due to the large number of exposed persons and the large periods of exposure. The city of Madrid constitutes a dense metropolitan area with over 2 million motor vehicles. The main outdoor noise source is road traffic, being attributed 80% of the overall noise exposure.

OBJECTIVES:
To quantify the short-term effects and the avoidable deaths resulting from reducing diurnal equivalent level noise levels (LeqD) in comparison with 24-hour average levels of particulate matter with a diameter less than 2.5 μm (PM2.5) on daily mortality (for natural, cardiovascular and respiratory causes) for those over 65 years old in the city of Madrid, Spain.

MATERIALS AND METHODS:
The health impact assessment of LeqD and $PM_{2.5}$ was carried out by using risk estimates previously reported (Tobías et al. 2014a, Tobías et al. 2014b), as relative risks for an increase of 0.5 dB(A) of LeqD and 10 $\mu g/m^3$ of $PM_{2.5}$, on municipal mortality rates and population figures for the study period 2003-2005.

RESULTS AND DISCUSSION:
The association found between diurnal noise levels exposure and mortality suggests an important health effect. The average population over 65 years during the study period was of 591,468. Table 1 shows the decline in 0.5 dB(A) in LeqD implies an avoidable annual mortality of 312 deaths by natural causes, 145 deaths by cardiovascular causes and 97 deaths by respiratory causes in the population older than 65 years. The magnitude of the health impact is similar to the change of 10 $\mu g/m^3$ in the annual average levels of $PM_{2.5}$.

Table 1 - Relative risk (RR) of mortality due natural, cardiovascular and respiratory causes for and annual number of attributable deaths for subjects aged over 65 years

	LeqD	PM2.5
Mortality Indicator	**RR (95% CI)**	**RR (95% CI)**
Natural causes (ICD9:1-799)	1.017(1.005 - 1.030)	1.017 (1.004 - 1.030)
Number of attributable deaths	312 (92 - 543)	312 (74 - 543)
Cardiovascular causes (ICD9: 390-459)	1.024 (1.003 - 1.046)	1.019(0.997 - 1.041)
Number of attributable deaths	145 (18 - 273)	116 (-19 - 244)
Respiratory causes (ICD9: 460-519)	1.031 (1.001 - 1.062)	1.033 (1.003 - 1.064)
Number of attributable deaths	97 (3 - 189)	103 (10-195)

CONCLUSION:

Our results strongly suggests the need to seriously consider the noise exposure levels reported in this study as an important public health issue, at least equally important as air pollution levels.

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Short-term effects of black carbon on human health in Barcelona

Authors: Aurelio Tobías[1], Angeliki Karanasiou[1], Cristina Reche[1], Fulvio Amato[1], Andrés Alastuey[1], Xavier Querol[1]

1 *Institute of Environmental Assessment and Water Research, Spanish Council for Scientific Research, Barcelona, Spain.*

Presenting Author: Email: aurelio.tobias@idaea.csic.es | Tel.: +34 934 006 100

INTRODUCTION:

Current air quality standards for particulate matter use the mass concentration with aerodynamic diameters ≤2.5 µm (PM2.5) and ≤10 µm (PM10). It has been suggested that particles from combustion sources are more relevant to human health than particles from other sources. Only few studies have assessed for adverse health effects of black carbon particles (BC) as an additional in air quality indicator in Europe.

OBJECTIVES:

To investigate the short-term effects of BC on daily mortality and hospital admissions due to cardiovascular (ICD10: I00-I99) and respiratory causes (ICD10: J00-J99) in the city of Barcelona, Spain.

MATERIALS AND METHODS:

We collected daily mortality and hospital admissions due to cardiovascular and respiratory causes, and daily 24h average levels of BC and PM (PM2.5 and PM10) between 2009 and 2011. We used a time-stratified case-crossover design with overdispersed conditional Poisson adjusting for temperature, public holidays and influenza epidemics. BC estimates are compared with those obtained with the usual indicators of PM mass (PM2.5 and PM10).

RESULTS AND DISCUSSION:

Estimated health effects of an interquartile range increase (IQR) were greater, and statistically significant ($p<0.05$) for BC than for PM2.5 and PM10, for which no effects were found. An IQR increase of 1.4 µg/m^3 in BC increased daily cardiovascular mortality by 5%, hospital admissions due to cardiovascular causes by 2%, and respiratory mortality by 10%, while no effects were found for hospital admissions due to respiratory causes. BC effects were mainly found for people older than 65 years. Multi-pollutant models, simultaneously adjusting BC for PM2.5 and PM10, showed that effect of the BC was more robust than effect of the PM mass.

CONCLUSION:

BC is a valuable additional air quality indicator to evaluate the health risks of air quality dominated by primary combustion particles.

ACKNOWLEDGMENTS:

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Musks are all around - A comprehensive review

Authors: Nuno Ratola[1,2], Vera Homem[2], José Avelino Silva[2], Lúcia Santos[2], Arminda Alves[2]

1 *Physics of the Earth, University of Murcia, Campus de Espinardo, 30100 Murcia, Spain.*
2 *LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal.*

Presenting Author: Email: vhomem@fe.up.pt | Tel.: +351 22 041 4947 | Fax: +351 225 081 449

INTRODUCTION:

Synthetic musks have been used for a long time as fragrance additives and fixative compounds in personal care (e.g. perfumes, lotions, shampoos, deodorants) and household (e.g. detergents, fabric softeners, air fresheners) products (Zhang et al., 2008). In recent years, their release into the environment has increased considerably, to the point that they have been recognized as emerging pollutants by the scientific community (Homem et al., 2013). Musks are bioaccumulative, have a lipophilic nature and are only partially biodegradable, hence usually not completely removed due to their persistence in the environment. This presents a hazardous potential to ecosystems even at low concentrations. The number of studies in literature describing their worldwide presence in several matrices is growing, and this review intend to assess the state of the science in this issue.

OBJECTIVES:

The objective of this review is to draw a comprehensive picture of the presence of musks in several environmental matrices (water, air, dust, soils and plants).

MATERIALS AND METHODS:

Scientific publications reporting the levels of musks in the target environmental matrices (from 1983 to 2013) were reviewed and compiled in this work. This systematic literature review was conducted using several available electronic databases such as Scopus®, Elsevier®, Taylor & Francis®, ACS Publications®, Springer® and Google® Scholar.

RESULTS AND DISCUSSION:

As can be seen in Table 1, the number of studies regarding the presence of musks in the environment is rapidly increasing. About 100 works could be gathered in total. The current decade already has a rate of 12.3 studies/year, the majority of them dealing with water matrices (almost 50%).

Table 1 - Studies in literature reporting the presence of musks in several environmental matrices (from 1983 to 2013)

Year	Surface water	Ground water	Sea water	Other waters	Ambient air	Indoor air	Dust	Soils/ plants	TOTAL/ year
1981-1990	1								0.1
1991-2000	9		2		1	1			1.3
2001-2010	25	3	5	4	5	6	3		5.1
2011-2013	13	4	2	3	3	4	3	5	12.3
TOTAL	48	7	9	7	9	11	6	5	

The two musks more often mentioned are galaxolide (HHCB) and tonalide (AHTN), and these are also the ones showing the highest concentrations overall. In surface waters, HHCB levels up to 20000 ng/L could be found and the levels in this type of water are generally much higher than in groundwater, sea water or snow (not higher than 754.1 ng/L for HHCB).

In air, studies are divided into ambient and indoor environments, and the latter present the highest incidence, as expected. Values up to 344306 ng/m^3 were found for HHCB in the indoor air (17223 ng/m^3 in the ambient

air) of a wastewater treatment plant (WWTP), one of the recognised sources of musk release into the atmosphere. In urban air, however, values not higher than 3500 ng/m^3 were obtained for the sum of HHCB and AHTN and an important urban-rural decreasing gradient was also observed.

As to the solid matrices, dust samples (collected in indoor environments) show the highest concentrations (max. of 893000 ng/g for HHCB), followed by soils (max. of 610 ng/g for AHTN) and plants (max. of 336 ng/g for ambrettolide in lettuce irrigated with reclaimed water).

CONCLUSION:

The levels of musks in several environmental matrices urge the continuous study of these chemicals, particularly in soils and vegetation, where information is extremely scarce.

ACKNOWLEDGMENTS:

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Integrating human health risks on air quality assessment to improve urban environments

Authors: Solange Costa[1,2], Carlos Silveira[3], Joana Ferreira[3], Hélder Relvas[3], Diogo Lopes[3], Carlos Borrego[3], Peter Roebeling[3], Ana Isabel Miranda [3], João Paulo Teixeira[1,2]

1 *ISPUP, Institute of Public Health, University of Porto, Porto, Portugal*
2 *National Institute of Health, Environmental Health Department, Porto, Portugal*
3 *Centre for Environmental and Marine Studies & Department of Environment and Planning, University of Aveiro, Aveiro, Portugal*

Presenting Author: Email: solange.costa2@gmail.com | Tel.: +351 223 401 100 | Fax: +351 223 401 149

INTRODUCTION:

Quantifying the impact of air pollution on the public's health has become an increasingly critical component in policy discussion. Over the years, air pollutants have been related to a wide range of adverse health effects usually expressed in several mortality and morbidity endpoints. Recent data indicates high levels of urbanization, over 70 percent of the population in Europe, Australia and Northern America live in urban areas. Numerous scientific findings show that current levels of air pollutants observed in European cities are associated with health risks. A growing body of epidemiological and clinical evidence has led to a heightened concern about the potential effects of outdoor air pollution on health, particularly regarding cardiovascular outcomes. Moreover, an increased risk of lung cancer associated to exposure to overall air pollution was consistently observed in epidemiologic studies worldwide. Based on these findings IARC has recently classified outdoor air pollution carcinogenic to humans. The impact of the effects at the individual level may appear low compared to other risk factors. However, since the whole population is exposed, this impact results in a non-negligible public-health burden. Any reduction in air pollution would therefore benefit a large number of people and would be associated with large health gains.

The European Directive 08/50/EC requires Member States (MS) to design appropriate air quality plans (AQP) for zones where the air quality does not comply with the established limit-values and to assess possible emission reduction measures to improve concentration levels. The new Directive encourages the use of numerical models and the incorporation of more 'integrated' approaches, that bring together air quality and health aspects in the current assessment methodologies of air quality plans. Despite the air quality improvements observed over the last years there is still a continued wide-spread of exceedances within Europe; Portugal is not an exception, particularly regarding particulate matter, namely, PM10, and nitrogen oxides (NOx). Porto Litoral is one of the several EU urban areas that had to develop and implement AQP to reduce particulate matter with a 50% efficiency cut-off at PM10 because of non-accomplished PM10 limit values. The AQP were initially designed based on a scenario approach and using an air quality model, which was applied over the study region for the reference situation with the current PM10 emissions, and for the reduction scenario with PM10 emissions re-estimated considering the implementation of abatement measures. Recently, significant associations were found between daily mortality and morbidity endpoints and short-term exposures to PM and NO_x in Lisbon city and Oporto district stressing the need of a close research..

OBJECTIVES / MATERIALS AND METHODS:

Aiming to cost-efficiently optimize Porto PM10 abatement measures and estimate health effects, the assessment procedure was repeated using an optimization approach based on the integrated assessment tool RIAT+.

ACKNOWLEDGMENTS:

This work is supported by Fundação para a Ciência e Tecnologia (FCT) under the project MAPLIA, grant PTDC/AAG-MAA/4077/2012.

Assessment of iron and nitrates concentration in drinking water, in the district of Bragança, Portugal between 2012 and 2013

Authors: Mendes A[1*], Souto F[1*],Faria P[1*], Pires B[2], Barros P[5], Nogueira A[3,4], Afonso A[2,3]

1 *Students of Clinical Studies and Public Health at the Polytechnical Institute of Bragança(IPB), Braganca, Portugal*
2 *Public Health Laboratory of Bragança, Bragança, Portugal*
3 *Department of Therapeutic and Diagnostic Technologies, Polytechnic Institute of Bragança (IPB), Bragança, Portugal*
4 *Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
5 *Clinical Pathology Laboratory, Hospital of Bragança, Portugal*
* *The authors had the same contribution in this work*

Presenting Author: Email: paulafaria92@hotmail.com | Tel.: +351 914 385 341
Email: flavia.souto.fcgs@gmail.com | Tel.: +351 914 806 781

INTRODUCTION:

Water is an essential resource for life. It is a crucial factor for all living beings' survival thus, it is necessary to promote means to obtain drinking water for human consumption.[1] The water intended for human consumption, according to the World Health Organization (WHO),is quality water as soon as it does not cause a significant threat to human health during its consumption.[2] The diseases related to the contamination of water for human consumption constitute a very high responsibility and importance to the Public Health. The nitrates have a high solubility and may reach the groundwater in the deep layers of the soil by processes as leaching, particularly in areas with intensive farming and livestock. High concentrations of nitrates can be very harmful to human health since they can be easily converted into nitrites which concentrations cannot exceed the parametric value of 50 mg/L in water for human consumption (Dec. Law n.º 306/2007). The greater effect of nitrite in human health is your involvement in the oxidation of hemoglobin (Hb) to methemoglobin (MetHb). High concentrations of nitrates cause severe methaemoglobinemia in infants and adults. The methemoglobinemia is a rare condition caused by excessive conversion of hemoglobin (Hb) in methemoglobin (MetHb), being unable to bind and transport oxygen.[3]The iron exists in low concentrations in natural waters and usually its presence results of processes such as leaching of soils or industrial pollution. The presence of iron in the water can consequently cause the corrosion of metal pipes or the use of iron salts as agents of coagulation/flocculation in water treatment and the parametric value of iron is 200µg/L (Dec. Law n.º 306/2007) in water for human consumption.

OBJECTIVES:

The objective of this study was to evaluate the concentration of nitrate and iron in drinking water in the district of Bragança in order to identify the sampling point that keeps concentrations of these ions above the parametric values.

MATERIALS AND METHODS:

The collection of drinking water samples in the district of Bragança occurred between the years of 2012 and 2013, with subsequent spectrophotometric determination of nitrate and iron concentration, in the Public Health Laboratory of Bragança (PHLB).Samples with concentrations above the parametric values were identified: above 200 µg/L to iron and above 50 mg/L to nitrates (Dec, Law n.º 306/2007). The collected data was statistically treated in program Microsoft Office - Excel and the analysis of the data was carried out using the normal distribution with levels of confidence of 95 %.

RESULTS AND DISCUSSION:

In the year 2012 were collected 624 samples and 8.8% of these were outside the parametric values with 8.7% corresponding to samples with values of increased iron and 0.1% corresponding to increased nitrate's

values. In the year 2013 it was collected 1208 samples in which 8.9% were above the parametric values stipulated, from which 8.6% corresponded to samples with increased concentrations of iron and 0.3% corresponded to samples with nitrate levels above the parametric values.

CONCLUSION:

Two regions exhibited a greater number of samples with concentrations above the established limits for the parameters studied in this work. One of the regions had 15.9% of the samples above the established limits and the other had 12.6%.Individuals that consumed this water with high levels of iron and nitrates may be associated with several potential health threats.

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Fluoroquinolones and tetracyclines in sludges and soils of a Portuguese wastewater treatment plant

Authors: André M.P.T. Pereira[1], Liliana J.G. Silva[1], Celeste M. Lino[1], Leonor M. Meisel[2,3], Angelina Pena[1]

1 *Group of Health Surveillance, Center of Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal*

2 *INFARMED, I.P. - National Authority of Medicines and Health Products, 1749-004 Lisboa, Portugal*

3 *Department of Pharmacology, Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal*

Presenting Author: Email: amptpereira@gmail.com | Tel.: +351 239 488 400 | Fax: +351 239 488 503

INTRODUCTION:

Residues of antibiotics can enter the environment via different pathways. Growing concern has arisen regarding their use and disposal since their environmental presence might potentially harm human and ecological health, particularly through the inducement and dissemination of antibiotic-resistant bacteria (Seifrtová, Pena, Lino, & Solich, 2008). Municipal wastewater treatment plants (WWTPs) are considered to be major point sources of antibiotic contamination of surface and groundwaters, being of ultimate importance to predict the concentration of these antibiotics and to evaluate the efficiency of the treatment (Seifrtová et al., 2008).

The sorption properties of fluoroquinolone (FQ) and tetracycline (TC) antibiotics favour their presence and possible accumulation in sewage sludge. FQs and TCs were reported to have long-term persistence (Golet, Alder, & Giger, 2002) being this matrix considered a possible environment reservoir. Since sludges are often applied to farmland or grassland soils as organic fertilizer, antibiotics can accumulate in soil or they can be rinsed off by rain into surface waters or may leach to groundwater (Seifrtová et al., 2008).

OBJECTIVES:

Our work focused on studying the presence and comparing of FQs (ciprofloxacin, difloxacin, enrofloxacin, norfloxacin and sarafloxacin) and a TC (oxytetracycline) during winter and summer seasons, in sludges and soil samples of a WWTP in Coimbra, Portugal.

MATERIALS AND METHODS:

Sludges and soil samples were collected in winter (March) and summer (July) of 2013, from a municipal WWTP of Coimbra, Portugal. This WWTP is designed for 213000 population equivalent and has a primary and a secondary treatment operating with trickling filters. It receives urban wastewaters (including domestic and hospital wastewaters). Sludges (n=8) were collected along the solid stream treatment process, whereas soils (n=12) were sampled in the first 10 cm from ground around the WWTP.

The method used for identification and quantification of these pharmaceuticals was based on accelerated solvent extraction (ASE) with phosphoric acid/acetonitrile (1:1) at 65 ºC and 1500 psi. Instrumentation analysis was performed in a LC-MS-MS (ion trap).

RESULTS AND DISCUSSION:

The results show that the contamination frequency in the winter season was higher, ranging between 60% (difloxacin) and 20% (norfloxacin and sarafloxacin), whereas, in the summer it ranged from 60% (ciprofloxacin) to 10% (enrofloxacin).

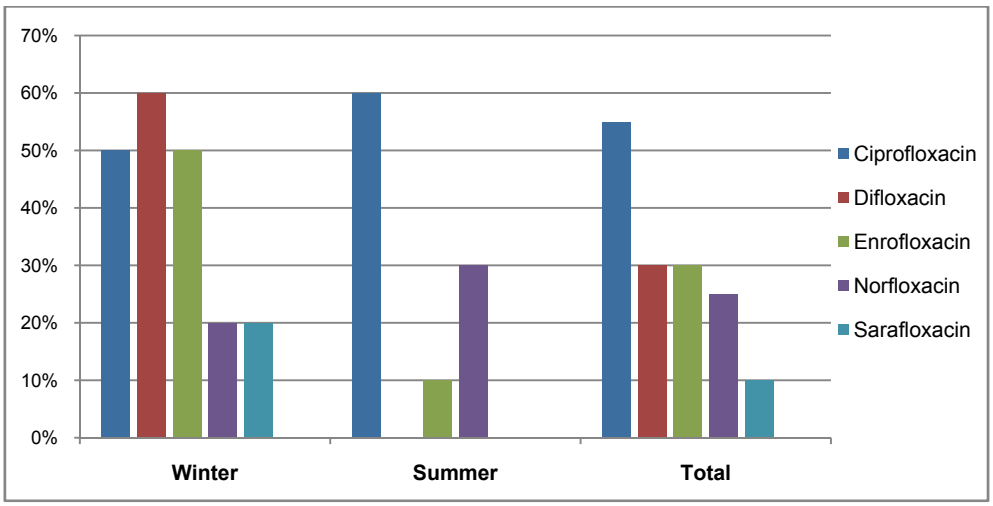


Figure 1 - Frequency of the selected antibiotics in WWTP sludges and soils.

Overall, the FQs prevalence decreased in the following order: ciprofloxacin (55%) > difloxacin and enrofloxacin (30%) > norfloxacin (25%) > sarafloxacin (10%). Oxytetracycline was never quantified, neither in winter nor in summer (Figure 1).

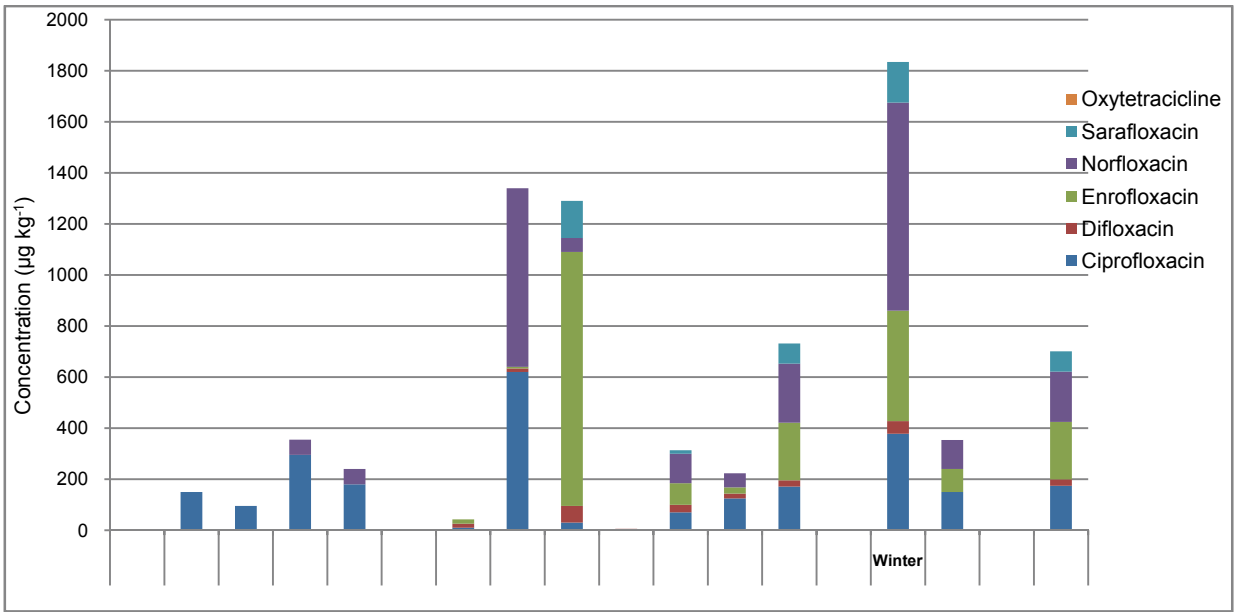


Figure 2 - Occurrence of the selected antibiotics in WWTP sludges and soils.

Although ciprofloxacin was the most prevalent, enrofloxacin was found in higher mean concentrations (225.4 µg kg-1), followed by ciprofloxacin (174.4 µg kg-1), norfloxacin, sarafloxacin and difloxacin (197, 79.5, 24.8 µg kg-1, respectively) (Figure 2).

Regarding seasonal influence, it was observed that total mean concentration was higher in winter (1834.3 µg kg^{-1}) when compared to summer (353.3 µg kg^{-1}). These results are explained by consumption and degradation. In the winter season, the increased consumption of antibiotics, lower temperatures and decreased photodegradation, favour accumulation of these compounds in sludges and soils (Figure 2).

The concentration levels in soil samples were approximately three times higher than in sludges, indicating that possible accumulation of antibiotics might occur in soils, due to sludges application as organic fertilizers and irrigation using WWTP effluents.

CONCLUSION:

Higher frequencies were observed in winter and for ciprofloxacin, nonetheless, enrofloxacin was found in higher concentrations. In the winter, in addition to higher frequencies, increased contamination levels were found. Soil samples were more contaminated than sludge samples. More monitoring and surveillance studies are needed at local level to determine exactly how the antibiotics and their metabolites reach the environmental compartments, and to obtain a better understanding of their transport and fate.

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Past environmental asbestos exposure reconstruction in Korea

Authors: Dong-Mug Kang[1,2,3], Kye-Sun Kim[1], Joo-Young Kim[1], Jong-Eun Kim[2]

1 *Environmental Health Center of Asbestos, Department of Occupational & Environmental Medicine, Pusan National University Yangsan Hospital, Yangsan, South Korea*

2 *Department of Occupational & Environmental Medicine, Pusan National University Yangsan Hospital, Yangsan, South Korea*

3 *Department of Preventive & Occupational Medicine, Pusan National University School of Medicine, Yangsan, South Korea*

Presenting Author: Email: kangdm@pusan.ac.kr | Tel.: +82 553 602 142 | Fax: +82 553 602 712

INTRODUCTION:

Since 2011, the Korean government has compensated victims of environmental asbestos exposure through the Asbestos Damage Relief Act. Assessment of the level of asbestos exposure is critical to ensure victims are compensated adequately, and to provide data for epidemiologic studies. Due to the long latency of many asbestos-related diseases, it is important to determine levels of previous exposure. However, scarce data are available that focuses specifically on environmental asbestos exposure in Korea.

OBJECTIVES:

To collect and analyze data on sources of environmental asbestos exposure to retrospectively estimate ambient air asbestos levels in Korea.

MATERIALS AND METHODS:

A systematic literature review (SLR) of ambient air levels of asbestos was conducted. The databases used for the SLR were PubMed for studies in English, and Research Institute for Social Service (RISS) for studies in Korean. The search terms used were (asbestos) and (environment or concentration or assessment or exposure or air or ambient or monitoring or matrix or lung cancer or mesothelioma). Studies containing concentrations of ambient air, including dissertations; journal articles; conference abstracts; and human studies were included. Non-human studies and experimental studies were excluded. Pooled analysis was performed using RevMan 5 Software. Fitting equation and line of among data of pooled analysis was constructed using EXEL. A total of 9,633 studies were found using search engines. After reviewing the literature, data from 14 papers (1-14) were used to simulate air dispersion of asbestos from sources such as asbestos mines and factories. Geometric means were converted to arithmetic means according to a previously published formula (15). In addition, other data regarding sources of asbestos exposure were gathered and reviewed.

RESULTS:

A database of former asbestos mines and factories was constructed (Fig 1).

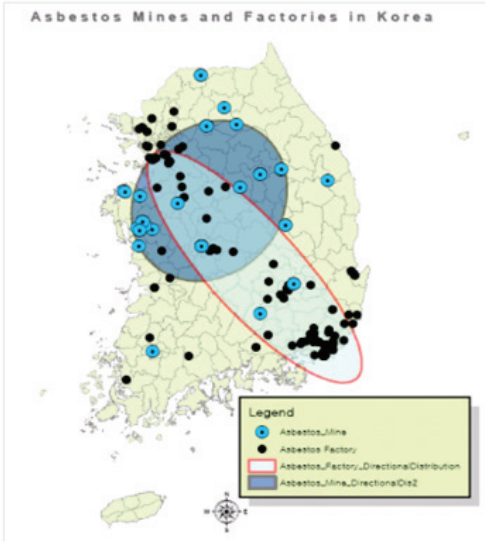


Figure 1 - Former asbestos mines and factories in Korea

After a pooled analysis of data from the 14 relevant papers, a dispersion equation of asbestos from source was estimated (Fig 2). The R^2 value for pooled data was 0.83.

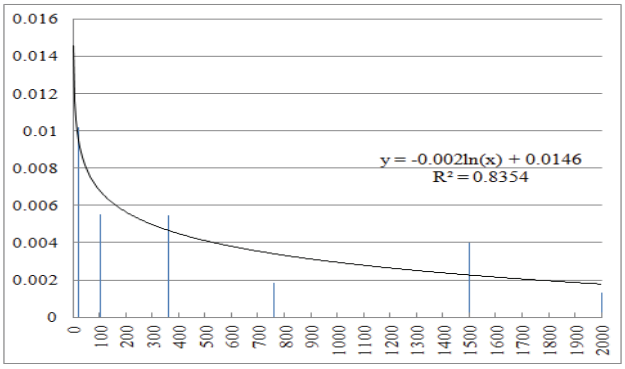


Figure 2 - Equation to estimate ambient air asbestos level by distance from asbestos source

There were several limitations in this study. Of the 14 studies identified by SLR, some used arithmetic, rather than geometric, means, and although we used an equation to convert these values, there is still the possibility of error. In addition, the methods used to analyze asbestos differed between studies, with some using light microscopy while others using electron microscopy. As asbestos concentration cannot be determined accurately using light microscopy, over-estimation or misclassification of the levels may have occurred. Further, the database of asbestos sources is still in formation.

CONCLUSION:

Data were collected on previous asbestos factories and mines. A pooled analysis was conducted to estimate air dispersion of asbestos from sources. Through the collection of data on sources of asbestos, additional information will be available on this topic. Data will be combined to estimate previous levels of environmental asbestos exposure in Korea. We aim to present our final data at the conference.

Keywords: asbestos, dispersion, reconstruction, past exposure, environmental

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Metals in ambient particulate matter - are we ready for the new EU air quality standards?

Authors: Carlos Soares[1], Rui Ferreira[1], Joana Silva[1], Patrícia Ramos[1], Edgar Pinto[2], Cristina Couto[1,3], Agostinho Almeida[1]

1 *REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal*

2 *REQUIMTE, Department of Chemical Sciences, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal*

3 *CICS, Research Centre on Health Sciences, Advanced Institute of Health Sciences-North, Gandra, Paredes, Portugal*

Presenting Author: Email: aalmeida@ff.up.pt | Tel.: +351 220 428 667 | Fax: +351 222 003 977

INTRODUCTION:

Several studies have demonstrated a direct relationship between long-term exposure to particulate matter (PM) and mortality, in particular mortality from respiratory and cardiovascular diseases (Pelucchi et al., 2009). Regarding specifically to cardiovascular diseases (CVD), recent experimental and epidemiologic studies show that PM10 (i.e., "particulate matter which passes through a size-selective inlet with a 50% efficiency cut-off at 10 µm aerodynamic diameter", which is essentially equivalent to "thoracic particles") is the air pollution parameter most consistently linked with acute and chronic cardiovascular effects (Braz Nogueira, 2009).

Most studies on the association between atmospheric PM and health effects simply rely on a gravimetric analysis (i.e., the determination of PM mass/m^3 air). However, it is increasingly recognized the importance of also consider the chemical composition of PM, especially the content in particular metals, and not simply its air concentration (Franklin, Koutrakis, & Schwartz, 2008).

In 1996, the Council Directive 96/62/EC (the *Air Quality Framework Directive*)[5] has defined the basic principles regarding ambient air quality assessment and management in the EU. In 1999, the Council Directive1999/30/EC (the *First Daughter Directive*)[2] has set a "limit value" for Pbin ambient air at 0.5 g/m^3. More recently, the Directive 2004/107/EC of the European Parliament and of the Council (the *Fourth Daughter Directive*)[3], recognizing that "scientific evidence shows that arsenic, cadmium, nickel (...)are human genotoxic carcinogens" and in order "to avoid, prevent or reduce harmful effects (...) on human health and the environment as a whole", also established "target values" for these elements in ambient air. Those target values were set at 6 ng/m^3 for As, 5 ng/m^3 for Cd and 20 ng/m^3 for Ni (as the total content of these elements and compounds in thePM10 fraction) and, according to the Directive, "Member States shall take all necessary measures not entailing disproportionate costs to ensure that, as from 31 December 2012", the target values are not exceed. All the relevant European legislation was revised by the current Directive 2008/50/EC on ambient air quality and cleaner air for Europe (please see footnote[4]).

[5]Transposed into Portuguese law by Decree-Law 276/99, of 23 July.[2] Transposed into Portuguese law by Decree-Law 111/2002, of 16 April.

[3] Transposed into the Portuguese law Decree-Law 351/2007, of 23 October.

[4] Directive 2008/50/EC adds a single act to the legal provisions of Directive 96/62/EC and the first three daughter directives (Directives 1999/30/EC , 2000/69/EC and 2002/3/EC) and Decision 97/101/EC of the Council, establishing a reciprocal exchange of information and data from networks and individual monitoring within the Member States. This Directive was transposed into national law by DL nº 102/2010 of 23 September, which also added the fourth daughter Directive (Directive 2004/107/EC), relating to arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air. The DL nº 102/2010 sets out the objectives of air quality taking into account the standards, guidelines and programs of the World Health Organization.

[5] Project (FCT) PTDC/SAU-ESA/108871/2008. (see at: http://projfct1088712008.blogspot.pt/)

OBJECTIVES:

This work was part of a more comprehensive Project intended to assess environmental risk factors for CVD at Porto Metropolitan Area (PMA).This part of the Project was focused on the metals /metalloids content in ambient air. Particular attention was paid to As, Cd and Ni, in order to assess compliance with the new EU requirements.

MATERIALS AND METHODS:

Using sampling recommended procedures, ambient particulate matter (PM10 and PM2.5) was collected at two different locations in PMA: #1-Centre of Porto city [41°09'19.7"N 8°37'14.0"W] (n=13 days; from 07-10-2011 to 7-12-2011); #2-A peripheral area of PMA, at Gandra-Paredes [41°10'54.5"N 8°26'43.7"W] (n=13; from 09-06-2012 to 28-07-2012). Latter, for comparison purposes, a third location, on the periphery of Amarante city [41°16'10.9"N 8°04'12.0"W] (n=6; from 15-04-2013 to 22-04-2013), was also studied (only for metals associated with PM10).A wide range of metals and metalloids (Be, Al, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Tl, Pb, Th and U) were determined in sampling filters extracts (obtained by sonication for 3 h with 20 mL of 4% HNO_3 at 69 ºC) by ICP-MS (ERG, 2005).

RESULTS AND DISCUSSION:

Results are summarized in Figure 1.At the three locations, the most abundant metals in PM approximately followed the same order (Al >Zn>Cu>Pb/Mn...), but the levels at location #1wereseveral times higher. At locations #1 and #2, a significantly higher metal content (1.2-2 fold) was found for PM10 than for PM2.5 samples. The most remarkable exception was Pb at location #2, which presented quite similar levels. This clearly indicates a different source for the air contamination, since PM10 mainly originate from soil dust resuspension, construction/demolition activities and non-exhaust vehicle emissions, whereas PM2.5 are mainly produced by combustion processes.

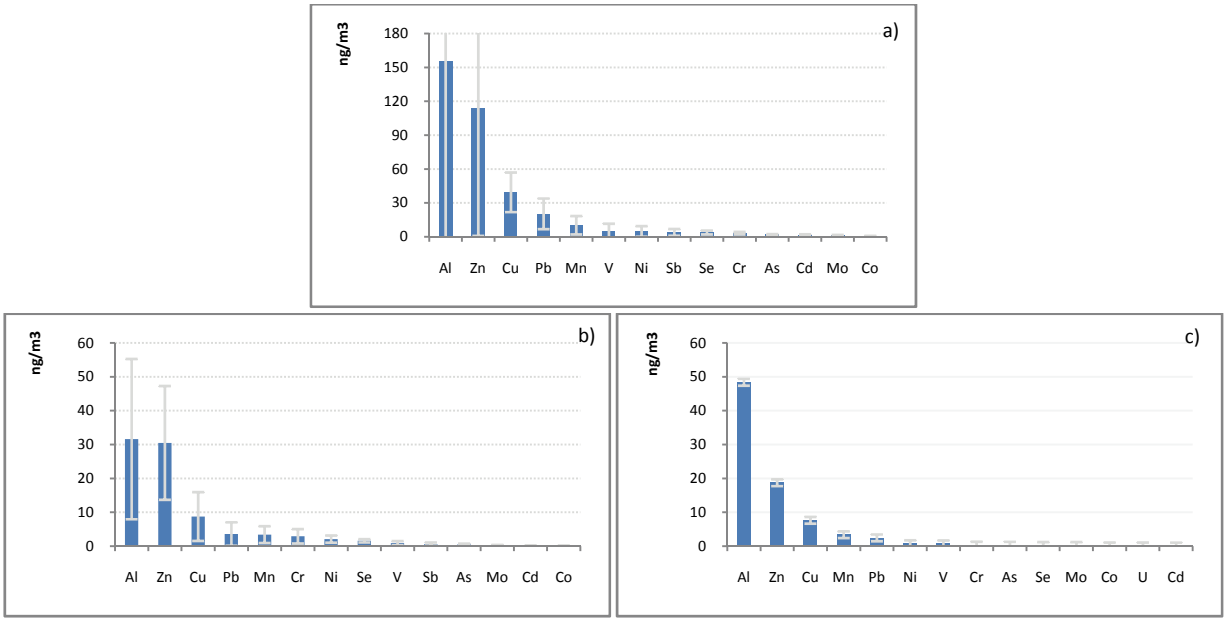


Figure 1 - Concentration (ng/m^3) of several trace elements (total content in the PM10 fraction)in ambient air at the three different locations studied: a) Centre of Porto city; b) Gandra-Paredes; c) Periphery of Amarante city.

Results for the "target" elements Pb, As, Cd and Ni are summarized in Table 1. Even at location #1 (*worst scenario*), they are quite well below the EU Air Quality Standards[6].

[6] http://ec.europa.eu/environment/air/quality/standards.htm

Table 1 - Concentration (ng/m^3) in ambient air of Pb, As, Cd and Ni (total content in the PM10 fraction)

Element	Porto (Centre)	Gandra-Paredes	Amarante (Periphery)	EU Air Quality Standard (ng/m^3)*
Pb	20.3±13.6	3.59±3.46	2.45±1.70	500[&]
As	1.76±0.52	0.56±0.21	0.28±0.28	6[#]
Cd	1.23±0.89	0.12±0.12	0.050±0.045	5[#]
Ni	4.77±4.65	2.08±1.06	0.71±66	20[#]

*Averaging period: 1 year. [&]**Limit value** entered into force 1.1.2005; [#]**Target value** entered into force 31.12.2012.

CONCLUSION:

Results from this study showed that ambient air at the three locations studied in North Portugal well complies with the health based standards and objectives for Pb, As, Cd and Ni.

ACKNOWLEDGMENTS:

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Burden of diseases from traffic noise in Seoul, Korea

Authors: Kyoung Sook Jeong[1], Jae Won Lee[2], Chang Sun Sim[3], Jiho Lee[3]

1 *Dongguk University Ilsan Hospital, Goyang, Rep. of Korea; Dept of Occupational and Environmental Medicine, School of Medicine*

2 *Indoor environment and Noise research division, Environment Infrastructure Research Department, National Institute of Environmental Research, Incheon, Rep. of Korea*

3 *Ulsan University Hospital, Ulsan, Rep. of Korea, Dept of Occupational and Environmental Medicine, School of Medicine*

Presenting Author: Kyoung Sook Jeong
Email: bandyoem@naver.com | Tel.: +82 319 617 519 | Fax: +82 319 617 039
Jiho Lee
Email: oemdoc@naver.com | Tel.: +82 522 507 288 | Fax: +82 522 507 289

INTRODUCTION:

In the making public and environmental policy and the planning of the intervention, it is necessary to quantify the impact of exposure to modifiable risk factors (Laaksonen, Knekt, Harkanen, Virtala, & Oja, 2010; WHO, 2011). World health organization (WHO) regional office for Europe reported burden of disease form environmental noise in 2011. At the review, the environmental noise by road, rail, airport, and industrial sites was causal relationship with cardiovascular disease, cognitive impairment, sleep disturbance, tinnitus, and annoyance (WHO, 2011).

OBJECTIVES:

We quantified burden of diseases from traffic noise to produce the basic data for grasping the current health impact of environmental noise in Seoul, Rep of Korea.

MATERIALS AND METHODS:

We estimated the burden of disease from environmental noise using disability-adjusted life years (DALY) method developed by the Global Burden of Disease (GBD) group of the WHO. We used the 3-D noise prediction model that contains information on geographical data, the height of buildings, road traffic data, etc, to calculate the population exposed to road traffic noise. We calculated the DALYs for depression (ICD-10 codes F32-F33), anxiety disorder (F41), sleep disorder (G47), hypertension (I10-I15), and ischemic heart disease (I20-I25) to quantify burden of disease from traffic noise. The death data in 2010 was used the mortality statistics of Korea National Statistical Office and prevalence data for target diseases in 2010 were used the data of National Health Insurance Service. For calculating DALYs, disability weights of diseases were used the previous Korean study (Do et al., 2004) and WHO data for disease that we could not be available for Korean data (WHO, 2011). (See Table 1) To calculate population-attributable risk (PAR) of traffic noise for health outcomes, we used the WHO's odds ratio for noise level ranging from 55 to 80 dB(A) (WHO, 2011).

Table 1- Disability weights for health outcomes by environmental noise

Health outcomes	Korea (Do et al., 2004)	WHO (WHO, 2011)
Depression	0.660	0.140
Anxiety disorder	0.433	
Sleep disorder	-	0.100
Hypertension	-	0.352
Ischemic heart disease	0.727	0.350

RESULTS AND DISCUSSION:

Approximately, 19.2% of the people in Seoul were exposed to road traffic noise level above 65dB(A) during daytime. The D district had the highest noise exposure that was 34.2% people were exposed to road traffic noise level above 65 dB(A). (See Fig 1) DALYs per 100,000 persons in male were 201 (depression), 114.8 (anxiety disorder), 11.6 (sleep disorder), 704.3 (hypertension), 166.2 years (ischemic heart disease) respectively, and in Seoul women, DALYs were 561.9 (depression), 223.0 (anxiety disorder), 21.8 (sleep disorder), 631.9 (hypertension), 118.4 years (ischemic heart disease) respectively. (See Table 2) The burden of disease from noise exposure was 230 DALYs per 100,000 persons for hypertension, cardiovascular diseases, severe annoyance and sleep disturbance in Netherlands in 2000 (Knol & Staatsen, 2005) and 280 DALYs for perceived sleep disturbance due to transportation noise in Switzerland in 1995 (WHO, 2005). The burden of disease from environmental noise in Seoul was much higher than DALYs at other studies for different period, disease categories, and higher disability weights of depression and ischemic heart disease.

This study have several limitations; First, the odds ratios of noise exposure were used the meta-analyzed result for cardiovascular diseases. Second, the disability weights in Korea were the values at ten years ago.

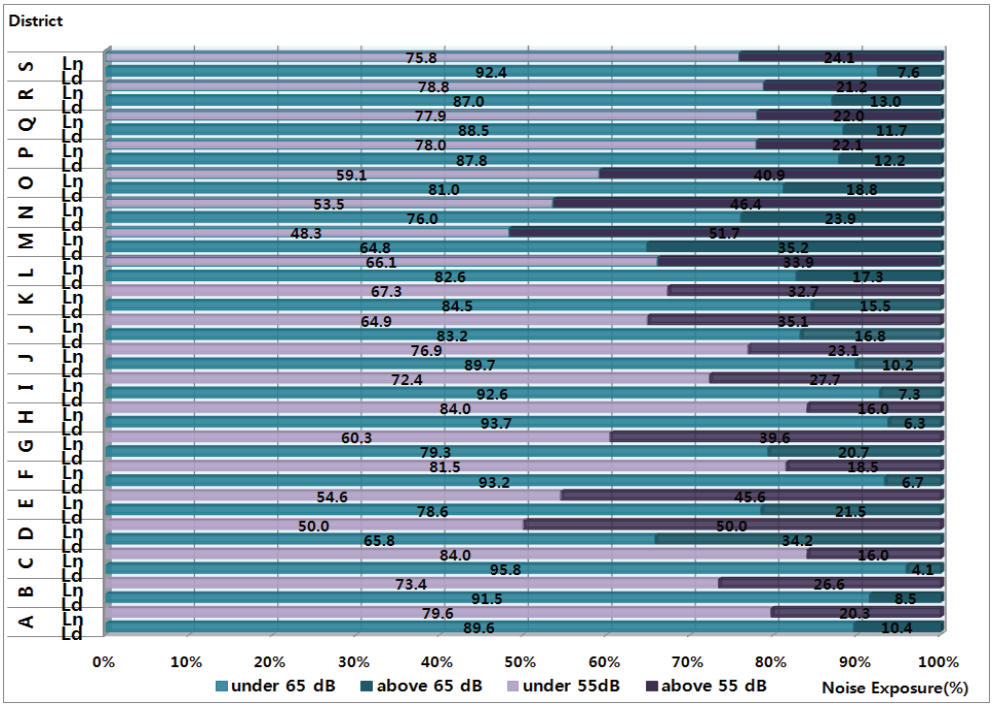


Figure 1 - Distribution of noise exposure by district in Seoul

Table 2 - DALYs per 100,000 persons from traffic noise in Seoul

Health outcomes	Male	Female
Depression	201	562
Anxiety disorder	115	223
Sleep disorder	12	22
Hypertension	704	632
Ischemic heart disease	166	118

CONCLUSION:

The burden of diseases from traffic noise in Seoul, Korea was calculated as 1,198 DALYs and 1,557 DALYs per 100,000 persons in men and women, respectively.

ACKNOWLEDGMENTS:

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Impact of forest fires on the levels of relevant outdoor air pollutants

Authors: Marta Oliveira[1,2], Klara Slezakova[1,2], Cristina Delerue-Matos[1], Maria do Carmo Pereira[2], Simone Morais[1]

1 *REQUIMTE, Instituto Superior de Engenharia, Instituto Politécnico do Porto, Porto, Portugal*

2 *LEPABE, Departamento de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Porto, Portugal*

Presenting Author: Email: martamadalena@gmail.com | Tel.: +351 228 340 500 | Fax: +351 229 321 159

INTRODUCTION:

Biomass burning has been recognized as an important source of atmospheric pollutants and, in a changing climatic scenario, this contribution has been dramatically increasing due to a larger area burned by forest fires (Carvalho et al., 2011). Concerning Europe, the Southern countries such as Portugal, Spain, Greece and Italy have been the most affected by forest fires. In Portugal, the Northern Region is one of the most disturbed areas. During the last decades the number of forest fires has been continuously increasing with longer fire seasons and more potent fires mainly due to the higher hot and dry conditions (Slezakova et al., 2013). Forest fires are devastating and have a great impact on the citizen's life. Despite aggravating the soil erosion, forest fires also cause desertification and present high economic costs to the governments. Apart from lost lives, burnt properties and forests, fires also release into the air large amounts of pollutants, such as carbon monoxide and dioxide, nitrogen oxides, particulate matter (PM), ammonia, methane and non-methane hydrocarbons (Carvalho et al., 2011; Slezakova et al., 2013). Indirectly, the forest fires can also influence the levels of atmospheric pollutants in air; it is known that higher ozone concentrations can occur in places where levels its precursors are currently high during episodes of very hot weather (WHO 2006). Most atmospheric nitrogen dioxide is emitted as nitrogen oxide, which is rapidly oxidized by ozone to nitrogen dioxide. In the presence of hydrocarbons and ultraviolet light, nitrogen dioxide is the main source of tropospheric ozone and nitrate aerosols, which form an important fraction of the ambient air $PM_{2.5}$ (particles smaller than 2.5 μm) (WHO 2006). The public health impact of PM consists in adverse effects, predominantly to the respiratory and cardiovascular systems, at exposures that are currently experienced by urban populations in both developed and developing countries (WHO 2006). In October 2013, International Agency for Research on Cancer (IARC) classified PM from outdoor pollution as carcinogenic to humans (i.e. Group 1; IARC 2013). The levels of the above referred pollutants are elevated during and after a forest fire season, thus increasing the potential adverse health impact in the general population.

OBJECTIVES:

The present work reports the concentrations of PM_{10}, $PM_{2.5}$, O_3, NO, NO_2 and NO_x observed in the North of Portugal during the hot season of the last three years (2011 to 2013). The trend of forest fire occurrences and burnt areas during the characterized period will be discussed. Pollutant levels will be compared between the several seasons. Results will be discussed according to the established national and international guidelines. The potential health risks to the exposed populations will also be evaluated.

MATERIALS AND METHODS:

Daily concentrations at different urban and rural sites were collected from monitoring network of Portuguese Environmental Agency. The data on occurrences of forest fires and burnt areas during 2011 to 2013 were obtained from the reports of the Portuguese Forest Fires Registry.

RESULTS AND DISCUSSION:

Portugal accounted 25 221 and 21 176 fire occurrences which resulted in total burned area of 73 813 and 110 232 ha, respectively in 2011 and 2012. 2013 was a critical year for forest fires, accounting with a total burned area of 140 944 ha in October. The total number of occurrences observed in 2011 increased 4.4%

compared to the average of the last decennium and 14.5% when compared with the number of occurrences accounted in 2010. In what concerns 2012, the number of occurrences was 16% and 12% lower than in 2011 and the average of the last decennium, respectively.

The burned area in 2011 and 2012 corresponded to 50% and 75% of the average of the last decennium, respectively (JCR 2013). In absolute terms, in 2011 there was an increase of 1 063 fire occurrences and a reduction of 74 056 ha of burned area to the decennium average while in 2012 a reduction of 2 810 occurrences and 33 789 ha of burned area to the average of the period 2002 to 2011 was observed. Compared to 2011, there was an increase of 49% (36 403 ha) of the burned area in 2012.

Bragança, Guarda, Viseu, Faro and Vila Real were the districts with the largest areas burned by forest fires in 2011 and 2012. Furthermore, the urban districts of Porto, Braga, Aveiro, Viseu, Viana do Castelo and Vila Real accounted for a total of 74% of the forest fire occurrences, Porto being the district with the higher number of occurrences. Despite the reduction in the burned area, in 2012 Portugal did not meet the target of the total burnt area lower than 100 000 ha per year (settled in the National Fire Plan) (JCR 2013).

The rural background monitoring station of Vila Real (part of network of Portuguese Environmental Agency) registered PM_{10} and $PM_{2.5}$ concentrations that ranged, respectively, up to 104 $\mu g/m^3$ and 39 $\mu g/m^3$ during the summer season. In Sobreiras (urban background monitoring station), the concentrations of these pollutants ranged up to 269 $\mu g/m^3$ and 64 $\mu g/m^3$ during the hot seasons of 2011 till 2013. Among the summer months considered, the O_3 levels exceeded the 200 $\mu g/m^3$ in some days at the rural zone while in the urban area levels reached a maximum of 170 $\mu g/m^3$ in the summer of 2013. NO, NO_2 and NO_x at the rural monitoring station reached maximum concentrations of 8.0, 94.6 and 104.7 $\mu g/m^3$ from July to October (2011 to 2013) while at the urban station levels were much higher 123.4, 121.6 and 415.4 $\mu g/m^3$, respectively. NO, NO_2 and NO_x maximum levels of 3.2, 30.4 and 32.0 $\mu g/m^3$ were observed during the respective winter season at the rural station. As NO, NO_2 and NO_x are also pollutants emitted by local traffic or other combustion sources, the urban station registered concentrations that ranged between 1.2 to 144.5 $\mu g/m^3$, 0.6 to 270.8 $\mu g/m^3$ and 2.9 to 538.4 $\mu g/m^3$ during the winter period.

CONCLUSION:

PM_{10} and $PM_{2.5}$ concentrations were usually higher at the urban monitoring stations. The maximum PM_{10} and $PM_{2.5}$ levels detected at both rural and urban areas during some days of the 2011 to 2013 hot seasons largely exceeded the 20 (for PM_{10}) and 10 (for $PM_{2.5}$) $\mu g/m^3$ settled by WHO (WHO 2006). In what concerns to O_3 and NO_2, it was observed that both urban and rural levels also exceeded in some days the guidelines of 100 and 40 $\mu g/m^3$ settled by WHO (WHO 2006), respectively. As expected O_3 levels were predominantly higher at the rural monitoring stations while NO_2 presented higher concentrations at the urban areas, mainly due to the higher traffic emissions. The present work revealed that during some fire incidents of the years of 2011 to 2013, both rural and urban populations from the North of Portugal were exposed to high concentrations of pollutants. These specific exposures may induce health effects (increased risk of asthma, allergies, pulmonary infections and cardiac diseases) in the population, especially of the most vulnerable groups (children, pregnant women and elderly).

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The context of environmental health in Metropolitan Region of São Paulo: conflicts, vulnerabilities and ecological restoration for management of river flows

Authors: Silvana A. Cutolo[1, 2], Ana Karina Merlin do Imperio Favaro[1, 2], Leandro L. Giatti[1, 2]

1 *Department of Environmental Health, School of Public Health, University of São Paulo, Brazil*
2 *Laboratory of Climatic Changes, Department of Environmental Health, School of Public Health, University of São Paulo, Brazil*

Presenting Author: Email: cutolosa@usp.br | Tel.: +55 11 30617898 | Fax: +55 113 061 7898

INTRODUCTION:
The metropolitan areas are matter of great interest in the field of public health, especially in developing countries. Irreversible impacts have been observed on the natural environment, including with respect to sustainability of renewable water resources and environmental services. Population growth, climatic changes, growing water uses, and ecosystems with a high weakness as watershed areas lead Metropolitan Region of São Paulo (MRSP) under high social and environmental conflicts due to irregular occupations, thereby causing damage to ecosystem services, loss of water availability, worsening public health and social degradation, further exacerbated by a perspective of the climate variability scenario.

OBJECTIVES:
We present the current scenario of conflict in the watershed due to water scarcity, loss of areas that supply ecosystem services and health iniquities in these areas of great vulnerability in the MRSP.

MATERIALS AND METHODS:
The MRSP is the highest urban concentration of population and economy of the Brazil in South America. It aggregates 39 cities distributed within an area of 7.9 thousand km^2 with about 19.6 million inhabitants. We analysed data from health indicators such as infant mortality rate, life expectancy, water availability and consumption of water and irregular occupation, not just pointing out the vulnerabilities and risks, but also aiming at building a strategic analysis for the ecological restoration and maintenance of the hydrological cycle to the environmental health of MRSP.

Data about health, water resources, population growth and ecosystems were obtained from exploring secondary data and indicators provided by some public institutes of São Paulo State (Foundation SEADE - State System of Data Analysis), Brazil (IBGE - Brazilian Institute of Geography and Statistics and DATASUS - the Ministry of Health's Information Department's database) and World Health Organization (WHO).We worked with the all cities that are located in sub-basin and we worked with number of the cities from each sub-basin, population (inh/Km^2), Infant Mortality Rate (death/1000 births), Life Expectancy at Birth (%) and Water Consumption (per capita/day) from IBGE (2010) and DATASUS (2009).

RESULTS AND DISCUSSION:
The accelerated economic development of the last decades is reflected in the demography of the region, in demands for environmental services and in pressures on the various ecosystems. In the MRSP the population grew by 10.1% between 2000 to 2010 and the demand of drinking water grew 14.2%. Table 1 shows data from population, infant mortality rate, life expectancy at birth and water consumption from 39 municipalities grouped into their respective sub-basins.

Table 1 - Data of indicators (average and standard deviation) in sub-basins for water consumption in Metropolitan Region of São Paulo (Brazil).

Sub-Basins	Number of Cities	Population (inh/Km^2)[1]	Infant mortality rate ≤ 5 years[2] Deaths/1000 births	Life expectancy at birth (%)[2]	Water consumption (per capita/day)[3]
Alto Tietê-Billings	6	3.432.58	15.88 (2.16)	75.52(1.12)	157.02 (34.25)
Tietê-Cabeceiras	10	2623.84	16.17(2.54)	75.43(1.34)	117.12(12.70)
Cotia-Guarapiranga	7	1106.98	16.07(3.40)	75.33(1.70)	138.90(25.03)
Juqueri-Cantareita	4	1149.35	16.43(3.65)	75.06(1.54)	109.44(12.23)
Penha-Pinheiros	5	8987.47	13.57(1.60)	77.05(0.92)	166.71(62.61)
Pinheiros-Pirapora	7	4860.28	16.2(2.11)	75.59(1.04)	145.24(83.35)

Note: [1]IBGE, 2010; [2]DATASUS (2009); [3]http://sigam.ambiente.sp.gov.br

In the context of MRSP, the watershed area has been occupied by disordered growth, making them vulnerable with loss of ecosystem services and population health, while life expectancy has increased over the last 15 years in Brazil. The values of infant mortality rate (≤ 5 years) report have been higher than the recommended by the World Health Organization (WHO) as 10 deaths per 1000 births, even with high levels of water consumption per capita per day but in areas without sanitation. The Figure 1 shows the map of MRSP and the sub-basins that provide water supply for many areas such as domestic, industrial, services and small farmers.



Figure 1 - State of São Paulo and the location of the sub-basins in MRSP

The Watershed Protection Areas (WPAs) represents 54% of the MRSP territory, which are undergoing into rapid population growth and with environmental and social degradation, where more than 2 million live people in risk areas as slums or squatter settlements (Cutolo & Giatti, 2014). The total area of MRSP is divided into six sub-basin, that provide water for many purposes, but the principal rivers are very polluted with industrial, agrochemicals and domestic sewage (Cunha et al, 2011). The river Tietê is a tributary of the Paraná River and runs through the MRSP, considered as a headwaters area for the production of water as one environmental service. However, non-point and point charge of domestic sewage, industrial and agricultural still high (Cunha et al., 2011).

CONCLUSION:

Currently the MRSP suffers from high water scarcity as a result of the metropolitan expansion on natural environmental areas with illegal occupation of nature reserves loss of ecosystem services, water availability, combined with climate changes scenarios posing a risk to the quality of life. It is essential to attain a stance of fairness within this context since there is an asymmetrical relation in the metropolitan context. The development of environmental governance policies must consider environmental health indicators as well as the need for ecological restoration, increasing living conditions.

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A survey of thorium (Th) and uranium (U) in the groundwater of NW Portugal

Authors: Edgar Pinto[1,2], Agostinho Almeida[3], Isabel M.P.L.V.O. Ferreira[1]

1 *REQUIMTE, Department of Chemical Sciences, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal*

2 *CISA - Research Center in Environment and Health, ESTSP-IPP*

3 *REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal.*

Presenting Author: Email: ecp@estsp.ipp.pt | Tel.: +351 916 941 708

INTRODUCTION:

Thorium (Th) and uranium (U)are both radioactive and chemical toxins and the role that radiation vs chemical effects play in its toxicology remains mysterious. In the last years, a great interest on the environmental toxicity of Th and U has been raised by two recent developments: (i) the global drive toward low-carbon energy which enhances the interest towards nuclear power, and (ii) its growing use as depleted uranium (DU) in military munitions [1]. A third important factor from an exposure perspective, and maybe the one receiving the least attention, is the increasing worldwide consumption of groundwater. Recent evidence points to the fact that groundwater in many parts of the world is contaminated with Th and U from natural and industrial sources [2]. Exposure to Th and U from drinking water is growing rapidly as more people increasingly rely on groundwater as their primary source of water [3].

OBJECTIVES:

The aim of the present work was to assess the Th and U contamination of groundwater used for irrigation and human consumption during 3 months.

MATERIALS AND METHODS:

The study involved a monitoring program in NW Portugal, in a region between Esposende and Vila do Conde. Groundwater samples were collected from 4 sites (S1, S2, S3 and S4) every 2 weeks during 3 months in a total of 6 sampling times. Groundwater physicochemical characteristics (pH, EC, salinity and TDS) and radioactives (Th and U) were determined according to standard methods. Water pH, EC, TDS and salinity were measured *in situ* by a portable pH/conductivity meter. Th and U were determined by ICP-MS.

RESULTS AND DISCUSSION:

With the exception of S4, Th and U were detected in all groundwaters. Groundwater from S1 had the highest mean values of Th (0.23 ± 0.02 µg/L) and U (11.5 ± 1.1 µg/L) compared with the other groundwaters. In the case of Th, groundwater from S1 was 7- and 12-times higher than groundwater from S2 (0.033± 0.004µg/L) and S3 (0.019± 0.003µg/L), respectively. In the same way, U concentrations in groundwater from S1 were 4- and 100-times higher compared to water from S2 and S3, respectively. Regarding the temporal variation, no significant differences ($p< 0.05$) were observed for Th and U concentrations along the studied period. The mean U concentration of all the studied groundwaters was below the threshold of 30 µg/L imposed by WHO. Currently, the WHO does not have a drinking water guideline for Th [4]. Despite the fact that U concentrations were always below the WHO guidelines and Th concentrations seem relatively small when expressed in µg/L, several trillions of Th and U atoms are present per liter of water. These trillions of Th and U atoms per liter of drinking water over a lifetime of exposure could pose a significant health risk, especially carcinogenic effects [5].

CONCLUSION:

These results highlight the urgent need for multiple metal testing of groundwater in NW Portugal and other areas where radioactive contamination is known to occur. To prevent the serious health effects of exposure to radioactive elements, remediation strategies must be considered in common water treatment facilities. In addition to the common elements monitored in drinking water, routine water testing must include analyses for other common groundwater contaminants such as Th and U.

ACKNOWLEDGMENTS:

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Association between air pollution and the Crude Death Rates in Turkey's most and less industrialized regions

Authors: Mehmet Sarper Erdogan

Istanbul University, Cerrahpasa Medical Faculty, Dept. of Public Health, Istanbul, Turkey

Presenting Author: Email: sarper@istanbul.edu.tr | Tel.: +90 537 244 0026

INTRODUCTION:

Sulfur dioxide is a major air pollutant and has significant impacts upon human health. Short-term exposures to SO_2 cause an array of adverse respiratory effects including bronchoconstriction and increased asthma symptoms. There is a connection between short-term exposure and increased visits to emergency departments and hospital admissions for respiratory illnesses. The largest sources of SO_2 emissions are from fossil fuel combustion at power plants (73%) and other industrial facilities (20%) (sulfurdioxide, EPA, 2014). The daily limit value was set at 125 $\mu g/m^3$ in Turkey (Regulation for assessing and managing the air quality, 2008). Particulate matter is the term for solid or liquid particles found in the air. The PM_{10} standard includes particles with a diameter of 10 micrometers or less. The daily limit value in Turkey was set at 50 $\mu g/m^3$. Major concerns for human health from exposure to PM_{10} include: effects on breathing and respiratory systems, damage to lung tissue, cancer, and premature death (Particulate Matter (PM-10), EPA, 2014). Crude death rate (CDR) indicates the number of deaths occurring during the year, per 1,000 population estimated at midyear. The 2012 CDR of Turkey is ‰ 5,0 (Regulation for assessing and managing the air quality, 2008).

OBJECTIVES:

This study aims to define the association between air pollution assessed with SO_2 and PM_{10} levels in the air and CRD, and investigate whether this relates with the industrialization level of the region.

MATERIALS AND METHODS:

The study has been conducted with the data provided by Turkish Statistical Institute and presented on the website of the institute. The most industrialized region of Turkey is the Marmara region comprising 14 cities and located on the northwestern part of the country. The less industrialized region is Southeast region comprising 9 cities. The air pollution is measured by the ministry of environment and urban planning by using two parameters: SO_2 and PM_{10}, levels are presented as monthly and yearly average values. CDR's are presented for each city and calculated by dividing the number of deaths to the midyear population. In the present study 2009 and 2010 data were used. To minimize the biases, possibly emanating from the locations of the measuring devices in the cities, the percentages of yearly changes were calculated and analyzed. To measure the association between two air pollution criteria and CDRs Pearson Correlation Test was used. Significance was set at 0.05. SPSS Ver.15 was used for statistical analysis.

RESULTS AND DISCUSSION:

The absolute levels of SO_2, PM_{10} and CDR in 2009 and 2010 in the cities of Marmara region and Southeast region of Turkey and percentages of changes from 2009 to 2010 are shown in Table 1. There is no correlation between SO_2 levels and CDRs, and between PM_{10} levels and CDRs as a whole (SO_2 & CDR: r: 0,286; p: 0,185 / PM_{10} & CDR: r: -,023; p: ,916). There is still no correlation if we separate two regions and do the analysis (Marmara= SO_2 & CDR: r: 0,131; p: 0,654 / PM_{10} & CDR: r: -,205; p: ,483. Southeast= SO_2 & CDR: r: 0,373; p: 0,323 / PM_{10} & CDR: r: ,174; p: ,655).

Table 1 - The SO_2 and PM_{10} levels and CDRs of the cities of Marmara and Southeast regions of Turkey in 2009 and 2010 and the changes in percentage from 2009 to 2010

Region	City	SO_2		Change	PM_{10}		Change	CDR		Change
		2009	2010	%	2009	2010	%	2009	2010	%
Marmara	Istanbul	10	7	-30	53	51	-4	4,2	4,0	-5
	Tekirdag	142	134	-6	88	80	-9	5,8	5,5	-5
	Edirne	52	45	-13	74	65	-12	7,5	8,0	7
	Kirklareli	29	23	-21	48	43	-10	7,6	7,9	4
	Balikesir	7	10	43	80	75	-6	7,7	7,8	1
	Canakkale	20	21	5	54	30	-44	8,3	8,1	-2
	Bursa	22	10	-55	87	61	-30	5,6	5,5	-2
	Eskisehir	4	6	50	49	36	-27	6,5	6,5	0
	Bilecik	20	12	-40	44	48	9	7,5	6,9	-8
	Kocaeli	19	20	5	73	64	-12	4,4	4,4	0
	Sakarya	13	12	-8	73	73	0	5,9	5,8	-2
	Duzce	10	16	60	88	81	-08	5,8	5,6	-3
	Bolu	52	67	29	100	100	0	7,2	7,1	-1
	Yalova	5	8	60	45	53	18	6,3	6,2	-2
Southeast	Gaziantep	16	19	19	87	71	-18	4,2	3,9	-7
	Adiyaman	3	14	367	84	96	14	3,7	3,9	5
	Kilis	17	6	-65	79	89	13	6,2	5,9	-5
	Sanliurfa	14	10	-29	78	85	9	3,6	3,6	0
	Diyarbakir	9	19	111	84	98	17	3,5	3,5	0
	Mardin	29	28	-3	114	89	-22	3,4	3,5	3
	Batman	9	13	44	126	143	13	3,3	3,2	-3
	Sirnak	96	128	33	96	99	3	3,4	3,1	-9
	Siirt	53	35	-34	114	124	9	3,6	3,8	6

Although prior work has shown clear association between particulate air pollution and daily mortality (Katsouyanni et al., 1997; Samet et al., 2000), we couldn't find any in our study. One of the reasons would be that the effect of each air pollutant on daily mortality should be estimated after controlling for meteorologic and seasonal influences. We didn't assess the meteorologic and seasonal influences. The other reason could be the mislocations of the measurement devices for SO_2 and PM_{10}. We tried to minimize this effect with using the change value of pollutants in the analysis. We also should have searched the association between air pollution and the CRDs after having categorized the population into age groups and the change in pollution levels should also be categorised as low, medium and high. The effect is apparent in older age groups and high pollution areas.

CONCLUSION:

Regarding the prior work it is not easy to claim that there is no correlation between air pollution and CDR based on our findings. There is a need for more in-depth analysis, especially after having controlled meteorologic and seasonal influences.

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Soil heavy metals potentially triggering human health risk based on elevated concentrations in the Niger delta

Authors: Richard Olawoyin[1, 2], Samuel A. Oyewole[2], Robert L. Grayson[2], Charles W. McGlothlin[1]

1 *Prevention Research Center, School of Health Sciences, Oakland University, Rochester, Michigan, 48309 USA*
2 *Environmental Health and Safety Engineering, Pennsylvania State University, University Park, Pennsylvania, USA*

Presenting Author: Email: olawoyin@oakland.edu | Tel.: +1 248-364-8653 | Fax: +1 248-364-8657

INTRODUCTION:

An analysis of the soil quality in the Niger Delta Area (NDA) was carried out to determine the severity of soil contamination. The United States Environmental Protection Agency (U.S. EPA) risk assessment model was used in determining the potential health risk due to lifetime exposure (by means of ingestion, dermal contact and inhalation) of the population to heavy metal contents in the soil. Significant levels of contamination were found indicating elevated levels of heavy metals above background concentrations in sampled areas. Median topsoil metal concentrations (0-15 cm) in the NDA measured in mg/kg were: Zn 58.3 ± 37.0, Cd 1.3 ± 1.0, Cr 13.2 ± 5.5, Cu 28.3 ± 31.5, Pb 895.1 ± 423.9, Ni 42.7 ± 20.3, Mn 201.8 ± 77.5 and Fe 1303.5 ± 2028.6. Pb and Fe were the most significant contaminants ($P < 0.005$). The comprehensive levels of total metals contamination exceeded international thresholds which could potentially affect human health. The collective total risk (carcinogenic and non-carcinogenic) for minors and adults were established from the model using the slope factors and reference dose of the compounds respectively. The study has shown that soil contamination in the industrial and residential regions are similarly significant; while the risk assessment proved that based on the pollutants concentration in the soil, the carcinogenic and non-carcinogenic risk are also significant, especially for children in the region. The chronic daily intake of the metals are of major concern as their cumulative effect could result to several health complications of children and adults in the region.

OBJECTIVES:

The potential effects on human health of individual and cumulative contaminants in the NDA was assessed and proven that there are various chemicals of concern in the area. A risk assessment adopted from the US EPA was used to calculate the carcinogenic and non-carcinogenic risks due to life time exposure through three pathways; accidental ingestion of soils, inhalation of soil particulate materials and soil-dermal contact.

MATERIALS AND METHODS:

Soil samples were obtained at variable depth (0-15 cm) in four different areas (Bonny, Eriemu, Odidi, and Ughelli). The Atomic Absorption Spectrophotometry (AAS) was used to analyze the concentration of heavy metals in each fraction of the sample by flame atomization (GBC Avanta version 1.33). The concentrations of Cu, Cd, Ni, Cr, Fe, Mn, Pb, and Zn in soils were determined. Sample analysis was done with duplicates. Blanks (indicated below detection limits) and multiple trials were conducted for the purpose of error correction and quality assurance. Recovery ratios for the heavy metals range between 92.5% - 101%.

The risk assessment involves a multi-level process with regards human health, such as; (a.) data gathering and analyzes, (b) exposure appraisal (evaluation of the extent of potential and/or actual contacts), (c) toxicity assessment (undesirable human health consequences due to chronic exposure to various substances are determined) and (d) the risk categorization (sums up the results of (b) and (c) above) (U.S. EPA, 2002).

RESULTS AND DISCUSSION:

The concentrations of heavy metals obtained from the soil sample analysis show that majority of the locations in this study have higher values as compared to natural background concentrations (HDOH, 2011).

These heavy metals are considered to be probable causes of carcinogenic risk to children and adults, some chronic health effects and affected human organs.

The human health effects due to accidental ingestion, inhalation of soil dust particulates and dermal absorption were derived by calculating the chronic daily intake (*CDI*) using the formulas in equations 1-3. Calculations for the simultaneous exposure to soil contaminants through the three exposure routes, with combined effects corresponding to hazard quotient of 1 and an incremental lifetime carcinogenic risk of 10^{-5} are presented. The calculated CDI values were used to derive both the cancer risk and the hazard quotient. Equation 1 is the lifetime cancer risk; it estimates the incremental likelihood of humans with a lifetime exposure to potential carcinogenic substances, while the cumulative total carcinogenic risk (*CTR*) due to simultaneous exposure to different substances is expressed in equation 2. For the non-carcinogenic effects, the systemic toxicity value is derived which is the hazard index (*HI*) - equations 3 and 4.

$Total\ Risk\ (TR) = CDI * SF$ …… …… Eq. 1; $CTR_k = \sum CDIk * SFk \quad k=1$ …… …… Eq. 2

$HI = \frac{CDI}{RfD}$… …… ……. Eq. 3; $CHI = \sum CDIk / RfDk \quad k=1$ ….. Eq. 4; Where; *TR* = probability of cancer affecting human health in a lifetime, i.e. child to adult (unitless), *CDI* = chronic daily intake ($mgkg^{-1}.day^{-1}$); *SF* = oral cancer slope factor ($mgkg^{-1}.day^{-1}$); CTR_k = risk estimate for the *k*th substance; *CDIk* = *k*th toxicant: chronic daily intake ($mgkg^{-1}.day^{-1}$); *SFk* = *k*th toxicant: oral slope factor ($mgkg^{-1}.day^{-1}$); *RfDk* = *k*th toxicant: chronic reference dose ($mgkg^{-1}.day^{-1}$).

This study shows that the measured soil concentrations from the different sites for the various elements contribute substantial lifetime carcinogenic risk on the human health of the population due to accidental ingestion and/or dust inhalation of soils in the area. From the results, the likelihood of having high carcinogenic threat in this region is evident. The heavy metals with chances of inducing carcinogenic risks to residents in the area include; Cd, Cr(VI), Pb, Ni and Mn. The risk values vary widely from the minimum *CTR* of 1.3×10^{-5} for Cd to the maximum cumulative total risk of 2.6×10^{-2} for Pb as shown in Fig. 1, which illustrates the median values for the calculated CTR

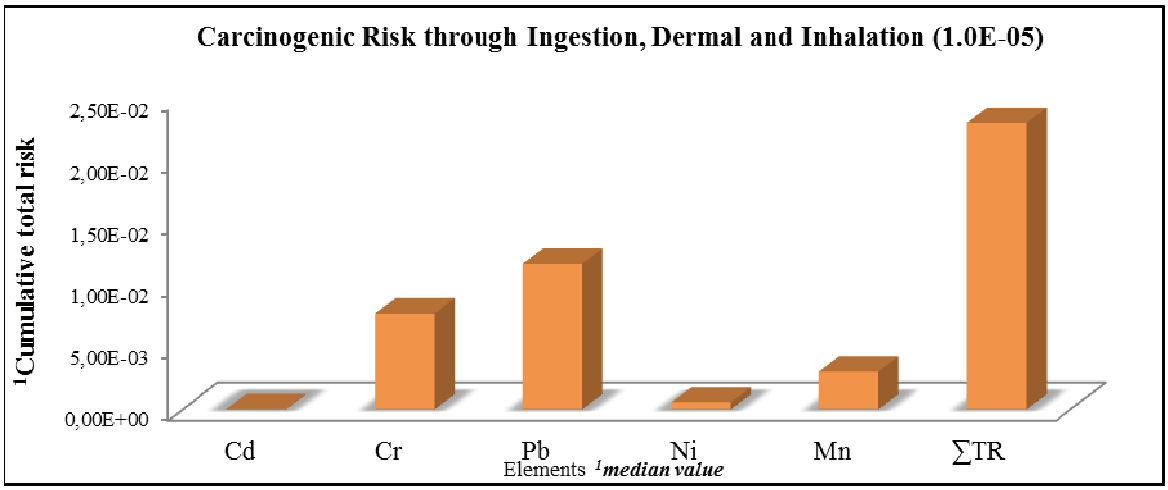


Fig. 1 - Potential carcinogenic risks induced by the heavy metals in the NDA.

The carcinogenic risk due to residents' exposure to soil heavy metals in the NDA is expected to be potentially significant due to the pollutants values. Conversely, hazard index (*HI*) which indicates the non-carcinogenic risk effect, is found not to be harmless. The *HI* of the individual elements seems not to pose any significant non-carcinogenic risk as shown in Fig. 2.

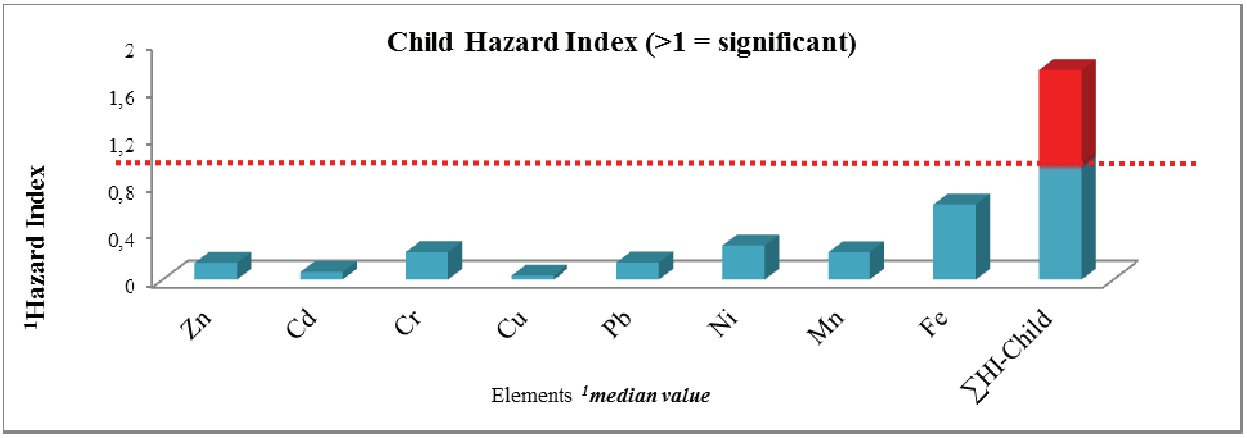


Fig. 2 - Potential non-carcinogenic risks induced a child (age < 6yo; avg. wt. =15 kg) by the heavy metals

CONCLUSION:

The potential effects on human health of individual and cumulative contaminants in the NDA was assessed and proven that there are various chemicals of concern in the area. This study has identified children as the most vulnerable in the population; and also the potential lifetime risks posed on children and adults in the Niger Delta area.

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Salinity variation: effects on two clam species, *Venerupis decussata* and *Venerupis philippinarum*

Authors: Vanessa Carregosa[1], Etelvina Figueira[2], Amadeu M.V.M Soares[2], Rosa Freitas[2]

1 *Department of Biology, University of Aveiro, Portugal*

2 *Department of Biology & CESAM, University of Aveiro, Portugal*

Presenting Author: Email: vanessacarregosa@ua.pt | Tel.: +351 234 370 350 | Fax: +351 234 372 587

INTRODUCTION:

The health status of marine benthic populations has been studied in the last few decades mainly in relation to the impacts caused by contamination. On the other hand, the effects of climate change in this group of organisms have received little attention. However, in the next hundred years, especially in estuarine and coastal areas, increases in temperature, water acidification and changes in seawater salinity are predicted (Booij, 2005).Salinity can be easily altered by heavy rainy periods and dry seasons, scenarios already foreseen in the context of climate change. Among environmental alterations, salinity changes are one of the main factors affecting marine bivalves due to their sedentary life, respiratory and feeding patterns. Therefore, salinity can restrict the spatial distribution of a given population. Tolerance to salinity differs between species which is especially important when assessing the spread of an invasive species into a new environment (Widdows and Shick, 1985). In lagoon systems, such as the Ria de Aveiro, events of heavy rain and hot periods may be combined with the ebb and flood of the tide and fresh water inputs from rivers, resulting in abrupt salinity changes. Thus, in these systems important effects may overcome from salinity alterations due not only ecological but also to their economic role.

OBJECTIVES:

The main objective of the present study was to understand how native (*Venerupisdecussata*) and invasive (*Venerupisphilippinarum*) clam species cope with salinity changes, by assessing the biochemical alterations of organisms submitted to a range of salinities that can be found in aquatic systems like the Ria de Aveiro.

MATERIALS AND METHODS:

Both clam species were collected in the Ria de Aveiro and depurated during 48 hours in the laboratory. A total of 63 individuals of each species were submitted to a range of salinities (0, 7, 14, 21, 28, 35 and 42) under continuous aeration, during 144 hours at 18 ±1ºC. Mortality rates were determined for both species after exposure.

Indicators of oxidant damage (Lipid Peroxidation, LPO (mol/g FW)), antioxidant enzyme activity (Superoxide dismutase, SOD (U/g FW)); glutathione-S-transferase, GST(U/g FW))and cellular redox status (total glutathione, GSH_t(µmol/g FW); reduced, GSH (µmol/g FW)) were quantified, according to Figueira et al. (2012) using clamssoft tissues.

RESULTS AND DISCUSSION:

The results obtained showed that both species can tolerate high salinities (14 to 42).For *V. decussata,* except for salinity 0, 100% of the individuals survived at all the salinities tested. *V. philippinarum* presented lower survival capacity at the lowest salinities tested, with 78% and 66% of mortality at salinities 0 and 7, respectively. Studies conducted by Kim et al. (2001), showed that the lower tolerance range for *Venerupisphilippinarum* was between salinities of 10 and 15, but the present study showed that this species can tolerate lower salinities as was also shown by Carregosa et al. (2014).

The biochemical parameters analysed revealed differences between both species (Figure 1), with *V. decussata* presenting higher values of all biomarkers. In both species, higher values were detected at salinity 14 (highlighted in Figure 1).

In general, for salinities lower and higher than 14, both species decreased the values of all biochemical parameters analysed (cf. Figure 1).

Since LPO is known as a mechanism of cellular injury and an indicator of oxidative stress lower values at salinities 21 and 28 indicate that both species are under lower oxidative stress at these salinities. On the other hand, the higher GSH/GSSG values found in *V. decussata* indicate that this species is experiencing lower oxidative stress than *V. philippinarum*, which is certainly achieved by the higher activity of SOD decreasing superoxide anion and thus decreasing oxidative stress (Figure 2). The higher activity of GSTs, detected at salinity 14 in both species, may also account for the conjugation of endogenous hydrophobic compounds, formed during stress and that are toxic if they accumulate in the cell (cf. Figure 2).

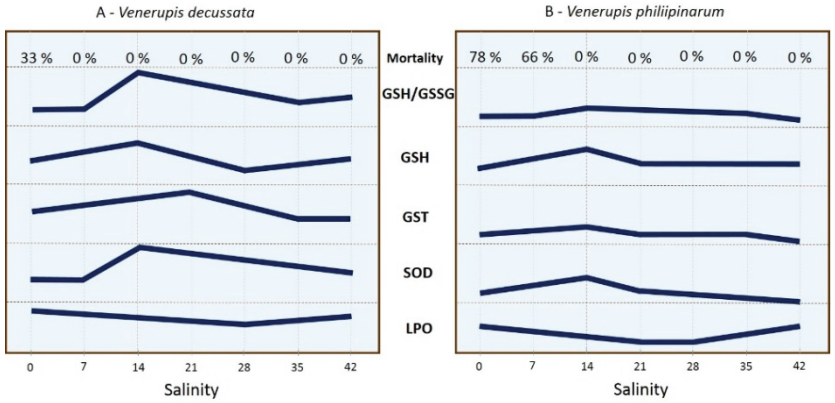


Figure 3 - Biochemical responses along a salinity gradient for Venerupisdecussata (A) and Venerupisphilippinarum (B). For both species, the increases and decreases in biomarker values, over salinity, are shown

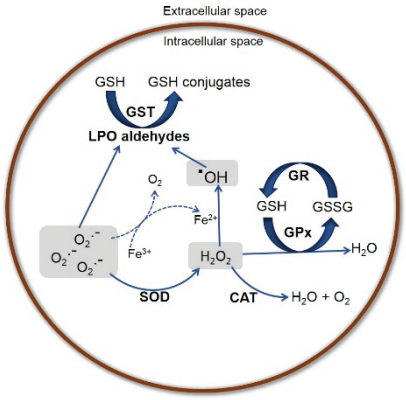


Figure 2 - Processes involved in oxidative stress.

CONCLUSION:

In the present work, *V. decussata* proved to be more tolerant to salinity changes, revealing lower mortality rates at salinities below 14. This response may be related to the lower oxidative stress, represented by the higher values of GSH/GSSG ratio displayed by *V. decussata*. Thus, this study demonstrated that in a scenario of climatic changes, namely salinity alterations, the native species (*V. decussata*) may have higher capacity to survive than the invasive species (*V. philippinarum*).

ACKNOWLEDGMENTS:

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Comparison of municipal waste management in Osijek (CRO) and Coimbra (PT)

Authors: Susana Paixão[1], Sven Bogdan[2]

1 *IPC, ESTeSC, Coimbra Health School, Environmental Health Department / CEGOT, Portugal*
2 *Environmental health, University of Applied Health Studies, Zagreb, Croatia*

Presenting Author: Email: supaixao@estescoimbra.pt | Tel.: +351 919 786 091 | Fax: +351 239 813 395

INTRODUCTION:

In this work we will try to compare the waste in Osijek and Coimbra, two cities with approximately equal numbers of residents, but totally different habits and social norms. We will also try to see whether the economic crisis has had any impact on the production of waste in the household and how much waste is properly separated and recycled.

OBJECTIVES:

The aim of this study is to raise awareness of waste management, waste production, recycling and proper disposal. These indicate that it is possible to take care of the environment very efficiently with very little effort.

MATERIALS AND METHODS:

During the study, we used informations available on the website. We watched the total amount of waste for each month of the 2012th and 2013th year, and we compared them between the two cities in each year, we also compared the differences between the two years for each city separately.

RESULTS AND DISCUSSION:

After analysis of informations, we concluded that the products of waste in Coimbra, on average, is twice as much than in Osijek. (Table1) In Osijek is also a noticeable decrease in the amount of waste in 2013th in relation to the 2012th which can be attributed to economical crisis, while in Coimbra we can notice larger quantities of waste in 2013th in relation to the 2012th.(Table1) If we analyze the amount of waste that is separated on paper, glass and plastic / metal for recycling, waste management in Coimbra is much more rational and pays more attention to the separation and recycling of waste. In Osijek may be noticed an increase of the quantity of separated waste in 2013th in relation to the 2012th which can be attributed to new laws which apply from accession to the European Union from July 2013th year.(Table2)

Table 1 - Comparison (total waste) 2012./2013.

City	Total watse (t)	Year
Osijek	30172 - 26273	2012 - 2013
Coimbra	58792 - 57497	2012 - 2013

Table 2 - Comparison (separated waste) 2012.2013.

City	Paper (t)	Glas (t)	Plastic / metal (t)	Year
Osijek	1278 - 1516	54 - 93	1038 - 942	2012 - 2013
Coimbra	2075 - 1646	2098 - 2018	33 - 133	2012 - 2013

CONCLUSION:

After we compared the results of total and separated waste in Osijek and Coimbra, we can conclude that many factors affect the production of waste in the household. Some of these factors may be social norms and

customs, leaving a large number of people on vacation out of town during the summer months. It is also necessary to point out that good waste management can greatly increase the recycling of waste if it is properly separated and treated, as we can see in the example of Coimbra.

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OCCUPATIONAL SAFETY AND HEALTH ABSTRACTS

Gender difference in hearing loss and hearing protection behavior among factory workers in high noise

Authors: OiSaeng Hong, Dal Lae Chin

University of California San Francisco, San Francisco, California, U.S.A

Presenting Author: Email: oisaeng.hong@nursing.ucsf.edul | Tel.: +1 415 502 5581 | Fax: +1 415 476 6042

OBJECTIVE:
To assess gender differences in the prevalence of noise-induced hearing loss (NIHL) and the use of hearing protective devices (HPDs) among factory workers exposed to high noise, above 85 decibels for an 8-hour time-weighted average. All workers at this exposure level are required to use hearing protective devices (HPDs) all the time (100%) to prevent hearing loss.

METHODS:
The study participants were workers included in worksite hearing conservation program at an automotive manufacturing plant in a Midwest state of the United States. The total of 2,579 who completed pre-hearing protection intervention surveys and annual audiometric tests were included in this study. Hearing ability was assessed by a standard pure-tone audiometric test in both ears at frequencies of .5- 8 kHz. Hearing loss at low (.5-3kHz) and high (4-6kHz) frequencies were determined using a cut-off of threshold level (HTL) 25dB in the worst ear. Workers' hearing protection behavior was assessed by a self-report survey on two questions regarding their use of HPDs in percentage of time at the plant for the past week and the past month.

RESULTS:
Male workers were older (44.6 vs. 40.5 years, p<.001), worked in the plant for longer periods (18.2 vs. 14.0 years, p < .001), and were more likely to perceive their hearing was poor than their female counterparts. Compared to female workers, males showed significantly higher prevalence of hearing loss at low frequencies (8.9% vs. 27.1%, p<.001) and high frequencies (23.4% vs. 64.9%, p < .001). The logistic regressions showed that, controlling for other covariates (years of work in the plant, noise level, ethnicity, and use of HPDs), male workers were three and four times more likely to have hearing loss at low frequencies (OR=2.73; 95% CI: 1.82-4.09) and at high frequencies (OR=4.22; 95% CI: 3.16-5.64), respectively. Yet, male workers were significantly less likely to use HPDs than female workers (77.3% vs. 87.5%, p<.001).

CONCLUSION:
The study found a significant gender differences in the prevalence of NIHL and hearing protection behaviors, higher rate of hearing loss and lower use of HPDs in male workers. More research is needed to understand better on contributing factors to high prevalence of NIHL and low usage of HPDs among male workers and thus to help develop effective targeted intervention programs for them.

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Keywords: Noise-induced hearing loss; Hearing protection; Gender difference

Assessment of the thermal environment in a textile plant for automotive components

Authors: Mariana Guise[1], Isabel Loureiro[2], Senhorinha Teixeira[2]

1 *Department of Production and Systems Engineering, University of Minho, Portugal*
2 *Department of Production and Systems Engineering, CGIT Research Centre, University of Minho, Portugal*

Presenting Author: Email: marianaguise@hotmail.com | Tel.: +351 914 604 967

INTRODUCTION:
The environment can be neutral, cold or hot. A combined action between four environmental parameters (air temperature, relative humidity, air velocity, radiant heat) and two individual parameters (clothing and metabolism) can lead to thermal comfort or to a thermal stress situation (Parsons, 2013). Thermal comfort can be defined as "that condition of mind that expresses satisfaction with the thermal environment" (ANSI/ASHRAE Standard 55-2010) and it is influenced by the six parameters (Parsons, 2013). The PMV index has been adopted as the International Standard method for assessing thermal comfort (ISO 7730:2005). It is used to check whether a given thermal environment complies with comfort criteria and to establish requirements for different levels of acceptability. ISO 7730(2005) refers that due to individual differences, it is impossible to specify a thermal environment that will satisfy everybody therefore, there will always be a percentage of dissatisfied occupants. As a function of PMV values, individual differences are measured by a method for predicting the percentage dissatisfied (PPD) with the environment. The analysis of thermal comfort can be achieved by the use of several techniques and simulating different environments using softwares of the human body (Teixeira et al., 2010).A practical approach to assess the effects of a heat stress situation on the workers was provided by the wet bulb globe temperature index (WBGT).

OBJECTIVES:
Definition of the thermal environment assessment in a textile plant, from the planning to the results.

MATERIALS AND METHODS:
Several steps were defined to perform this study: (1) characterization of the activity and plant, (2) the measurements of the environmental parameters and (3) the analysis of the worker's perceptions. The characterization was possible through direct and indirect observations on the field. To measure the parameters of the thermal environment, several instruments were used: a globe thermometer, a thermo anemometer model 8330 from *TSI VelociCheck* and a *Data Logger* model EL-USB 2 from *Lascar* that gives the values of air temperature and relative humidity. The measurements were made at 29 points identified on the available layout at three different levels: 0.10m, 1.10m and 1.70m. The levels were defined according to the ISO recommendations for standing work. In order to ensure that thermal variations among the different shifts were identified, the strategy comprised measurements in different periods of the three shifts. To the calculation of the metabolic rate, physical activity' observations and conversations with workers and supervisors were taken into account.

The calculation of the PMV/PPD and WBGT index was done using the model developed by Malchaire and Piette (1999). This model was used as it allows rapid data handling. Finally, a questionnaire was developed to assess workers' thermal sensations and the value of the clothing insulation. The questionnaire was divided in three main parts: workers characterization, workers thermal sensation evaluation and activity characterization. Each worker fulfill the questionnaire only once during labor time.

RESULTS AND DISCUSSION:
The study was carried out in a plant of a textile industry for automotive components, located in Vila Nova de Famalicão, Portugal. The company belongs to a Corporation that produces tires and other automotive

systems and that have many factories all over the world. The workload is continuous: 24 hours a day, 7 days per week, with various work schedules: normal rotation, fixed rotation and continuous labor. The industrial building has 22 twisting machines which are a source of radiant temperature.

After data collection, a descriptive analysis of the questionnaire was performed. On average, workers have 33 years old (standard deviation=11.78) and a vast majority were male (87.76%). The effects of the thermal environment were mainly felt by the workers of the shift 14h00 to 22h00(78.72%)(Figure 1).

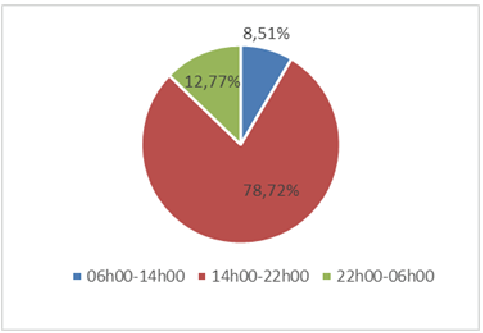


Figure 1 - Worker's thermal environment sensation, by shift

Nearly half of the respondents reported that they were feeling slightly warm (48.94%) while 40.43% were feeling warm. Only 10.64% of the workers marked the neutral category. None of the respondents choose the options "cold", "cool", "slightly cool" and "hot" (Figure 2).

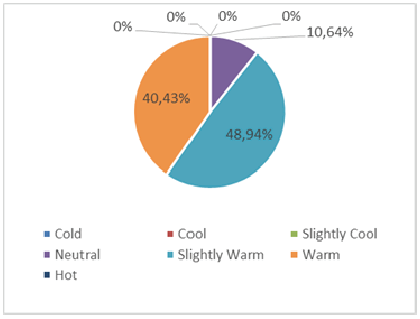


Figure 2 - Workers' Thermal sensation

After measurements, PMV/PPD and WBGT indexes were calculated. Results are summarized in Table 1. Even though the WBGT limit value was not exceeded, results indicate that actions are needed as the action value was exceeded. This can be achieved by engineering controls and/or working practices. The PMV/PPD results indicate that there is a long-term constraint; discomfort and health risk after several hours of exposure. The shift with the highest value of WBGT and PMV was the shift from 14h00 to 22h00.These results are in line with the information gathered through the questionnaire which identified this shift as the critical one. The analysis of PPD values show that all the workers are uncomfortable which also indicates the need of improvements in the industrial plant.

Table 1 - PMV/PPD and WBGT values according day and night shifts

	PMV	PPD (%)	WBGT	WBGT limit	WBGT action
06h00 - 14h00	3.76	100	21.1		
14h00 - 22h00	3.98	100	21.7	23.3	18.9
22h00 - 06h00	3.47	100	19.1		

CONCLUSION:

Is important to study thermal comfort since a comfortable environment is more willing to worker perform his activity, increasing the company productivity. Results showed that the shift with the highest WBGT is the

shift from 14h00 to 22h00 which confirms the answers given by workers in the questionnaire. All the workers are uncomfortable with the thermal environmental. Further studies are required in order to study with more detail the reaction of the human body to the thermal environments parameters. This will provide the efficiency of the prevention actions.

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Exposure to *Penicillium glabrum* in cork industry: the real scenario assessment

Authors: Carla Viegas[1], Mateus dos Santos[1], Daniel Botelho[1], Anita Quintal Gomes [1, 2]

1 *Environmental Health RG - Lisbon School of Health Technology - Polytechnic Institute of Lisbon, Portugal*
2 *Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon.*

Presenting Author: Email: carla.viegas@estesl.ipl.pt | Tel.: +351 218 980 430 | Fax: +351 218 980 469

INTRODUCTION:
Portugal is the world's leading producer of cork with 54.0% of the market (Basílio et al., 2006). There are several fungal species that were already been found in this industry, however *Chrysonilia sitophila* and *Penicillium glabrum* are the most prevalent species (Winck et al., 2004). Moreover, the presence of *P. glabrum* in this kind of industry is an indicator of respiratory diseases such as suberosis, the most prevalent disease among workers from this context, being *P. glabrum* the etiologic agent of this disease (Winck et al., 2004).

OBJECTIVES:
The aim of this study is to assess fungal contamination due to *Penicillium glabrum* by conventional and molecular methods in three cork industries (Plants A, B and C) from the outskirt of Lisbon city.

MATERIALS AND METHODS:
Regarding conventional methods, air samples of 50-100L were collected from10 indoor sampling sites (agglomeration, sanding, cutting, selecting, baking, tracing, grinding, pressing, mixing and sawing) through an impaction method onto malt extract agar (MEA) supplemented with chloramphenicol (0.05%), using the Millipore Air Tester (Millipore). An outdoor sample was also collected to be used as a reference. Surface samples were collected from the same indoor sites by swabbing the surfaces with a 10 by 10 square cm stencil, disinfected with 70% alcohol solution between samples in accordance with the International Standard ISO 18593 (2004). The obtained swabs were then plated onto MEA. All collected samples were incubated at 27ºC for 5 to 7 days. After laboratory processing and incubation of the collected samples, quantitative (colony-forming units - CFU/m^3 and CFU/m^2) and qualitative results were obtained with identification of the isolated fungal species. Morphological Identification was achieved through macro and microscopic characteristics as described by specific atlas for fungal identification. Regarding molecular methods, air samples of 250L were collected using the impinger Coriolis µ air sampler (Bertin Technologies), at 300 L/min airflow rate. Samples were collected onto 10ml sterile phosphate-buffered saline with 0.05% Triton X-100, and the collection liquid was subsequently used for DNA extraction using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research) according to the manufacturer's instructions. Molecular identification of *P. glabrum* is ongoing. Extracted DNA will be quantified by Real Time PCR (RT-PCR) using the Rotor-Gene 6000 qPCR Detection System (Corbett).

RESULTS AND DISCUSSION:
In Plant A *C. Sitophila* was identified in all the six air and surfaces samples. Only in one surface sample we were able to find 20000 CFU/m^2 from species belonging to *A. fumigatus* complex (Table 1).

In Plant Ba total of six samples were collected (three air samples and three surface samples) from three different sites and the species *Chrysonilia sitophila* and those belonging to *A.fumigatus* complex were detected. In Plant C, the most prevalent species in the air was *Penicillium* sp. (76.5%), followed by *Geotrichum* sp. (11.8%). Regarding surfaces, the most identified was *Trichoderma* sp. (52.9%), followed by *Penicillium* sp. (29.4%) (Table 1).

Table 1 - Most common fungi in the three cork industries

Fungal species	Plant A		Plant B		Plant C	
	Air (CFU/m^3)*	Surfaces (CFU/m^2)*	Air (CFU/m^3)*	Surfaces (CFU/m^2)*	Air (CFU/m^3)*	Surfaces (CFU/m^2)*
C.sitophila	TNTC	TNTC	TNTC	TNTC	40	-------
Penicillium sp.	-------	-------	-------	-------	780	50000
A.fumigatus	-------	20000	10	20000	------	10000
Geotrichum sp.	-------	-------	-------	-------	120	-------
Trichoderma sp.	-------	-------	-------	-------	-------	90000

* Total of colonies count; TNTC (too numerous to count)

The exclusive use of conventional methods can underestimate mold quantification and, on some samples, fungi with higher growth rates, such as *C. sitophila* (Francuz et al., 2010), may hide the presence of other species (Francuz et al., 2010) such as *P. glabrum*. Discrimination of *Penicillium* species from each other or from closely related species is difficult when using conventional methods which are based mainly on morphological features, and requires considerable expertise. In order to overcome this limitation, there has been an increasing use of molecular methods in detection and quantification of fungal DNA. However, despite the fact that molecular methods are more sensitive, specific, and fast (Viegas et al., 2014), conventional methods are still needed whenever it is important to distinguish between non-viable and viable organisms. The last ones pose higher risk for cork industry workers (Samson et al., 2000), since exposure occurs mainly through air inhalation. Thus, these two methodologies, when used in parallel, provide additional information that is useful in the evaluation of occupational exposure to fungi (Viegas et al., 2014).

CONCLUSION:

Regarding the fungal contamination already assessed it is recommended the application of preventive and protective measures intended to minimize workers fungal exposure. It will be possible to assess occupational fungal exposure due to *P. glabrum* in this setting through the concomitant use of conventional methods and molecular biology.

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Occupational exposure to Aflatoxin B_1 in waste management

Authors: Susana Viegas[1], Luísa Veiga[3], Paula Figueiredo[3], Ana Almeida[3], Carla Viegas[1]

1 *Environment and Health RG. Lisbon School of Health Technology - Polytechnic Institute of Lisbon*
2 *Center for Malaria & Tropical Diseases (CMDT) - Public Health and Policy, Escola Nacional de Saúde Pública, Universidade Nova de Lisboa, Portugal.*
3 Lisbon School of Health Technology- Polytechnic Institute of Lisbon

Presenting Author: Email: susana.viegas@estesl.ipl.pt | Tel.: +351 914 484 676 | Fax: +351 218 980 460

INTRODUCTION:
All the most common waste treatment methods implicate workers exposure to several risks such as virus, bacteria, fungi and their metabolites and, also, to dust (Domingo and Nadal, 2009).However, and despite the optimal conditions for fungi grow only few articles were dedicated to study occupational exposure to mycotoxins in this occupational setting (Degen et al., 2003). Recently, published work by Viegas and colleagues showed fungi contamination in this setting related with species that are recognized as AFB1 producers (Viegas et al., 2014).

Aflatoxin B1 (AFB1) is normally predominant in cultures as well as in food products and was shown to be genotoxic and a potent hepatocarcinogen.

OBJECTIVES:
Assess AFB1 exposure in workers from waste management industry.

MATERIALS AND METHODS:
The waste company enrolled in the study is constituted by three units that are related with the waste management chain: a sorting unit, a composting unit and, an incineration unit. Forty one workers accepted to participate in the study (26 from sorting; 9 from composting; 6 from incineration). As no apparent information was available on AFB1background levels for the Portuguese population, a control group (n = 30) was included. This group was composed of subjects without any type of contact with waste or other activities described as responsible for AFB1exposure. All participants signed a consent form and were provided with the study protocol. In order to assess occupational exposure to AFB1, a biomarker of internal dose that measures AFB1 in serum was used. For AFB1 quantification, the RIDASCREEN Aflatoxin B1 30/15 enzyme-linked immunosorbent assay (ELISA; R Biopharm) was used, and was calibrated with aflatoxin standards from1 to 50 ng/ml. Values below 1 ng/ml were considered nondetectable since these are below the method detection limit.

RESULTS AND DISCUSSION:
All the workers showed detectable levels of AFB1 with values ranging from 2.52 ng/ml to 25.99 ng/ml and with a median value of 9.75 ± 5.35 ng/ml (Table 1). In the control group, the AFB1 values were all below 1 ng/ml.

Table 1 - Aflatoxin B1 results (ng/ml)

Group	Median/Stdev/range
Workers (n=41)	9.75/5.35/ 2.52 - 25.99
Control (n=30)	<1ng/ml

The results obtained based on AFB1 quantification are related to intensity of environmental contamination and absorption rates. All the workers presented detectable levels of AFB1, in contrast with the individuals used as controls who showed no detectable values. These findings corroborate the hypothesis that

occupational exposure to AFB1 occurs in waste management industry. Moreover, the results obtained are eight times higher than the ones recently obtained in workers from animal production (Viegas et al., 2013). Two important factors contribute to explain the results: the continuous fungi contamination that occurs during all the waste management chain that results in continuous waste mycotoxin contamination; mycotoxins are resistant to adverse environment factors such as high or low temperatures and can be present in the environment or waste long after death and disintegration of the producer (Halstensen, 2008).

Mycotoxins are not volatile but when found in respirable air are associated with fungalspores or particles. In this occupational setting, exposure is probably occurring through inhalation and particles are the carrier for the workers respiratory system. In agreement with this postulation, Brera and colleagues (2002) found aflatoxin in airborne dust samples from different occupational settings.

It is also important to consider possible chemical interactions in the risk assessment process due to exposure to more than one mycotoxin simultaneously, since the presence of other mycotoxins, such as ochratoxin A has been already reported (Degen et al., 2003).

CONCLUSION:

Results obtained suggest that exposure to AFB1 occurs in waste management industry and inhalation is probably the most important exposure route.

ACKNOWLEDGMENTS:

The study would not have been possible to develop without the financial support given by Lisbon School of Health Technology.

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Occupational exposure to FA. Genotoxicity, immunotoxicity and susceptibility

Authors: Solange Costa[1,2], Sandra Carvalho[1], Julia García-Léston[3], Carla Costa[1,2], Patrícia Coelho[1], Susana Silva[1], Blanca Laffon[3], Beatriz Porto[4], João Paulo Teixeira[1,2]

1 *National Institute of Health, Environmental Health Department, Porto, Portugal*
2 *ISPUP, Institute of Public Health, University of Porto, Porto, Portugal*
3 *Toxicology Unit, Department of Psychobiology, University of A Coruña, A Coruña , Spain*
4 *ICBAS, Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal*

Presenting Author: Email: solange.costa2@gmail.com | Tel.: +351 223 401 100 | Fax: +351 223 401 149

INTRODUCTION:

Formaldehyde (FA) is a high-volume production chemical produced worldwide with a large range of industrial and medical uses. The highest level of human exposure to FA occurs in occupational settings. Over the last two decades, several epidemiological studies have revealed an increased risk of cancer development among workers exposed to FA, namely nasopharyngeal cancer and myeloid leukaemia (1).Based on these findings plus supporting evidence from animal studies and data on mechanisms of carcinogenesis FA status was recently revised and classified as a human carcinogen (2). FA's genotoxicity is confirmed in a variety of experimental systems ranging from bacteria to rodents. However, data from human studies is conflicting and needs further investigation in particular the biological evidence of FA's ability to induce (geno) toxicity on distant-site tissues. Published studies on the immunological effects of FA indicate that this compound may be able to modulate immune responses, although data in exposed subjects are still preliminary (3). Several studies point to pathology and anatomy laboratories as one of the occupational settings where workers are frequently exposed to levels of FA near or superior to recommended limit values.

OBJECTIVES:

The aim of the present study was to evaluate the occupational exposure to FA in a multistage approach relating the exposure with different biomarkers and individual susceptibility.

MATERIALS AND METHODS:

Eighty-five workers from hospital anatomical pathology laboratories exposed to FA and eighty-seven non-exposed controls took part in the study. Air monitoring was performed in worker's breathing zone for representative working periods and the level of FA-exposure in workplace air was estimated. Genotoxicity was evaluated in peripheral blood lymphocytes (PBLs) by means of cytogenetic alterations, DNA damage (comet assay, percentage of tail DNA, %TDNA) and T-cell receptor (TCR) mutation assay. Percentages of different lymphocyte subpopulations were selected as immunotoxicity biomarkers.In addition, the effect of polymorphic genes of xenobiotic metabolising enzymes (*CYP2E1*, *GSTM1, GSTT1, GSTP1*) and DNA repair enzymes (*FANCA, RAD51, XRCC2, XRCC3, XRCC1, PARP1, MUTYH, BRIP1*) on the endpoints studied were determined.

RESULTS AND DISCUSSION:

The mean level of FA-exposure was 0.38±0.03 ppm, exceeding recommended limit values, as observed in other studies. All cytogenetic endpoints and DNA damage evaluated by comet assay were significantly increased in PBLs of FA-exposed workers compared to controls. The exposed group also presented significant alterations in the percentage of cytotoxic T lymphocytes, natural killer (NK) cells and B lymphocytes in comparison with control group. Concerning the effect of susceptibility biomarkers on the different endpoints studied, results suggest that polymorphisms in *CYP2E1*, *GSTP1* and *GSTM1* metabolic genes are associated with increased genetic damage in FA-exposed subjects. Furthermore, a polymorphic gene involved in FANC repair pathway, *FANCA*, significantly altered the level of genetic damage induced by FA exposure, revealing

a potential novel repair pathway involved in the repair of genetic lesions caused by FA-occupational exposure.

CONCLUSION:

Data obtained in the present study show that workers in anatomical pathology laboratories are exposed to air-levels of FA greater than recommended criteria, indicating a potential risk to workers' health. Further, FA-exposed workers presented higher levels of genetic damage compared to controls. Genotoxic endpoints analysis are of great interest in risk assessment of occupational carcinogens because they precede by a long time the potential health effects, thus offering a greater potential for preventive measures. The results of the present biomonitoring study emphasise the need of more effective measures in order to protect the workers from potentially hazard health effects due to occupational exposure to FA. Implementation of security and hygiene measures, such as periodic air sampling and medical surveillance, as well as good practice campaigns may be crucial to decrease risk. The obtained information may also provide new important data to be used by health care programs and by governmental agencies responsible for setting the acceptable levels for occupational exposure to FA.

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Potential occupational toxicity of respirable crystalline SiO2 from HydroFrac. Operations in the US shale plays.

Authors: Richard Olawoyin[1], Charles McGlothlin[1], Patrick Frazee[1]

1 *Prevention Research Center, School of Health Sciences, Oakland University, Rochester, Michigan, USA*

Presenting Author: Email: olawoyin@oakland.edu | Tel.: +1 248 364 8653 | Fax: +1 248 364 8657

INTRODUCTION:

Respirable crystalline silica (RCS) is a known human carcinogen and a contaminant of potential concern (CoPC)(IARC, 2009). Proppants are used during the process of hydraulic fracturing as additives in the fluid cocktail and sand is often used as proppants (Olawoyin et al., 2013)which contains high percentage of silica determined by the quartz content. Empirical occupational exposure risk models were employed in this study to assess the potential health consequences from chronic RCS exposures based on RCS data from NIOSH and risk assessment formulas. The prevalence of elevated RCS exposure exceeding 0.1 mg/m3 (OSHA- PEL) increases the possibility of workers developing lung cancers or other forms of tumors and also elevates the chances of increased cases of silicotic related diseases. Evaluating the lifetime excess cancer risk (LCR) potential, based on a risk target of 10^{-4}, The job titles that are ***likely*** to experience any substantial potential effect of cancer induction are the sand mover (LCR = 1.61 X 10^{-4}) and transfer belt (LCR = 1.92 X 10^{-4}) operators. While the cumulative LCR effect for all job functions was theoretically estimated to be about 6 carcinogenic disease burden cases per 1000 workers. While the sand truck driver, Data Van operator etc. are among the job functions with a cumulative disease burden of 7.2%, that are ***unlikely*** to be affected by < 2% carcinogenic disease burden. The chemical truck, sand mover transfer belt (T-belt) operator may potentially be at risk of other occupational non malignant respiratory diseases with hazard quotient (HQ) of 0.65, 11.79, and 2.13 respectively.

OBJECTIVES:

To determine the potential occupational carcinogenic and non-carcinogenic disease burdens associated with exposures to respirable crystalline silica (RCS) during high volume hydraulic fracturing (HVHF) processes.

MATERIALS AND METHODS:

The job titles and exposure levels at risk of potential adverse health complications as a result of RCS was identified in a NIOSH study (Esswein et al., 2013). The study evaluated the occupational exposures to RCS during the use of hydraulic fracturing techniques (HF_{tech}). Eleven sites with HF_{tech} operations were investigated and one hundred and eleven (111) samples were collected from worker's personal breathing zone (PBZ) during a complete 12 hour shift. Sample collection techniques and analyses were presented in details in the published literature (Esswein et al., 2013).

Theoretical Occupational Carcinogenic Risk and Hazard Quotient: The inhalation reference exposure (I_r) value of 0.003 mg/m3 (ISO 1995) was used in this study to predict the total lifetime carcinogenic risk (L_{CR}) in the hydraulic fracturing industry, provided the worker continuously inhaled the RCS dust at the calculated dust concentration (C_j) during the exposure period. The product summation of the total occupational exposure to the calculated median 8-hr TWA for each job title (C_j) throughout the employment period for the job (T_j) gives the cumulative lifetime dust concentration intake (C_{Dc}) as given in equation 1, $C_{Dc} = \{\sum_{j=1}^{n}(C_j * T_j)\} = mg/m^3 - yrs \ldots\ldots\ldots \quad eq.1$; Where; T_j was calculated using equation 2; $T_j = \left\{\frac{(EF * ED)}{(LT)}\right\} = yrs \ldots\ldots eq.\ 2$. The exposure frequency (EF) and worker's lifetime were assumed to be 45 years and 70 years respectively. While the exposure duration (ED) was calculated as; $\left\{\left(\frac{8}{24}\ hours\right) X \left(\frac{5}{7}\ days\right) X \left(\frac{48}{52}\ weeks\right)\right\}$. Therefore; the potential excess lifetime carcinogenic risk (L_{CR}) using theHF_{tech} was determined using equation 3; $L_{CR} = C_{Dc} * I_r \ldots\ldots\ldots \quad eq.\ 3 \qquad L_{CR} > 10^{-4} = "Risk"$ Exposure effects with the potentials of

non-carcinogenic health complications can be estimated using the hazard quotient equation (eq. 4); $H_Q = \frac{C_{Dc}}{I_r}$ …… $eq.\ 4\ H_Q > 1\ = "Risk"$

Table 1 - Potential carcinogenic and non-carcinogenic occupational risk due to RCS dust

Occupational Exposure Guidelines				
Component	Units	OSHA PEL	ACGHI TLV	NIOSH REL
RCS	mg/m^3	8-HR TWA		10-HR TWA
		$\left\{\frac{10\ mg/m^3}{\%\ SiO_2 + 2}\right\}$	0.025	0.05

The adjusted OSHA PEL exposure and severity value (Adapted fromEsswein et al., 2013)

	Job Titles															
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Totals
#Sample	16	3	2	5	3	1	1	1	4	10	1	50	6	7	1	111
Median TWA	0.10	0.14	0.04	0.04	0.07	0.04	0.02	0.01	0.02	0.06	0.04	0.38	0.45	0.06	0.01	0.11
C_{Dc} *10^{-2}	1.44	1.96	0.61	0.62	0.98	0.61	0.3	0.18	0.28	0.86	0.58	0.54	6.40	0.79	0.09	21
L_{CR} * 10^{-5}	4.32	5.89	1.82	1.86	2.92	1.82	0.89	0.55	0.85	2.59	1.74	16	19	2.37	0.28	63
$HQ = 1$	0.48	0.65	0.20	0.21	0.32	0.20	0.10	0.06	0.09	0.29	0.19	1.79	2.13	0.26	0.03	7.03

"A=Blender Operator, B=Chemical Truck Operator, C=Fueler, D=Hydration Unit Operator, E=Mechanic, F=Operator, Data Van, G=Pump Truck Operator, H=QC Tech, I=Roving Operator, J=Sand Coordinator, K=Sand Truck Driver, L=Sand Mover Operator, M=T-belt Operator, O=Water Tank Operator, P=Wireline Operator"

The potential occupational carcinogenic and non-carcinogenic risks from continuous exposure to RCS through the use of the HF_{tech} are presented in Table 1. This risk outcomes did not take into account the synergistic effects from other potentially deleterious substances the workers may have been exposed to at the same period as the RCS exposure. The results presented herein also assumed that the workers continued on the same job with constant exposure levels until the end of the follow up year.

RESULTS AND DISCUSSION:

Within the field workers, the T-Belt and Sand Mover Operators have the relative highest exposures to RCS based on the adjusted 8-hr TWA calculated following the OSHA PEL guidelines. The prevalence of high RCS exposure exceeding 0.1 mg/m3 increases the possibility of workers developing lung cancers or other forms of tumors and also elevates the chances of increased cases of silicotic related diseases. Evaluating the lifetime excess cancer potential, based on a risk target of 10^{-4}, the lowest possibility of cancer development was determined to be for the water tank operator with a rare classification of about 0.3 occurrence per 1000 workers (See Table 2). The sand truck driver (K), fueler (C), Data Van (F) and the hydration unit (D) operators with a cumulative disease burden of 7.2%, are among the job functions that are ***unlikely*** to be affected by < 2% carcinogenic disease burden per the risk target of 10^{-4} as determined. In general, the possibility of 2.6% of the LCR prevalence among the following; water tank operator (O), QC tech (H), roving operator (I) and the pump truck operator, fall under the rare category. This gives the magnitude of safety related to each job function, based on the possibility of toxic dust contacts.

Table 2 - Likelihood of Occurrence of LCR based on Occupational RCS using the HF_{tech}

	< 1% (1.0 X 10^{-4})				< 2% (1.0 X 10^{-4})				< 4% (1.0 X 10^{-4})			> 4% (1.0 X 10^{-4})			
Job Title	O	H	I	G	K	C	F	D	N	J	E	A	B	L	M
LCR	0.3	0.6	0.8	0.9	1.7	1.8	1.8	1.9	2.4	2.6	2.9	4.3	5.9	16.1	19.2
Likelihood of Occurrence	Rare Possible $\sum = 2.6\%$				Unlikely Possible $\sum = 7.2\%$				Possible $\sum = 7.9\%$			Likely $\sum = 45.6\%$			

The cumulative dust exposure may result to other health complications other than lungs cancer. The potential effects of RCS leading to non-carcinogenic human health problems was assessed using the hazard

quotient (HQ); which shows the same trend as the lifetime cancer risk, based on the overall RCS exposure. HQ > 1 poses significant health risk to humans, at the current level of RCS exposure, only the chemical truck operator, the sand mover operator and transfer belt (T-belt) operator may potentially be at risk of other occupational non malignant respiratory diseases with HQ of .65, 11.79, and 2.13 respectively.

CONCLUSION:

In this study, the intervention epidemiology analysis was used to appraise the potential health effects due to occupational exposures to RCS during the use of the $\boldsymbol{HF_{tech}}$. The sand mover operator and the transfer belt operator both have LCR values of 1.6 X 10^4 and 1.9 X 10^4 respectively; and HQ of 1.79 and 2.3 respectively. These values are higher than the LCR target risk level of 10^4 and HQ = 1, therefore, these two job functions are considered to have the likelihood of disease burden occurrence. The projected risks were based on some occupational assumptions and also considering the PEL exposure levels for each job function. It is quintessential to underscore the benefits obtainable from preventing workers from any potential continuous RCS dust exposures, which is substantial in reducing the potentiality of either a lifetime excess risk or other nonmalignant health conditions.

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Assessment of arterial hypertension risk using results of vessel wall condition examination among potassium salts mining workers

Authors: Dmitriy Shlyapnikov, Pavel Shur, Vadim Alekseev, Elena Vlasova, Anna Khasanova

FBSI «FSC for Medical and Preventive Health Risk Management Technologies», Perm, Russian Federation

Presenting Author: Email: shlyapnikov@fcrisk.ru | Tel.: +79 124 866 461

INTRODUCTION:

Potassium salts mining work is cyclic and characterized by different levels of mechanization and noise exposure [1]. Noise is considered as one of the risk factors of cardio-vascular diseases, in particular of arterial hypertension [2]; as arterial hypertension precursors intima-media thickness level and endothelial dysfunction are determined [3].

OBJECTIVES:

To conduct quantitative risk assessment of arterial hypertension precursors formation among potassium salts mining workers including characterization of dependences between health effects and occupational factors exposure and length of employment.

MATERIALS AND METHODS:

Study group comprised 139 male underground mining workers (average age - 36.36±1.02 years, average length of employment - 7.29±0.89 years). Control group - 53 male surface workers (average age - 40.23±2.72 years, average length of employment - 5.75±1.95 years). Both groups were exposed to different noise levels. Noise levels were measured by noise level-vibration meter, spectrum analyzer system ECOFIZIKA - 110 A.

Ultrasound examination was performed with scanner «Toshiba VIAMO»; intima-media thickness estimation - according to standard methodology; endothelium vasculomotor function - according to update technique Celermajer D.S. (1992). Towards assess indexes change dynamic from length of employment increase, control group workers were divided into 3 subgroups in terms of length of employment (less than 5 years, 5.1-10 years and more than 10 years).

Smoking prevalence as a factor influencing on endothelium function [3] was evaluated using special questionnaires.

Primary statistical data procession was conducted with SPSS 16.0, Stata/SE 12.1 for Windows.

Mathematical modelling of dependences between health effects and occupational factor exposure and length of employment was performed on the basis of 3D-model “exposure - length of employment - effect” in order to individual and population risk estimation.

Credibility of variables and model adequacy assessment were based on single-factor analysis of variance by Fisher’s ratio test.

RESULTS AND DISCUSSION:

Noise level for underground mining workers varied from 83 to 94 dBA; for group of surface workers (control group) it was 73 dBA.

Percentage of smokers in study group was 28.9%, in control group - 29.8%. Average smoking status length in study group was 16.8±0.88 years, in control group - 15.9±1.96 years.

There was no statistically significant difference (p>0,05) between groups in terms of average daily nicotine intake among habitually smoking individuals that allowed to carry out assessment of noise exposure impact on vessel wall characteristics.

Endothelium function decline is associated with age increment and for men without risk factors is 0.2% per year [4], comparison of different length of employment subgroups taking into account difference of average age showed that after 5 years of employment yearly rate of endothelium function decline was higher and reached 0.62% per year.

It was determined that exposure to higher than in control group noise level lead to increase of intima-media thickness ($p<0.05$). Results of comparison of different length of employment subgroups indicated statistically significant increase of intima-media thickness from 5 years employment period ($p<0.05$). According to scientific data, increase of intima-media thickness has to be less than 0.0124-0.0138 mm per year [5]. Taking into account age difference between different length of employment subgroups increase of intima-media thickness exceeded standard value from 5 years employment period and reached 0.016 mm per year.

When performing mathematical modelling "exposure-length of employment-effect" dependence, it was estimated that individual risk level for parameters characterizing vessel wall status increased with increase of length of employment and noise level (Figure 1).

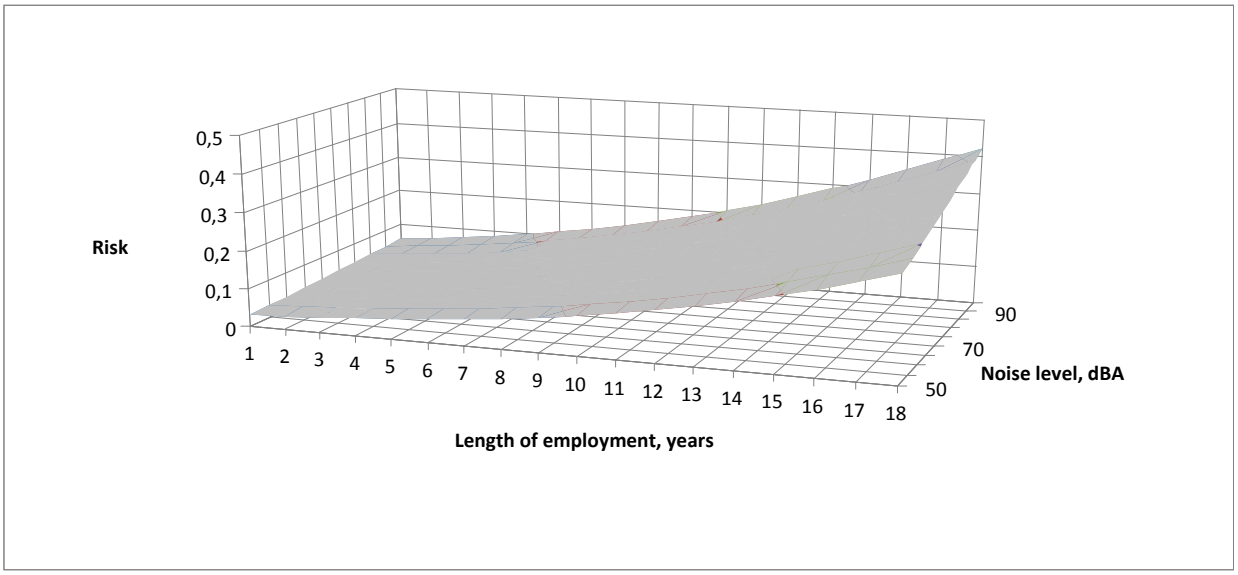


Figure 1 "Exposure-length of employment-effect" dependence model for parameters characterizing vessel wall status

Under higher than in control group noise level (94 dBA) risk of parameters characterizing vessel wall status decline was 15% higher by 1 year of employment.

Work in the same noise level condition could lead to decline of parameters characterizing vessel wall status even on the noise level of 83 dBA: 11 workers per year by 5 years length of employment and 22 workers per year by 10 years length of employment; under the 94 dBA noise level: 13 workers per year by 5 years length of employment and 25 workers per year by 10 years length of employment.

CONCLUSION:

It was determined that noise causes vessel wall disturbances. Under noise exposure of 83 dBA and higher yearly rate of endothelium function decline increase, increase of intima-media thickness growth rate per year and increase of intima-media thickness were determined. Risk of parameters characterizing vessel wall status decline increased with increase of noise level and exposure duration.

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Lighting conditions and optical filters effects on visual performance in speleologists in the cave environment

Authors: Carla Costa Lança[1], Nádia Fernandes[1], Ana Monteiro[2]

1 *Orthoptic Department, Lisbon School of Health Technology, Portugal*
2 *Enviromental health Department, Lisbon School of Health Technology, Portugal*

Presenting Author: Email: ana.monteiro@estesl.ipl.pt | Tel.: +351 218 980 430 | Fax: +351 218 980 460

INTRODUCTION:
Visual function in speleologist's workers has not been documented. Speleologist's workers often perform their activity in demanding visual conditions of very low luminance, because the cave environment is surrounded by similar conditions to the night vision. There are many visual tasks involving resolution of detail that must be performed under conditions of low luminance and contrast. Contrast sensitivity and visual acuity are fundamental descriptors of visual systems and play a role in determining the quality of vision (Kang et al., 2009; Kohnen et al., 2005). Good visual acuity is crucial for a several numbers of professions like law enforcement officers, driving instructors, drawbridge operators or correctional officers (Johnson & Casson, 1995). Visual acuity typically measured under optimal viewing conditions with appropriate refractive correction will be altered by different environmental conditions and refractive properties in the work environment (Johnson & Casson, 1995). Poor lighting conditions may also increase the risk of accidents (Reinhold & Tint, 2009) and could also cause a variety of symptoms of visual discomfort.

OBJECTIVES:
The aim of this study was to evaluate lighting conditions and optical filters effects on the visual status of speleologist's workers who were directly involved in caving and/or are exposed to the cave environment.

MATERIALS AND METHODS:
A cross-sectional study was conducted. A questionnaire was administered to respondents to describe their socio demographic data, previous and current work history, workstation, detailed medical and ocular history, current use and type of medication, the use of protective eye wear, lighting conditions, activities performed, accidents, time and length of stay in the cave. The first step involved a visual function examination at the Clinical Orthoptic Lab of Lisbon School of Health Technology. Twenty-three speleologist's volunteers were submitted to an evaluation of visual acuity, contrast sensitivity, stereo acuity, auto-refraction and tonometry, ocular alignment and near convergence point. The second step involved a visual function examination (visual acuity, contrast sensitivity and stereo acuity) and evaluations of headlamps lighting levels in 16 speleologists at two caves in Portugal deprived of natural lightning. Two organic filters (450nm and 550nm) were used to measure and compare visual function (distance visual acuity, near visual acuity and contrast sensitivity) with and without filters in the cave environment. The illuminance measurements regarding lighting levels (headlamp on the helmet or head) were made using a meter lux brand Gossenr, model Mavolux 5032C. In the evaluations of the illuminance levels, the timings chosen for the determination of these levels was not fixed a priori. In each point the measurements were carried out for a sufficient time in order to stabilize the value of the light levels. These measurements were conducted in different light conditions used by workers at a distance of half a meter and in a lower plane, simulating the walking position. The lamps had a maximum of four positions and had a diffuser. All positions of the lamps were measured with and without diffuser.

RESULTS AND DISCUSSION:
The mean age of the workers was 40.65(±10.93) years. The majority of the subjects were males (65.2%). The mean number of years of experience in caving was 15.20±11.20 (median=14.00) years. The subjects practised

this activity in approximately 12 days per year. The mean time spent in the cave was 4.30±2.49 hours per day during the daytime. Accidents during this activity were reported by 21.7% of the participants.

We detected 26.1% (n=6) participants with visual impairment of which refractive error (17.4%) was the major cause. During the cave activity blur vision was the most referred visual symptom with 62.5% (n=10). However, in 3 subjects we found out that these symptoms could be related with the presence of a refractive error, in other 2 subjects we detected a history of retinal detachment and keratoconus. The second symptom most referred was visual irritability with 43.8% (n=7). In this group one subject had a refractive error and other one had a history of retinal detachment.

In the first step of the study all vision tests in the lab were administered under artificial lighting mounted at ceiling level (illuminance 443 lux to 568 lux). For near vision tests additional local lighting as used with a total illuminance of 1552 lux to 2390.

Binocular visual acuity in the cave environment was -0.05±0.15 LogMAR (20/18) and all subjects had visual acuities LogMAR of 0.3 or higher. Binocular visual acuity for distance without filter was not statistically different from the visual acuity with the 550nm or 450nm filters (p=0.093). Improved contrast sensitivity was observed with the use of 450 optical filters, but this difference was only significant for the 6 cpd (p=0.034) and for the 18cpd (p=0.026) spatial frequencies.

Workers preferred the 450nm filters (68.8%) when compared with the 550nm filters (6.3%) or without filter (25.0%). For the type of activity performed, in most cases, the developed tasks consisted in: walking (vertical progression in galleries, sub-vertical and horizontal), topography, cartography, bats observation and photography. In the cave environment the majority of the workers used a headlamp on the helmet or head (26.1%), especially at position 2 (or medium) without diffuser (47.8%). Is this position the illuminance values were 451.0±305.7 lux (Table 1). According to the DIN 5035 - 2: 1990 Artificial Light norm, this level of illuminance can be used for normal visual tasks with medium details (500-750 lux) which is in accordance in the most of the tasks performed by the workers. However, these illuminance levels are the focus of the flashlight from a distance of half a meter and no light in the surroundings.

Table 1 - Evaluation of illuminance levels (lux) in different positions of the equipment.

	headlamp in position 1 without diffuser (n=15)	**headlamp in position 2 without diffuser (n=14)**	**headlamp in position 3 without diffuser (n=11)**	**headlamp in position 4 without diffuser (n=7)**	**headlamp in position 1 with diffuser (n=3)**	**headlamp in position 2 with diffuser (n=2)**	**hand lantern (n=1)**
Mean ± Std. Deviation	250.2 ± 414.3	451.0± 305.7	1551.3± 1165.9	1670.2± 1275.4	134.0± 108.6	210.0± 127.3	28.0
Median	100.0	400.0	840.0	2000.0	170.0	210.0	28.0
Minimum	16.8	50.0	365.0	92.0	12.0	120.0	28.0
Maximum	1600.0	1300.0	3000.0	4000.0	220.0	300.0	28.0

CONCLUSION:

In this study we observed two subjects with a history of a visual pathology, a retinal detachment and a keratoconus. These reported pathologies were not related to the cave environment. Although the cave environment was very humid there were no signs and symptoms of visual pathologies related to the exposition to the cave in this workers. Also none of the workers had previous history of acute or chronic conjunctivitis, which leads us to conclude that occupationally associated eye diseases and disorders were not present among the speleologist's workers. Most of the visual symptoms referred by subjects during the cave activity were related with a presence of a refractive error or an ophthalmic pathology.

In the cave environment binocular visual performance was not impaired by the lighting conditions of the cave. The illuminance levels for the workers who used the headlamp in the position 2 without diffuser were adequate to the majority of the activities/tasks performed. However, some of the workers need to acquire appropriate equipment with a lighting regulator suitable for different activities, mainly for visual tasks with medium details. The enhancement in contrast sensitivity with the 450nm filters could be beneficial to the cave operational environments to potentially improve the tasks related to bats observation. More investigation is therefore needed to better understand the influence of cave lighting conditions in the visual symptoms referred by the workers. It is also important to observe the improvement in contrast sensitivity involving tasks with longer time spent in the cave.

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Magnetic resonance occupational safety zones and standards

Authors: Vítor Silva[1], Isabel Ramos[2], J. Agostinho Moreira[3], Manuel B. Marques[4]

1 *Doctoral Program on Occupational Safety and Health, Faculty of Engineering of University of Porto; Magnetic Resonance Imaging Department, Centro Hospitalar São João, EPE; Morphological Sciences Department, School of Allied Health Sciences, Polytechnic Institute of Porto, Portugal.*
2 *Faculty of Medicine, University of Porto.*
3 *IFIMUP and IN-Institute of Nanoscience and Nanotechnology, Department of Physics and Astronomy, Faculty of Sciences, University of Porto.*
4 *Department of Physics and Astronomy, Faculty of Sciences, University of Porto.*

Presenting Author: Email: vitorsoft@gmail.com | Tel.: +351 917 401 305

INTRODUCTION:
Nowadays, magnetic resonance imaging (MRI) is widely accepted in Medicine. However, the use of this imaging technique opens new questions regarding the hazards and new safety standards are discussed. There are potential risks in MRI environments, not only for patients and workers, but also for patients' family members, other healthcare professionals and others who find themselves only occasionally or rarely in MRI environments, like housekeeping personnel, policemen. The increase of MRI exams and the advances of this technique demand the definition of safety zones and standards.

OBJECTIVES:
In this contribution we shall present a general overview of the occupational safety zones and standards of MRI environments.

MATERIALS AND METHODS:
A brief literature review on relevant databases will be presented, using some key-words, such as occupational safety zones, MRI standards, hazards, risks and effects.

RESULTS AND DISCUSSION:
There are potential hazards and risks associated to MRI clinical environments, not only for patients and MRI workers, but also to accompanying family members, attending health care professionals, security and housekeeping personnel, etc.

All clinical and research MRI sites must maintain MRI safety policies and procedures, which have to be established and maintained and routinely reviewed and updated. All of procedures should be in place to ensure that any adverse events and incidents are reported.

MRI use three types of magnetic fields to create images: a) a strong static magnetic field (SMF); b) tome-varying gradient field (TVGF); c) radiofrequency (RF) fields. All of these types of fields bring different kinds of hazards and effects (Coskun, 2010; McRobbie, 2012). There are been reports in medical literature detailing MRI incidents involving patients, equipment and personnel (Kanal, 2013).

Due to the powerful SMF and TVGF used in MRI equipments, MRI departments establish four conceptual zones around MRI scanner (figure 1). These safety zones were introduced by the American College of Radiology (ACR) (Kanal, 2013). Since the static magnetic field extends in three dimensions, some zones may extend into other areas or floors of hospital facilities. The four safety zones have personnel restrictions:

a. Zone 1: includes areas that are freely accessible to general public and typically outside MRI environment. This zone is not controlled by MRI personnel;

b. Zone 2: it is the interface between publicly accessible (zone 1) and the strictly controlled zones 3 and 4. Patients and other personnel are screened in this zone;
c. Zone 3: all access to this zone have to be strictly restricted, with access to regions within it (zone 4) controlled by, and entirely under the supervision of MRI personnel (typically the MRI technologists);
d. Zone 4: this area is where MRI equipment is installed and located. Zone 4 should be clearly marked with warning panels.



Figure 1 - MRI safety zones.

All of these zones have associated hazards, as described on table 1.

Table 1 - Four MRI safety zones

ACR Zones	Occupants	Hazards
Zone 1	General public	Negligible MRI hazards
Zone 2	Unscreened MRI patients	Immediately outside area(s) of hazard
Zone 3	Screened MRI patients / personnel	Potential biostimulation interference, access to magnet room
Zone 4	Screened MRI patients under constant direct supervision of trained MRI personnel	Biostimulation interference, radiofrequency (RF) heating, missile effect, cryogens

Formation, training and knowledge on MRI safety standards are vital for better practices. By definition, there are non-MRI personnel and MRI personnel. Patients and their family members, for example, are non-MRI personnel and they have to pass to a MRI safety screening process to enter in zone 3. This screening is made by MRI personnel (Kanal, 2013). MRI personnel are divided into two levels: I) Level 1 MR personnel are those who have to have basic understanding of MRI safety, such as the magnet is always on, basic understanding of access restriction. These include housekeeping, fire fighters, non MRI physicians and technologists; II) Level 2 MR personnel need to have comprehensive understanding of MRI safety standards and magnetic field interaction with implants and foreign bodies. They are ultimately responsible for the screening process and have authority to independently enter in zone 4 and safely permit other to do so. They include MRI technologists, MRI radiologists and nurses (Kanal, 2013).

With the increasing advances and use of higher static magnetic fields, users need to recognize that one should never assume MRI compatibility or safety information about a device if it is not clearly documented in writing. Nowadays, the majority of medical implants and devices are MRI compatible, but all patients and non-MRI personnel with a history of surgeries must undergo further investigation prior to being permitted entrance in zones 3 and 4. A patient with a history of a ferromagnetic foreign object penetration must be investigate too. X-ray films are acceptable methods of screening.

Nonresponsive and unconscious patients need to be well screened. Family members can provide medical history of those patients. Otherwise, the prescribing physician's exam must take responsibility of patients' screening under supervision by level 2 MRI personnel. For example, all areas of scars and deformities that might be anatomically indicative of medical implants and devices should be subject of further investigation before zone 3 and 4 entrance (Kanal, 2013).

MRI departments have their own compatible materials, such as wheelchairs, stretchers, ventilators. Non compatible medical materials should not enter in zone 4.

As stated above, some zones may extend to other areas and floors. Zone 4 is an example of that because is where MRI equipment is located. MRI scanner has a SMF with a certain field strength that produces and propagates a 5 - Gauss line which is the dispersion magnetic field (Karpowicz, 2006). This line is very important to take into account because all implanted cardiac pacemakers, auto defibrillators, diaphragmatic pacemakers or other electrical and mechanical devices can interact with SMF, causing malfunction and damages to medical devices and consequently to patients.

Housekeeping personnel have to be screened before working at MRI departments. Personnel with implanted medical devices should avoid MRI environments. These personnel need to have basic understanding on MRI safety procedures, such as the compatibility of cleaning supplies. This staff does not possess the required training to safely enter zone 4 unattended.

CONCLUSION:

A well knowledge of safety zones, standards and practices in MRI environments can avoid many adverse events, incidents and accidents. There are risks and hazards not only for patients and MRI personnel, but also for non-MRI personnel. All patients and non-authorized personnel that require entering into MRI zones must be screened before do so. Non technology is absolutely safe, bringing new paradigms with regard to safety aspects and a better knowledge is synonymous of good practices.

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Characterizing illuminance levels and perceived work environment in an automotive industry

Authors: Carlos Carvalhais[1,2], Betty Abreu[3], Joana Santos[1,2], Manuela Vieira da Silva[1,2]

1 *Environmental Health Department, School of Allied Health Sciences of Polytechnic Institute of Porto (ESTSP-IPP), Portugal*
2 *Research Centre on Environment and Health (CISA), School of Allied Health Sciences of Polytechnic Institute of Porto (ESTSP-IPP), Portugal*
3 *Health and Safety Department, FicoCables, Lda, Maia, Portugal*

Presenting Author: Email: caa@estsp.ipp.pt

INTRODUCTION:

Automotive Industry in Portugal, is these days a dynamic and competitive industry. For components production such as cables, comfort systems or others is require observation, human touch and must be done manually using man power capability (Ismail et al, 2010). Thus the quality of the product heavily depends on worker's comfort in the working environment. Environmental factors are considered to have influence for acceptability and performance on the occupant's premises (Ismail et al, 2010). One of them, such lighting, is determined by the satisfaction of three basic human needs: visual comfort, where the workers have a feeling of well-being, in an indirect way also contributing to a high productivity level; visual performance, where the workers are able to perform their visual tasks, even under difficult circumstances and during longer periods and safety (EN 12464-1, 2011).

Proper lighting is a prerequisite for obtaining a good working environment. Poor lighting may increase the risk of accidents and could also cause a variety of symptoms. The variables that determine a good working visual environment are: the even distribution of luminance, proper lighting, the absence of reflections, the right direction of light, no flicker, appropriate choice of lamp color and availability of natural light (Calleja and Pérez, 2011).

OBJECTIVES:

Considering that proper lighting could be one way to improve productivity and well-being of workers (Juslén et al, 2007), this study has the main objective to assess and characterize the illuminance levels of several workstations of an automotive industry and obtain workers perception of light conditions.

MATERIALS AND METHODS:

A descriptive study was developed to assess occupational lighting levels in an automotive industry. One hundred and forty four tasks/workplaces were evaluated, and more than seven hundred and sixty measurements (task and surrounding areas) were performed. The tasks/workplaces evaluated were assembly lines, laboratory tasks and warehouses.

In the evaluations of lighting levels, the measurements were carried out for a sufficient time for the stabilization of illuminance levels. The measurements followed the methodology suggested by the EN 12464-1:2011 and Portuguese Institute of Accreditation (IPAC) clients' information nº 3/2008. Some variables, such the day period when the measurements were carried out and weather, were taken into account, due to their possible influence on measurements results. The equipments used to determine the illuminance levels, were two light meters, brand Gossen, model MAVOLUX 5032C USB. These devices are calibrated annually by an accredited calibration laboratory, being able to measure the mentioned variables by complying with the applicable acceptance criteria. The results were compared with the lighting requirements for interiors (areas), tasks and activities given by the EN 12464-1:2011. To analyze the perception of workers in their work places, it was adapted and applied a questionnaire, in order to

characterize work conditions and comfort. This questionnaire was an adaptation of a questionnaire developed by Lofberg & Staffan (1997).

RESULTS AND DISCUSSION:

In some workplaces evaluated the type of activity implies performing demanding visual tasks with medium details and others that do not require high visual acuity. The reference values of required illuminance for these tasks, varied from 100 to 500 lux.

It was found that 49,3% of the measured values were below the normative recommended values. Concerning the illuminance of immediate surroundings, 13,9% of the workplaces had values below the recommended.
A representative sample (n=57) of workers answered the questionnaire. The majority of the subjects were females (63,4%). The results obtained through the questionnaire, shows that relating to the satisfaction level of the existing illumination, 88,0% of workers were satisfied. The perception of workers contrasts with the measured levels of illuminance, since they consider that the illuminance levels are adequate or sufficient to consider the accomplishment of their task. This was evidenced by Juslén et al (2007), in their study in a food industry that assessed the perception of workers about the lighting before and after implementation of local lighting at workstations. They concluded that the workers before the change were satisfied with the lighting levels and that after the implementation, admitted that the lighting levels were better and helpful for them to perform their tasks more effectively and efficiently.

Concerning health outcomes, 44,0% of the participants, reported that suffer from eye pathology (mostly astigmatism and myopia) and 37,0% use glasses. Only 19,1% of workers reported visual fatigue at the end of the work day. It would be expected that most participants felt visual fatigue, not only because the low illuminance levels, but also because the majority of the tasks requires looking closely at small objects increasing the possibility of eyestrain (Kroemer and Grandjean, 2005).

CONCLUSION:

Results show that lighting conditions are below recommended guidelines. According to the observations made corrective measures should be taken. In addition to the natural lighting should be provided artificial lighting adequate for the type tasks performed in this kind of industry, taking into account that lighting levels shall not exceed the recommended, due to the increasing possibility of occurrence of accidents and also excessive energy consumption.

A maintenance program of luminaries is also important to consider for maintaining proper lighting conditions.

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Exposure to fungi in Cork industry - case study

Authors: Carla Viegas[1], Mateus dos Santos[1], Márcia Meneses[1], Tiago Faria[1]

1 *Environmental Health RG - Lisbon School of Health Technology - Polytechnic Institute of Lisbon, Portugal*

Presenting Author: Email: carla.viegas@estesl.ipl.pt | Tel.: +351 218 980 430 | Fax: +351 218 980 469

INTRODUCTION:

The world production of cork is focused in southern Europe, with Portugal being the most important producer followed by Spain. Cork workers are at risk for developing diseases of the respiratory tract such as occupational asthma and suberosis, a form of pulmonary hypersensitivity due to repeated exposure to mouldy cork dust (Winck et al., 2004).

OBJECTIVES:

This study intended to characterize the fungal contamination in one Cork industry located in the outskirts of Lisbon city.

MATERIALS AND METHODS:

Air samples of 50L were collected from 4 indoor sampling sites (agglomeration, grinding, pressing, mixing and sawing) through an impaction method with a flow rate of 140 L/min onto malt extract agar (MEA) supplemented with chloramphenicol (0.05%), using the Millipore air Tester (Millipore). An outdoor sample was also collected since this was the place regarded as reference. Surfaces samples were collected by swabbing the surfaces of the same indoor sites, using a 10 by 10 cm square stencil disinfected with 70% alcohol solution between samples according to the International Standard ISO 18593 (2004). The obtained swabs were then plated onto MEA.

All the collected samples were incubated at 27ºC for 5 to 7 days. After laboratory processing and incubation of the collected samples, quantitative (colony-forming units - $CFU.m^{-3}$ and $CFU.m^{-2}$) and qualitative results were obtained with identification of the isolated fungal species. For species identification, microscopic mounts were performed using tease mount and lactophenol cotton blue mount procedures. Morphological Identification was achieved through macro and microscopic characteristics as noted by specific atlas for fungal identification.

RESULTS AND DISCUSSION:

Sawing was the sampling site with the highest fungal load in air and agglomeration and grinding had the highest fungal load in surfaces samples (Figure 1).

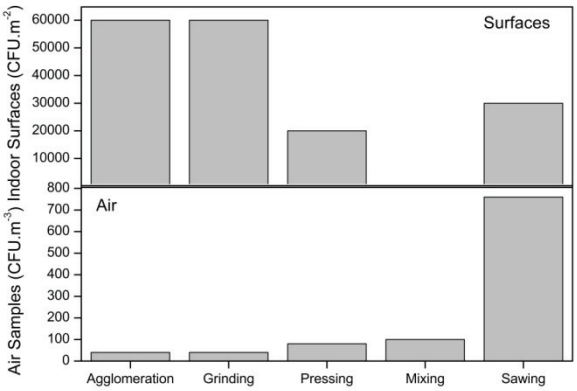


Figure 1 - Fungal load distribution in the different workplaces

All the sampling sites presented more CFU.m^{-3} than the outdoor sample, and also different species from the ones found outdoor suggesting the presence of indoor fungal contamination sources (Goyer et al., 2001).With respect to the health risks derived from exposure to fungi, nowadays, there are no guidelines set by the National Institute of Occupational Safety and Health (NIOSH) concerning the allowable load at the workplace (Vilavert et al., 2009). The World Health Organization (WHO) considers the value of 150 CFU.m^{-3} as a reason for concern, being this value surpassed only in the sawing workplace.

Six different species of filamentous fungi were identified in air samples with a total of 1020 isolates. *Penicillium* genus presented the highest prevalence (76.5%) followed by *Geotrichum* genus (11.8%). *Chrysonilia* sp., *Aureobasidium* sp., *Cladosporium* sp. and species from *Aspergillusniger* complex were also identified. Regarding surfaces samples, four different species were isolated with a total of 170000 isolates. *Trichoderma* genus presents the highest prevalence (52.9%) followed by *Penicillium* genus (29.4%). Species from *A. fumigates* complex and *Aureobasium* sp. were also isolated.

According to the American Industrial Hygiene Association (AIHA 1996) in the Field Guide for the Determination of Biological Contaminants in Environmental Samples, the identification of species from *A. Fumigates* complex, although identified only in surfaces, requires implementation of corrective measures, since fungal species aerosolization is dependent of several environmental variables (Goyer et al., 2001).

Penicillium genus was the most prevalent in air samples and the second most found in surfaces samples, corroborating results from previous studies in this kind of industry (Basílio et al., 2006). Considering conventional methods limitations(Viegas et al., 2014) and since the species *P. glabrum* is one of the most common found in cork industry and also the etiologic agent of suberosis disease (Winck et al., 2004) it is essential to apply molecular biology methodologies to characterize the real scenario regarding fungal contamination.

CONCLUSION:

The results obtained highlight the need to apply molecular methods to assess occupational fungal exposure in this setting. Data obtained from fungal contamination could represent an occupational problem and it is recommended the application of preventive and protective measures to avoid or minimize workers's exposure.

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Characterization of OSH hazards in the Portuguese furniture sector

Authors: Matilde A. Rodrigues[1,2], Pedro Arezes[2], Celina P. Leão[3]

1 *Research Centre on Environment and Health, Allied Health Sciences School of Polytechnic Institute of Porto, Portugal*
2 *CGIT, Department of Production and Systems, Engineering School, University of Minho, Guimarães, Portugal*
3 *R&D Centro Algoritmi, Engineering School, University of Minho, Guimarães, Portugal*

Presenting Author: Email: mar@estsp.ipp.pt | Tel.: +351 918 043 393 | Fax: +351 222 061 001

INTRODUCTION:
The furniture sector is one of the most important economic sectors in the north of Portugal, comprising 5 798 companies and employing more than 32 000 workers (Eurostat, 2012). However, this is a sector with several economical and organizational constraints. For example, in these companies most of the workforce is unqualified, the professionalization in terms of their management, marketing and trade policies is reduced and the occupational accidents remain high (EGP, n.d.; Eurostat, 2012).

The high number of accidents in this sector is the result of several factors, namely the sector's risk, stakeholders' low safety concerns, lack of machine maintenance and the lower qualifications of workers in the sector. Furthermore, the current economical situation in Portugal can also contribute to the small decrease of the number of accidents in this sector, which may be associated to the lack of investment on Occupational, Safety & Health (OSH) domain (Loureiro *et al.*, 2014).

Considering this scenario, it is important to know and characterize the hazards in this sector in order to identify the key risks of the sector, helping the competent authorities and the companies in the definition of a strategy of intervention in this crisis period.

OBJECTIVES:
This work aims to characterize the OSH hazards in the furniture sector, identifying the key risks for workers, as well as, some possible risk reduction measures.

MATERIALS AND METHODS:
A safety audit was performed to 14 furniture companies. The analysed companies varied in size, from micro- (less than 10 employees) to small- (from 10 to 50 employees), and medium- (from 50 to 250 employees) sized companies as well. To support this process, a checklist was formulated and applied based on safety legislation and guidelines. It included a set of 112 items related to workplace conditions involving health and safety issues, tasks, equipment and machinery. The items were selected according to the major risk factors in the furniture sector. These items were evaluated based on a 5-point Likert scale, in which 1=very deficient and 5=excellent, to characterize the level of deficiency of each feature under analysis. At the end of the safety conditions analysis, all results were discussed with the companies' management and supervisors and the risks related to each identified hazard were characterized.

RESULTS AND DISCUSSION:
The obtained results showed that in furniture industries the main risks are related to contact with saws, drill and milling cutter. Furthermore, noise and particles exposure, as well as, the risk of musculoskeletal disorders also appear as prevalent risks in the studied companies. These risks were related to: machines without protection or protection raised; non-use of the driving-bar for cutting small pieces; pieces blocked in machines removed with the hands; inadequate use/stowage of manual tools; non-use or inexistence of sound proofing protections and a large number of tasks with manual handling of loads.

One of the more critical situations was the saw used for panel dimension. Only in two companies the protection was used. However, even in these situations it was not always used and in some cases it was adjusted to the upper limit, and not carrying out the supposed protection function. Furthermore, only these two companies had a plan of the saws' maintenance and have adjusted the same in accordance to the wood height. Also, only in two companies workers always use the driving-bar for cutting small pieces. Taking this into account and considering the continuous exposure of the operator, the probability of a cut in the hand when operating these machines is high. In the other machines, as manual crosscuts and guillotines, the protections were used in some cases.

Another important risk in this sector is the projection of some components of the machine. Without a suitable protection and maintenance, saws, milling cutter, drills and particles, can be projected and cause severe damages in workers (Miguel, 2005).

The continuous high noise levels exposure verified in some workplaces, as well as, the constant manual materials handling, particularly in tasks of wood pieces and furniture transportation and in support of the pieces when supply the machinery were also identified as important risk factors in this sector.

CONCLUSION:

This study highlighted that in the furniture sector the risks are diverse; however, most of them can be prevented through correct work procedures and training. Some of the actual level of deficiency can be related to the current pressure that companies put on workers, denoting a lower interest in safety comparing with the production. In fact, it was observed that the tasks are being performed with a minimum of workers number (e.g. furniture handling) and the workers are susceptible to unsafely behaviors, where most of them can be related to an attempt to increase production. Therefore, the intervention in this sector needs to be performed not only at workers' level, but also at the management level, in order to avoid unsafe behaviors and promote safe procedures with a better and more efficient resources' management.

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Postural dysfunctions and musculoskeletal disorders in Portuguese spelunkers

Authors: Luísa Pedro

(PhD) - ESTeSL-IPL

INTRODUCTION:
The Speleological develops a set of actions that require the preparation of a good physical condition, such as: walking on a varied terrain, swimming, vertical techniques, acquire positions of muscle imbalance, etc.

Thus, prevention of musculoskeletal injuries, practitioners of these is a priority. The scientific evidence (Stenner, et al 2006; Townes, 2005) demonstrates that the most common injuries are: sprains, ligament injuries in tibial-tarsal and knees, meniscal injuries due to movements patellofemoral overload, spine injuries due to prolonged flexion and polytrauma due to walk inside caves with little light that can lead to serious accidents, such as falls from great heights, although these situations are more related to practitioners who venture without the guides (Hooker & Shalit, 2000) .

OBJECTIVES:
The objective of this study is to assess musculoskeletal disorders and postural dysfunctions in Portuguese spelunkers.

MATERIALS AND METHODS:
This study is exploratory and transversal. In this study to make a descriptive analysis of changes musculoskeletal disorders in Portuguese spelunkers.

This study involved 15 Portuguese spelunkers, between 18 and 56 years in M = 36.6, 66% were men, 73% are single or divorced, exercised caving as a hobby.

Using a questionnaire to characterize the historical injuries or musculoskeletal pain, and the postural assessment, developed by these authors (Lippert, 2013).

We used nonparametric statistics to assess data. We was used descriptive analysis

RESULTS AND DISCUSSION:
The results show that 46% of people referred lumbar or thoracic pain, indicating that long bending movements of the spine, increased pain symptoms. No elements of the sample reported performing physical therapy at this time. 20% report taking medication for pain relief. 46% report that the home improves symptoms 73% of the sample elements refer to perform walking, over 30 minutes daily. 40% in sport. Postural assessment no significant change of posture were recorded, however about 40% showed an asymmetry of the shoulders. 46% have changes of curvature of the lumbar and thoracic spine, predominantly correction of the thoracic spine and lordosis of the lumbar spine.

CONCLUSION:
The results indicate that most of the elements of this sample did not show significant musculoskeletal injuries, however, and this was indicated in the literature, the changes of the spine are the most evident symptoms suggestive for training postural correction which should be professional by an expert area.

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Is the crisis affecting the companies' commitment on the OSH issues? (The workers' perceptions)

Authors: Cláudia Vale[1], Isabel F. Loureiro[2], Matilde A. Rodrigues[1], Rui Azevedo[3]

1 *Research Centre on Environment and Health, Allied Health Sciences School of Polytechnic of Porto, Portugal*
2 *Research Centre for Industrial and Technology Management, School of Engineering of the University of Minho, Guimarães, Portugal*
3 *Centro de Apoio Técnico à Segurança no Trabalho CATST, ISMAI, Portugal*

Presenting Author: Email: vale.claudia@hotmail.com | Tel.: +351 911 784 462

INTRODUCTION:

Portugal is undergoing an economic crisis affecting the European Union since 2007. The financial unsustainability of the country required foreign aid and austerity measures have been implemented. In consequence, it is expected more financial difficulties to the companies at different levels, particularly in issues related with safety. In this context, Leahy *et al*. (2012) pointed out that, due to the crises, companies have to adjust their priorities regarding the investments namely, on the Occupational Safety & Health (OSH) issues.

Even though OSH is now one of the most important and most highly developed aspects of EU's policy on employment and social affairs, Kompier (2006) e Papadopoulos *et al*. (2010) confirmed that in the past few years, the work environment have suffered significantly changes. The most important ones are the workload, the schedule and worker flexibility, the extension of working hours for the weekend, the inconsistent schedules, the precarious work (Kompier 2006; Papadopoulos *et al*., 2010). These changes are responsible for some effects on the workers' wellbeing, take as example the exposure to the psychosocial risks and stress at work.

Nowadays organizations need to be increasingly more competitive, in part due to the financial crises we are facing, but also due to the emergence of new competitors. In Portugal the most affected dimension was the Small and Medium enterprises (SME). This factor has a negative impact in the economic competitiveness as this type of companies is responsible for the employment of two millions people.

It is authors' believes that the effects of an economic crisis on safety and health are a challenging topic nevertheless, studies in this area, can stress new strategies to then OSH issues.

OBJECTIVES:

The aim of this study was to analyze the employment population feelings about the influence of the crisis on the companies' commitment on the OSH issues.

MATERIALS AND METHODS:

In order to perform this study, an observation tool was developed and data collection was carefully defined. The study adopted a quantitative methodology using a self-completed questionnaire named External Environment Questionnaire (EEQ). The EEQ included several questions to characterize the respondents profile and study their opinion about the current situation of Portugal and how does it affect their perception about security issues (Loureiro *et al.,* 2014).

The EEQ was divided into two different parts: (1) characterization of the respondent and (2) perception of the respondent regarding the engagement of the organization for which they work in relation to security issues. Briefly, the first part refers to the respondent characterization and gathers information such as age, gender, level of education and characterization of household. In part (2) of the EEQ gathers information about: the

perception of respondents regarding the current financial situation of the organization, the organization' awareness to security issues and the recovery workers on safety at work.

The target population for this survey was the working population. This survey was conducted in 8 cities of the north of Portugal. The questionnaires were sent by mail and / or email through a list of contacts or by hand from June 14 to July 11, 2013.

After data gathering a descriptive analysis was performed on all questions and depending on the relevance, correlations between variables were tested.

RESULTS AND DISCUSSION:

This study surveyed 600 people of which 510 were considered to be valid questionnaires. Nearly half of the respondents were male. On average, respondents have nearly 39 years old (SD=11.40; interval range 17-76 years old). The respondents aged above 65 years old have a par-time job. It is important to notice that this age is considered to be the retirement age in Portugal. The majority of the respondents are from Portugal (98.6%).Most of them received primary education (37.7%), 32.7% received senior high school education, 28.6% went to college and 1.0% received an unspecified type of qualification. The respondents developed their professional activity mainly in the industry sector and services.

Almost 80% of the respondents agree that the companies that they work for are being affected by the economic crisis. Even though the crisis, 60% of the respondents reported that their companies are still investing in the training actions regarding the OSH issues and 51.6% remarked that companies value those who comply with the safety rules. This seems to indicate that there is a will on investing in this area. A vast majority of the respondents (80% of the respondents, on average) agrees that the crisis is responsible for the non-investment in protection equipment, increasing of the occupational psychosocial risks at work, excessive workload, lack of involvement, conflicting demands, mismatch between the workloads demands and production demands. In general, respondents feel that the crisis is responsible for the worst working conditions.

CONCLUSION:

As pointed out by Kousenidis *et al*. (2013), companies are facing economic constraints due to the crisis that is affecting all the Europe. Results of this survey indicate that workers are feeling job insecurity in addition to the non-investment in the OSH issues and these factors may contribute to profound changes in the working conditions. Indeed, competitiveness of the trade market is leading to the fact that companies are prioritizing the production in relation to compliance with the safety rules. The respondents' perceptions about the companies' commitment on the OSH issues is in line with the studies by Papadopoulos *et al*. (2010) that highlighted the increase of the worst working conditions in the past years till the current days.

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Occupational Exposure to Particulate Matterin Grill Houses

Authors: Susana Viegas[1], Patrícia Neves[2]

1 *Environment and Health RG. Lisbon School of Health Technology- Polytechnic Institute of Lisbon; Center for Malaria & Tropical Diseases (CMDT) - Public Health and Policy, Escola Nacional de Saúde Pública, Universidade Nova de Lisboa, Portugal.*

2 *Lisbon School of Health Technology - Polytechnic Institute of Lisbon, Portugal*

Presenting Author: Email: patriciamvneves@gmail.com

INTRODUCTION:

The different processes that can be applied in cooking such as frying, roasting, grilling, boiling and broiling contribute to pollutant emissions and can be affected by different factors, namely ingredients, recipes and procedures, fuel types, temperature and, also, extraction/ventilation equipment available (Abdullahi et al., 2013). All these processes are responsible for generating large quantities of particulate matter, polycyclic aromatic hydrocarbons, volatile organic compound sand carbonyls. Most fire-based cooking is based on the combustion of fuel (charcoal)and is the main cause of air contamination (Taner et al., 2013) due to the fact that the grillroom restaurants used extensively charcoal for barbecuing. Moreover, recent studies of indoor air have identified cooking as one of the most significant particle generating activities indoors (Buonanno et al., 2009; Massey et al., 2012).Therefore, restaurant employees can be exposed to respirable suspended particles that results from grill activities (Taner et al., 2013).

The adverse health effects related with the combustion of biomass fuels like charcoal show us an increased risk of respiratory tract infections, exacerbations of inflammatory lung conditions, cardiac events, stroke, eye disease, tuberculosis and cancer (Fullerton et al., 2008).

OBJECTIVES:

Characterize the particulate matter (PM) emissions produced during grilling and recognize the factors that have impact on the emissions.

MATERIALS AND METHODS:

The study was developed in one take-away grill house located in Lisbon. Environmental monitoring was developed in a single day of April 2014.Measurements were performed using a portable direct-reading hand-held equipment (Lighthouse, model 3016 IAQ). This equipment gives detail information about mass concentration in 5 different sizes ($PM_{0.5}$; PM_1; $PM_{2.5}$; PM_5; PM_{10}). Additionally, data related with particle number concentration by each diameter size is also available. In this case, particles generated were given six different diameters sizes, corresponding: 0.3 µm, 0.5 µm, 1 µm, 2.5 µm, 5 µm and 10µm.The measurements were conducted near the workers respiratory apparatus during tasks performance.5 minutes measurements were considered and also collected data during each task developed.

RESULTS AND DISCUSSION:

Statistically significant differences were detected (Kruskal-Wallis test) between the different sampling locations for both metrics used, With respect to mass distribution by size, $PM_{2.5}$, PM_5 and PM_{10} presented higher values. Regarding particle number concentration results, data showed that 0.3 µm obtained higher counts in all the measurements (more statistical data will be presented later). Additionally, it was possible to observe that the values were higher when the worker was near the grill changing the meat and when he added more meat on the grill. This last situation was particularly relevant when he added meat with higher amounts of fat (pork meat for instance: 2 fold higher). The same tendency in the results was obtained in a previous study developed by Buonanno and colleagues (2009): foods containing a higher percentage of fat generating higher emission rates.

Previously Nygaard and colleagues (2004) reported the need to monitor the size distribution and particle number concentration, in addition to mass, particularly when trying to predict the biological effects of PM (Nygaard et al., 2004). Our results showed higher counts of the smaller particles (0.3 µm) and this can mean that is possible those particles reach deeper in the respiratory system and, besides local effects, also systemic effects can be expected. However, these systemic effects are also dependent of the PM chemical characterization.

A recent study (Taner et al., 2013) has showed that charcoal combustion is an important source of various toxic element emissions, such as metals, that can be carried to the respiratory system by the PM emitted.

CONCLUSION:

Additional studies should be developed aiming on characterizing in more detail the emissions and also the morphological and chemical characterization of the PM emitted. This data will allow a better understanding of the possible negative effects to the workers' health and define the most suitable preventive measures.

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Hematologic changes and DNA Repair System in gas station attendant in the ABC region, São Paulo - Brazil.

Authors: Roseli Corazzini[1], Thaís Moura Gascon[2], Diego Martinez Garcia[1], Marcio Augusto Zanon Martins[1], Jéssica Feliciana Codeceira[1], Fernando Luiz Affonso Fonseca[2], Cristina Valletta de Carvalho[1]

1 *Biology, Centro Universitário Fundação Santo André, Santo André, Brazil*

2 *Hematology and Oncology, Faculdade de Medicina do ABC, Santo André, Brazil*

Presenting Author: Email: roselicorazzini@gmail.com | Tel.: +55 11 991 897 077

INTRODUCTION:

We are constantly exposed to different chemicals that are present in food, medicine, herbicides, fuels, pesticides and even in the air. Many of these substances may have toxic effects on the body, especially in DNA, such as cellular changes that, if not repaired, can result in gene mutations, chromosome aberrations, apoptosis regulation in uncontrolled cell growth and even the development of diseases (Kauffer & Vincent, 2006). Among the chemicals that cause toxic effects to human body, we can include petroleum products such as gasoline, which is in its chemical composition benzene. Due to the existence of several types existence of several types of DNA lesions, there is a repair system, which allows for enzymatic mechanisms reverse the effects caused, they are natural or not. This system allows the integrity of the genome, a fundamental role in preventing several diseases that could arise and especially in cancer prevention. The XRCC1 protein is a nuclear platform, despite having enzymatic action, interact with key enzymes involved in the repair of single strand breaks as part of the DNA-binding protein complex. Consecutive stages coordinates and interacts with several enzymes, staying until the last stage of binding, regulating and managing the whole process of base excision repair (Horton et al., 2008). Genes need to be considered polymorphic alleles present in their stable, changes in protein activity relative to wild-type protein. One example is the gene polymorphism of the XRCC1 gene polymorphism at codon 194 has (Arg /Trp) to 399 (Arg /Gln) generating a protein with greater efficiency and less repair, respectively, when compared with the wild alleles (Wang et al. 2003).

OBJECTIVES:

Evaluate hematological alterations and the DNA repair system in workers exposed to gasoline (attendants).

MATERIALS AND METHODS:

The clinical demographic questionnaire was administered to gas station attendants. After finding exposure to gasoline, about 20.0 mL of peripheral blood was obtained by venipuncture. Hematologic parameters were Evaluated by cytometry flow and then the DNA was extracted for determination of polymorphisms of the gene repair: XRCC1 (Arg194Trp and Arg399Gln).

RESULTS AND DISCUSSION:

73 workers were evaluated in haematological parameters and showed 64,38% presented by two changes, 8,21% (3 changes) and 27,39% (4 a 11 changes**)**. Of these, 41 were evaluated for genetic polymorphism of two genes and no changes in homozygote genotype with the genetic variant were found. (Tables 1, 2).

Our preliminary studies (n=33) demonstrated the analysis of an index of severity of changes which were segmented into 3 distinct levels: low severity (0-2 haematological disorders); average severity (3 haematological disorders) and high severity (4-5 changes). This index was formed as an explanatory variable with the exposure time of the attendants (number of months worked in gas stations) of the dependent variable called number Hematologic Changes. The linear regression analysis resulted significant at 0.001 with haematological disorders (R2 = 0.904) and suggestive of a deleterious effect of inhalation of volatile organic compounds in fuel handled by attendants. Similar analysis is being processed in this study.

Table 1 - Frequency of allelic variants of the gene Arg399Gln polymorphism XRRC1 the attendants and control groups.

Genótipos XRCC1				
Study groups	**Arg/Arg N(%)**	**Arg/Gln N(%)**	**Gln/Gln N(%)**	**Total**
Gas station attendants	2 (4,9)	13 (31,8)	26 (63,4)	41
Control	21 (17,8)	62 (52,5)	35 (29,7)	118
TOTAL	24	75	61	159

The results obtained for the attendants group, we can see that there were only 2 subjects genotyping without the genetic variant that leads us to suggest that this group really has a protection in DNA repair. The same pattern is presented to the heterozygous genotype between the two groups, supporting the work previously seen in literature. When we pay attention to and homozygous genotype with the genetic variant we observed a large predominance among the attendants, a result that is consonant with the now published as Wang et al. (2003). According to Wang et al. (2003), 399 polymorphism (Arg/Gln) has lower efficiency of repair compared with wild alleles without the variant.

Tabela 2 - Frequency of allelic variants of the gene Arg194Trp polymorphism XRRC1 the attendants and control groups.

Genótipos XRCC1				
Study groups	**Arg/Arg N(%)**	**Arg/Trp N(%)**	**Trp/Trp N(%)**	**Total**
Gas station attendants	26 (63,4)	15 (36,6)	0 (0)	41
Control	88 (73,9)	19 (16)	12 (10,1)	119
TOTAL	114	34	12	160

By analyzing Table 2, we first verify that for the homozygous without the genetic variant there is a higher frequency of appearance in the control group compared with the attendants of posts, which leads us to believe that we have a relationship of protection for individuals lacking the gene variation.

CONCLUSION:

Exposure to gasoline may alter hematological parameters however this exposure can be time dependent as the genes studied to check the DNA repair system. After the analysis conducted for the genetic variation of the repair gene XRCC1 (Arg194Trp) we can conclude that for the homozygous without genetic variation the control group shows a higher frequency (63.4%) showing a possible protection of the absence of polymorphism. Now to XRRC1 repair gene (Arg399Gln) with homozygous genetic variant we observed a large predominance among attendants (63.4%) indicating 399 polymorphism (Arg/Gln) has lower efficiency of repair compared with wild alleles without the variant. This study revealed that 63% of workers exposed to pollutants in fuels, showed the homozygous genotype with genetic variation. Thus, we can check genetic changes more frequently among attendants when compared to control. There is a need to correlate the changes in the repair system with those reported in blood cells. Thus, we suggest increasing the sample size of workers of the gas stations and conducting logistic regression in order to clarify the involvement of the repair system with haematological disorders interfering with the health of workers exposed to fuel at gas stations.

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Evaluating the necessity of harmonization of occupational risk assessment methodological approaches in the Russian Federation and European countries

Authors: Nina Zaitseva[1], Pavel Shur[1], Anna Khasanova[1,2], Dmitriy Shlyapnikov[1]

1 *Federal Budget Scientific Institution "Federal Scientific Center for Medical and Preventive Health Risk Management Technologies", Russian Federation, Perm*

2 *Perm State National Research University, Russian Federation, Perm*

Presenting Author: Email: sharaeva@fcrisk.ru | Tel.: +79 129 807 560

INTRODUCTION:

In Russia and the European Union the general scheme of occupational risk assessment including hazard identification (work types and workplace hazards), exposure assessment (measurement or calculation of exposure level), and risk characterization is used [1]. However there are differences in implementation of these steps, and that is why Russian and European methodologies have their own strengths and weaknesses. The advantage of the method used in the Russian Federation is that it allows to identify cause-effect relationships between health effects and work activities and its results give a possibility to estimate health risk level and to compare it with the standard [2, 3]. However its implementation requires laboratory data as well as instrumental measurements of occupational exposure factors. Experts identify exposure factors then quantitive assessment of exposure is performed and cause-effect relationships are determined. All that makes Russian methodology very laborious and expensive [3].

The advantage of the European methodologies is that the majority of them are easy-to-use, do not require deep knowledge and measurements, so assessment procedure is quick and cost-efficient. In many countries special risk matrices are developed and on hazard identification stage health risk assessors use questionnaires that provides risk information distribution among workers and allow to involve them in risk management process [1]. Also, in these questionnaires such risks as, for example, a wide range of traumatic factors which vary depending on the specific working conditions are presented, but these factors are not considered in Russia. Quantitive assessment of exposure in case of risk matrices usage is not necessary. Risk level is determined by combinig the probability that event should occur and the severity of possible health impact. According to risk assessment results, the urgency of preventive measures is determined [4]. At the same time, these methods do not require special equipment and skilled professionals, but the final result of the assessment is highly dependent on expert opinion, who performs hazard identification and risk ranking [1]. Being guided by international experience in occupational risk assessment it is necessary to harmonize methodological approaches to occupational risk assessment in Russia and European countries.

OBJECTIVES:

Approbation of methodological approaches used in European countries on the Russian enterprise, in order to assess feasibility of harmonization of methodological approaches to occupational risks assessment in Russia and European countries.

MATERIALS AND METHODS:

For carrying out a research a chemical and metallurgical enterprise located in Perm region was selected. Occupational risk assessment was performed for workers of production (chemical and metallurgical and melt shops) and administrative-and-managerial personnel. For hazard identification specially developed questionnaires were used, on the basis of the obtained data risk levels were determined. Obtained risk levels were compared with workplace assessment data. Questionnaires were based on guide "Risk Assessment at workplace" used in Finland and consisted of 36 questions. Questions referred to chemical factors, noise and vibration, non-ionizing radiation, microclimate, lighting, injury prevention, emotional and physical demands.

To estimate risk level the following answers were used - if you know that a hazard exists, select "YES", if you know that there is no danger, select "NO" [4]. Also, questionnaires contained an open-ended question in which the employee could specify an occupational factor, which in his or her opinion was harmful. Risk was determined as a combination of event occurrence probability and severity of possible health impact according to tables of risk level determination for certain groups of factors. Risk levels varied from a minimum value of 1 (non significant risk) to a maximum value of 5 (unacceptable risk) [4]. In order to provide comparability of the risk assessment results of Russian and European methods, questions were grouped according to type of factors which were assessed in workplace assessment. Risk level was assessed on each question and the total risk level for a group of questions combined into one category of factors was estimated by the maximum value. An overall score for workplace conditions was a result of risk level estimation.

RESULTS AND DISCUSSION:

According to workplaces assessment data, the workplace conditions of chemical and metallurgical manufactory were defined as harmful, hazard degree - 3 (working conditions class 3.3) and 4 (working conditions class 3.4) and characterized by the combined exposure to chemical factors, noise, general vibration, increased level of microclimate indicators and physical demand. Labor conditions of melt shop were characterized by the combined exposure to dust, noise, general vibration, increased levels of thermal load and the physical demand; working conditions class was evaluated as 3.4 - harmful. When comparing the data obtained from questionnaires and the workplace assessment with respect to working conditions it was found that in both cases, chemical factor was limiting hazard factor, health risk levels due to noise, microclimate and physical demand were high too. However differences in assessment results were also observed. According to questioning results, the majority of workers did not identify lighting as a hazard factor, although the results of workplace assessment showed that it belonged to harmful factors. The reason of such a difference was probably the fact that the hazard class for lighting was estimated due to light pulsations that workers did not notice. In addition, there were differences in the injury risk class determination that might be related to usage of workplace regulations to determine injury risk class in workplace assessment while in questionnaires a wide spectrum of real injury risk factors (electrical installation, moving parts of equipment, slippery surfaces, etc) was comprised. Based on the questionnaires analysis, additional risk factor - overcrowding of workers - was introduced. This risk factor could not be estimated in workplace assessment, however, according to questioning results it was identified as unfavorable by workers of certain types of work.

CONCLUSION:

According to study results, general principles of occupational risk assessment allow using basic elements of hazard identification and risk characterization for formation of harmonized approaches to prior qualitative occupational risk assessment. Approbation of these approaches shows that the use of questionnaires to identify hazards together with the Russian method of risk assessment allows to identify additional occupational risk factors, and the use of instrumental measurements together with the European approaches to risk assessment gives an opportunity to expand risk factors list as well as to go to the quantitative occupational risk assessment. Thus harmonization of occupational risk assessment methodologies is expedient, because it will allow to assess occupational risks comprehensively.

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Biomonitorization in hospital settings with cytostatics occupational exposure

Authors: Carina Ladeira[1,2], Susana Viegas[1,2], Elisabete Carolino[1], Manuel C. Gomes[3], Miguel Brito[1]

1 *Escola Superior de Tecnologia da Saúde de Lisboa, Av. D. João II, lote 4.01.69, 1990-096 Lisboa, Portugal*
2 *CMDT - Public Health and Policy, Escola Nacional de Saúde Pública, ENSP, Universidade Nova de Lisboa,1600-560 Lisboa, Portugal*
3 *Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisboa, Portugal*

Presenting Author: Email: carina.ladeira@estesl.ipl.pt | Tel.: +351 218 980 445 | Fax: +351 218 980 460

INTRODUCTION:
Exposure in a hospital setting is normally due to the use of several antineoplastic drugs simultaneously. Nevertheless, the effects of such mixtures at the cell level and on human health in general are unpredictable and unique due to differences in practice of hospital oncology departments, in the number of patients, protection devices available, and the experience and safety procedures of medical staff. Health care workers who prepare or administer hazardous drugs or who work in areas where these drugs are used may be exposed to these agents in the air, on work surfaces, contaminated clothing, medical equipment, patient excreta, and other surfaces. These workers include specially pharmacists, pharmacy technicians, and nursing personnel. Exposures may occur through inhalation resulting from aerosolization of powder or liquid during reconstitution and spillage taking place while preparing or administering to patients, through Cytokinesis-block micronucleus test (CBMN) is extensively used in biomonitoring, since it determines several biomarkers of genotoxicity, such as micronuclei (MN), which are biomarkers of chromosomes breakage or loss, nucleoplasmic bridges (NPB), common biomarkers of chromosome rearrangement, poor repair and/or telomeres fusion, and nuclear buds (NBUD), biomarkers of elimination of amplified DNA.

OBJECTIVES:
The aim of this study is to compare the frequency of genotoxicity biomarkers, provided by CBMN assay in peripheral lymphocytes between pharmacists, pharmacy technicians, and nurses occupationally exposed to cytostatic drugs with a control group; understanding if there are some differences by job activity.

MATERIALS AND METHODS:
The group of cases was constituted by 46 workers exposed occupationally to cytostatics: 6 pharmacists, 13 pharmacy technician, and 27 nurses; and 46 unexposed individuals (controls), from whom both peripheral blood was collected in order to measure the genetic endpoints proposed in this study.

RESULTS AND DISCUSSION:
All the genotoxicity biomarkers studied presented statistical significant differences between exposed and non-exposed subjects to cytostatics (Mann-Whitney test, $p \leq 0.05$). In what concern to professional category, the distribution of the genotoxicity biomarkers - MN, NPB and NBUD, were: pharmacists (7.82±1.30; 1.09±0.39, 2.82±0.58), pharmacy technicians (10.83±2.10; 0.50±0.22, 2.17±0.70), and nurses (10.11±2.05; 0.48±0.15, 2.41±0.57), respectively. There were no significant differences in the genotoxicity biomarkers under study between the three categories of workers exposed occupationally to cytostatics (Kruskal-Wallis test, $p \geq 0.05$).

CONCLUSION:
Occupational exposure to cytostatics drugs is associated with genotoxic effects that can be evaluated by biomarkers. In this study, the results reveal that workers exposed occupationally to cytostatics showed increase frequency of genotoxicity biomarkers in comparison with non-exposed., however no differences were found in what concern to job categories, and therefore, tasks.

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Occupational hematopoietic cancer surveillance system in Korea in 2013

Authors: Jong-Eun Kim[1], Yoon-Ji Kim[2], Dong-Mug Kang[2]

1 *Department of Occupational & Environmental Medicine, Pusan National University Yangsan Hospital, Yangsan, South Korea*
2 *Department of Preventive and Occupational Medicine, School of Medicine, Pusan National University, Pusan, South Korea*

Presenting Author: Email: kim-jongeun@hanmail.net | Tel.: +82 55 360 2142 | Fax: +82 55 360 2712

INTRODUCTION:
Most occupational cancer systems rely on existing data or passive surveillance system. Hematopoietic cancer is the 3rd largest occupational cancer following lung cancer and malignant mesothelioma. National occupational hematopoietic cancer surveillance system has been operated in Korea since 2011. This is active surveillance system including intensive interview.

OBJECTIVES:
This study is conducted to prevent work-related cancer by investigating (examining) the causes of hematopoietic cancer in terms of occupational and industrial classification. Furthermore, it will be beneficial to establish the government's policy or recommendation for prevention and improvement in case of dealing with materials that could cause the occupational cancer.

MATERIALS AND METHODS:
Study subjects were 422 patients over twenty years old, and confirmed hematopoietic cancer in 18 university hospitals in Korea from May 1 to August 31, 2013.The questionnaire consists of age, gender, smoking, diagnosis, histological type, and occupational history including life-long occupations; working hours, type, process, and exposed materials related to the hematopoietic cancer. Structured interviews were conducted by trained nurses. The job-relatedness was classified as 6 categories of Definite, Probable, Possible, Suspicious, None and Undetermined in terms of the exposed duration, dose, intensity and material designated by the International Agency of Research on Cancer (IARC) classification.

RESULTS AND DISCUSSION:
Numbers of male and female were 241 (57.1%) and 181 (42.9%) respectively. All definite cases were benzene exposed (n=3, 0.7%). Number of definite and probable cases were 14 with 11 benzene exposed (2.6%), and 3 others (0.2%) which were comprised of X-ray, gamma ray, and formaldehyde exposed. Disease subgroups of definite cases were acute myeloid leukemia, multiple myeloma, and idiopathic aplastic anemia. And those of probable cases were chronic myeloid leukemia, acute myeloid leukemia. Standardised incidence ratio (SIR) of standard industrial code (SIC) was highest in the tobacco industry (44.8, 95% CI: 32.2 ˜ 57.9) followed by coal mine (24.3, 95% CI: 9.1 ˜ 21.6), non-metal mine (13.4: 95% CI: 9.3 ˜ 18.2), research and development (11.8, 95% CI: 9.3 ˜ 14.8), and electric and gas (11.1, 95% CI: 9.0 ˜ 13.6) in order. SIR of standard occupational code (SOC) was highest in science expert (54.1, 95% CI: 44.0 ˜ 65.5) followed by medical service (13.8, 95% CI: 10.6 ˜ 17.1), social service and religion (12.4, 95% CI: 9.9 ˜ 15.4), and security service (9.9, 95% CI: 7.5 ˜ 10.5) in order. In result, the most dangerous material of the occupational hematopoietic cancer was benzene in Korea. Average working period of exposing benzene was 23 years. Benzene may be exposed more often and long time and it may cause the cancer.

CONCLUSION:
There are various materials which cause the occupational hematopoietic cancer. Especially benzene may be the most dangerous factor, and it is needed for benzene related workers to be under strong surveillance system and to improve the working conditions.

Keywords: Hematopoietic cancer, Occupational, Surveillance, Korea

Characterization of commuting accidents that occurred in workers of Portuguese health institutions

Authors: Matilde Martins[1], Maria do Céu Barbieri[2], Teresa Correia[3]

1 *School of Health Sciences, Polytechnic Institute of Bragança, Research Centre for Sport Health and Human Development; Institute of Biomedical Sciences Abel Salazar University of Porto, Bragança, Portugal.*
2 *School of Nursing of Porto, Institute of Biomedical Sciences Abel Salazar University of Porto, Porto, Portugal.*
3 *School of Health Sciences, Polytechnic Institute of Bragança, Research Centre for Sport Health and Human Development, Braganca, Portugal.*

Presenting Author: Email: matildemartins@ipb.pt | Tel.: +351 962 777 141

INTRODUCTION:
According to Portuguese legislation in itinere work accident is something that occurs while traveling outside of the workplace on work travelling between their home and place of work and vice versa. (Law No. 102/2009).

Commuting accidents have been little investigated by the scientific community possibly because they are associated with multiple circumstances.

These health institutions occupy the fourth place in the scale of industrial accidents, and it has been in these that were observed the largest average growth rates in the period 1997-2007, 23.27 %. (ACSS, 2009).

OBJECTIVES:
Characterize in itinere workplace accidents in workers of Portuguese public health institutions between 2009 and 2010; Describe the epidemiological profile of the injured; Identify the main causes and consequences of accidents while traveling.

MATERIALS AND METHODS:
Retrospective cross-sectional study using the information of records, anonymously, data based in Excel of the Central Administration Health Services program, upon request and subsequent approval of the President of the institution. We have obtained a sample of 999 workers who were hurt during the route between home and the workplace, in the period from January 1st 2009 to December 2010. Data were selected, transported and analyzed by the identification number, on the computer program Statistical Package for Social Sciences version 21.0 for Windows respecting the confidentiality of the participants.

RESULTS AND DISCUSSION:
During the study period there were 999 in itinere accidents representing 8.4 % of all accidents occurred during the 2 years of the study. The year 2010 registered the highest prevalence of notifications, 58.0 % and ARS Lisbon and Tagus Valley 55.0 %. The most affected professional category was Operational Assistants Auxiliary Medical Action and Nurses 28.4% , the females 84.4%, among the age group 50-54 years 17.2%, with service time superior to 10 years 52.0%, below the level of 12th grade education 57.0%, with an employment contract in public functions 48.2 % and fixed practice hours 53,8%. Although there are no studies that make comparisons, these data are similar to those found in other studies on occupational accidents in health institutions and typical accidents. (ACSS, 2009; Martins, Silva and Correia, 2012, Secco et al, 2008). This rugged profile may be due to the fact professional group of nurses and auxiliary medical action is mostly made up of women and represent the largest contingent workers in health institutions. A higher prevalence of accidents on Monday 19.4 %, 1 hour before of onset labor 65.2%, between 07-08 am 44.7%, on day 3 after weekly rest 31.7% and on emergency service 86.6%. Most workers in health institutions start their work schedule at 8 o'clock, which justifies the high prevalence between 7 and 8

o'clock, data that doesn't agree with the found on typical accidents in other studies, where the highest prevalence was found between 9 and 12 hours. (ACSS, 2009; Ribeiro, Ribeiro, Jr., 2010; Martins, Silva and Correia, 2012).

The main cause was the fall of workers 52.8%, fact which may be due to the rush not to be late for work. The main effects were sprains / strains 27.1% and fractures 12.2%, *have reached multiple parts of the body 25.1% and led to absenteeism 70.4% with 27,463 days lost work.*

CONCLUSION:
There has high prevalence of in itinere accidents from workers' falls, with high rates of absenteeism and consequently with enormous economic, personal, familiar, social and institutional burdens. We emphasize the need for further research on this issue to deepen the knowledge about in itinere accidents that will lead to prevention interventions of these accidents, which can be traumatic to the worker.

ACKNOWLEDGMENTS:
Accidents at work; Initinere; Occupational Health; Health promotion.

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Occupational and environmental exposure to anticancer drugs

Authors: Luís Teles, Sara Fernandes; Agostinho Cruz; Manuela Silva; Manuela Amorim; Mónica Vieira; Piedade Barros

1 *CISA-Centro de Investigação em Saúde e Ambiente; ESTSP-IPP, Escola Superior de Tecnologia da Saúde do Instituto Politécnico do Porto, Portugal*

Presenting Author: Email: pgb@estsp.ipp.pt

INTRODUCTION:

The use of anticancer drugs is not restricted to a single type of drug (Connor *et al.*, 1999; Suspiro & Prista, 2012). These are varied and present diverse mechanisms of action, which may be causes of occupational exposure. Despite the restricted and controlled use of cytotoxic agents, the risk involved is not confined to individuals who administer them but also to all health care professionals and general population directly or indirectly in contact with them (Suspiro & Prista, 2012).

The presence of pharmaceuticals products in natural surface waters and in the effluent sewage treatment plants have been reported since 1980s. More recently, these products have been detected in ground and drinking water. However, there is little information about the risks these drugs and their metabolites in the water, have, for the human health (Ferrando-Climent*et al.*, 2013).There are few published studies with detailed information about the significance of pharmaceuticals compounds in water for humans (Ferrando-Climent*et al.*, 2013). The major concern with genotoxic/carcinogenic pharmaceuticals compounds is a cancer risk, at any level of exposure because there is no threshold dose below which no carcinogenic effect may occur (Garcia-AC *et al.,* 2011). The pharmaceutical compounds are excreted to wastewater and become potential water contaminants. Currently, very little is known about the presence and elimination process of cytostatic drugs in wastewater treatment plants. The lack of methods and knowledge about the specific risk of pharmaceutical products in aquatic environment may be consider a public health problem.

OBJECTIVES:

The aims of this work was to identify and characterize the most commons anticancer agents involved in occupational exposure and environmental contamination, as well as to identify the most common methods in the assessment of occupational and environmental exposure.

MATERIALS AND METHODS:

A literature review has been performed using articles published in several electronic databases, namely the National Center for Biotechnology Information (NCBI) and B-On on the subject using as key-words: Cytostatic drugs; occupational exposure; environment; cytostatic metabolites and cytostatic measurement methods in water. Articles were selected due to their relevance to their proposed aims.

RESULTS AND DISCUSSION:

Based on the literature on this subject, we can highlight some drugs as the most commonly evaluated in terms of their occupational exposurerisk and environmental contamination: cyclophosphamide, ifosfamide, cisplatin, methotrexate, 5-Fluorouracil (5-FU), gemcitabine, vincristine, vinolrrebine, etoposide, doxorubicin and epirubicin. Currently, a new set of drugshas emerged on which no studies are yet available, as far as the risk of occupational exposure and environmental contamination is concern.

Given the serious toxic effects which may result from exposure to cytotoxic agents, several authors have been addressing the risk assessment of occupational exposure of health workers to these agents. In Hospital, site of primary consumption of such drugs, there are various professional classes and patients in contact with

anticancer agents; among them, nurses stand out - tasked with administration of such drugs— and pharmacy professionals, responsible for preparation of formulations. Although these two classes are the most exposure, risk of exposure may occur at any given point during handling of these drugs, affecting all professionals involved. Forms of exposure to anticancer agents are varied, the most typical being inhalation, dermal or oral exposed.

Assessment of occupational exposure is made using different methods. Among these, chromatographic ones stand out. HPLC-MS/MS analysis or, in some cases, other chromatographic techniques are also considered (e.g. GC-MS/MS).

We observed a scarcity of information about the concentration of these drugs in the environment, more precisely in water. This fact is due to the lack of analytical methods for environmental control. The instrumental methods of analysis more used in wastewater, are gas chromatography, capillary electrophoresis, and high-performance liquid chromatography (HPLC). The confirmation and qualification of these techniques is commonly performed by mass spectrometry or tandem mass spectrometry because these methods are sensitive and selective for the typical levels of these pollutants in environment. However, one can also use the UV and fluorescence detectors because, usually are much more accessible.

CONCLUSION:

Evaluation of occupational exposure to anticancer agents is widely documented, but studies conducted in this field do not cover all types of anticancer drugs available and used. Health professionals, are the most affected population group, a fact that is due to direct contact with patients and affects all professionals, especially those who handle, prepare and/or administer such drugs.

Despite the presence of cytostatic drugs and its metabolites in the environment have been considered a public health problem, few or any solutions have been adopted.

The costs of methodologies and difficulties to identify the metabolites are the most important problems. There is no information available about the drugs metabolization and metabolites form in the environment, when wastewaters containing these drugs are dumped in effluents.

ACKNOWLEDGMENTS:

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Sound exposure of garage rock bands musicians

Authors: João Almeida[1], Daniel Silva[2], Paulo Carmo[2], Paula Neves[1,3], Matilde Rodrigues[1]

1 *Research Centre on Environment and Health, Allied Health Sciences School of Polytechnic Institute of Porto, Portugal*
2 *Department of Audiology, Allied Health Sciences School of Polytechnic Institute of Porto, Portugal*
3 *Department of Environmental Health, National Health Institute, Dr. Ricardo Jorge, Porto, Portugal*

INTRODUCTION:
Previous studies have been shown that musicians are exposed to high sound levels in the course of rehearsals and performances (Rodrigues *et al.*,2014), which can be the source of hearing damages (Jansen *et al.*, 2009). However, other musical groups can also be exposed to high sound levels, as is the case of the garage rock bands musicians. This particular group of musicians, due to the style of the music played, can be exposed to dangerous sound levels. This situation can be even worse if considered the conditions of the rooms where the rehearsals and performances are being done. In fact, in most of cases, rock musicians play in rooms with small dimensions and with poor acoustic conditions. Despite the importance of this issue, few studies analyzed this issue, particularly integrating two important issues, the sound exposure and to hearing damages.

OBJECTIVES:
This study aims to analyse the sound exposure of garage rock bands musicians and to analyse their hearing damages.

MATERIALS AND METHODS:
Three garage rock bands were analyzed, including a total of nine musicians. All the band musicians were evaluated with an acoustic dosimeter during the entire rehearsal. Values of equivalent continuous sound pressure levels ($L_{p,A,eqt}$), peak sound pressure level ($L_{p,Cpeak}$) and octave frequencies were measured. The rooms where the rehearsal was performed were characterized and the isolation index determined.At the final of the rehearsals a questionnaire to analyze the perception of noise exposure, the use of hearing protection and health effects was applied to each musician. The level of hearing loss was also analyzed. In view of this, an air tone audiogram was then carried out, where hearing thresholds were tested at high frequencies at 12000hz.

RESULTS AND DISCUSSION:
The results of this study show the analyzed musicians are exposed to high sound levels in the course of rehearsals. Values of $L_{p,A,eqt}$ higher than 100 dB(A) were found for all musicians and $L_{p,Cpeak}$ values higher than 135 dB(C) too. The rooms presented poor acoustic conditions, where most of them were small, with the use of egg cartons as absorption material on the wall and ceiling, which has been referred as inadequate material for this purpose. Furthermore, as no sound reduction material or protection was used to the drums, amplifiers had to be used to the other instruments. The results also showed that tinnitus occurs with high frequency, and some musicians presented a certain level of changes in hearing threshold in the high frequencies.

CONCLUSION:
The results obtained in this study reflect the need to implement measures to prevent and control exposure levels of garage rock bands musicians and increase their awareness of the risks to which they are subject. In fact, these musicians are in a critical risk situation, particularly considering the high number of hours that they spend in the rehearsals and concerts.

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Assessment of biological risk in packaging glass sorting facilities

Authors: Vasconcelos Pinto, Marta[1]; Veiga, José [2], Ramos, Carla[2]; Gonçalves, Sérgio[2]; Vaz Velho, Manuela[2]; Fernandes, Paulo [2]; Santos Guerreiro, Joana [2]

1 *Department of Environmental Health from Coimbra Health School, Polytechnic Institute of Coimbra*

2 *College of Technology and Management of Viana do Castelo, Polytechnic Institute of Viana do Castelo*

Presenting Author: Email: martavasconcelos@estescoimbra.pt | Tel.: +351 239 802 430 | Fax: +351 239 813 395

INTRODUCTION:

Numerous sectors are potentially exposed to biological agents. However since they are rarely visible, the accompanying risks are rarely recognized. The waste management sector, where there is an increased risk of handling material in decomposition, is one of the 10 main emergent activities associated with exposure to biological risks according to the European Agency for Occupational Health and Safety. In this context, the previous knowledge of the risks to which workers are exposed is of crucial importance, and preventive actions for the accurate identification and quantification of those risks should be performed.

OBJECTIVES:

This research aimed the assessment of occupational risk exposure to biological agents (bacteria and fungi) in the waste sorting industry.

MATERIALS AND METHODS:

The experimental component in this study comprised environmental collections in strategically selected and distinct places at waste sorting cabins (critical area), administrative services (non critical area) and outdoors (control point). Simultaneously, collections at surface were carried out in order to evaluate the microbiological contamination and transmission of biological risks through contact surfaces (fomites). The biological contamination of the operators hands was equally assessed. Bacterial and fungi species were identified with microscopic and biochemical methods.

RESULTS AND DISCUSSION:

As a result of the 40 air samples collected in the packaging glass industry it was completed 220 bacterial identifications and 143 mold identifications. Environmental samples revealed the existence of high environmental contamination by bacteria (1,6 x10^4ufc/m ^{3}) and fungi (1,5 x10^4ufc/m ^{3})(Table 2).

Table 2 - Minimum, maximum and average of total counts of bacteria and fungi by collection point

Collection Point		Bacteria Total Count (ufc/m^3)			Fungi Total Count (ufc/m^3)		
		Min.	Max.	Avg.	Min.	Max.	Avg.
Critical Area	Sorting Cabin	1660	26000	16248	160	26000	14978
Non Critical Area		420	2690	1173	90	5010	1591
Control Point		90	1220	516	60	4870	1265

The predominant bacterial genus was *Staphylococcus* spp. (negative coagulase) with values ranging between 29.6% and 60.0% (% on the total count of bacteria). The genus *Penicillium* spp. is the most frequent with an approximate value of 95% of the total count of fungi at the waste sorting cabin (Table 3).

The evaluation of biological risk in surfaces and manipulators shows the existence of considerable bacterial contaminations on the surfaces studied and in the hands of the collaborators of the productive sector. The

compared analysis of the contamination of contact surfaces with the hands of the collaborators revealed the inexistence of a correlation pattern between the bacterial and fungal microflora of manipulators and surfaces.

Table 3 - Average Total Counts (air - CFU/m^3; manipulator and surface - ufc/dm^2) of each kind of bacteria and fungi identified at each sampling point

Collection Point	Bacteria				Fungi		
	Gram	Genera	Classification	Total	Genera	Classification	Total
Critical Area Sorting Cabin	(-)	*Serratia* spp.	NC	1990	*Penicillium* spp.	NC	11866
	(-)	*Acinetobacter* spp.	NC	1920	*Rhodotorula* spp.	NC	2440
	(-)	*Proteus* spp.	2	1340	*Ulocladium* spp.	NC	1020
	(-)	*Citrobacter* spp.	NC	620	*Cladosporium* spp.	NC	773
	(-)	*Enterobacter* spp.	2	587	*Candida* spp.	NC	300
	(-)	*Chryseobacterium* spp.	NC	480	*Aspergillus* spp.	NC	64
	(-)	*Klebsiella* spp.	2	300	*Rhizopus* spp.	NC	60
	(-)	*Shewanella* spp.	NC	120	*Trichophyton* spp.	2	40
	(-)	*Chromobacterium* spp.	NC	60			
	(-)	*Stenotrophomonas* spp.	NC	20			
	(-)	*Pseudomonas* spp.	2	20			
	(+)	*Corynebacterium* spp.	2	6040			
	(+)	*Micrococcus* spp.	NC	1360			
	(+)	*Staphylococcus* spp. (negative coagulase)	NC	916			
	(+)	*Arthrobacter* spp.	NC	320			
	(+)	*Bacillus* spp.	NC	206			
Hands of Operator	(-)	*Pantoea* spp.	NC	2100	*Candida* spp.	NC	1925
	(+)	*Staphylococcus* spp. (positive coagulase)	NC	9175	*Penicillium* spp.	NC	1100
	(+)	*Staphylococcus* spp. (negative coagulase)	NC	7025	*Rhodotorula* spp.	NC	600
	(+)	*Micrococcus* spp.	NC	5243	*Cladosporium* spp.	NC	500
	(+)	*Bacillus* spp.	NC	4069	*Aspergillus* spp.	NC	250
	(+)	*Streptococcus* spp.	2	130	*Saccharomyces* spp.	NC	100
	(+)	*Corynebacterium* spp.	2	100			
Locker	(-)	*Acinetobacter* spp.	NC	800	*Cladosporium* spp.	NC	2100
	(+)	*Staphylococcus* spp. (negative coagulase)	NC	1231	*Candida* spp.	NC	1700
	(+)	*Micrococcus* spp.	NC	600	*Penicillium* spp.	NC	400
	(+)	*Cellulomonas* spp. / *Microbacterium* spp.	NC	250	*Rhodotorula* spp.	NC	100
	(+)	*Bacillus* spp.	NC	240			
Door Knob form toilets	(+)	*Aerococcus* spp.	NC	1925	*Rhodotorula* spp.	NC	30000
	(+)	*Micrococcus* spp.	NC	1525	*Candida* spp.	NC	2600
	(+)	*Staphylococcus* spp. (negative coagulase)	NC	1133	*Saccharomyces* spp.	NC	400
	(+)	*Bacillus* spp.	NC	225	*Cladosporium* spp.	NC	350
					Chrysosporium spp.	NC	300
					Penicillium spp.	NC	250
Door Knob from adminis-trative services	(+)	*Staphylococcus* spp. (conegative coagulase)	NC	1163			
	(+)	*Bacillus* spp.	NC	440			
	(+)	*Micrococcus* spp.	NC	300			
Water tap	(-)	*Klebsiella* spp.	2	2800	*Saccharomyces* spp.	NC	1500
	(+)	*Aerococcus* spp.	NC	10333	*Candida* spp.	NC	433
	(+)	*Staphylococcus* spp. (negative coagulase)	NC	1642	*Penicillium* spp.	NC	400
	(+)	*Micrococcus* spp.	NC	800	*Rhodotorula* spp.	NC	200
	(+)	*Bacillus* spp.	NC	563	*Rhizopus* spp.	NC	200
	(+)	*Cellulomonas* spp. / *Microbacterium* spp.	NC	500	*Cladosporium* spp.	NC	150
					Aspergillus spp.	NC	100

CONCLUSION:

The results obtained in this research indicate the existence of a significant environmental contamination of viable bacteria and fungi in the air, work surfaces and hands of employees involved in the sorting of packaging glass.

ACKNOWLEDGMENTS:
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Analytical and subjective interpretation of thermal comfort in hospitals: a case study in a sterilization service

Authors: Vítor Barbosa[1], Carlos Carvalhais[1,2], Joana Santos[1,2], Manuela Vieira da Silva[1,2]

1 *Environmental Health Department, School of Allied Health Sciences of Polytechnic Institute of Porto (ESTSP-IPP), Portugal*
2 *Research Centre on Environment and Health (CISA), School of Allied Health Sciences of Polytechnic Institute of Porto (ESTSP-IPP), Portugal*

Presenting Author: Email: caa@estsp.ipp.pt

INTRODUCTION:
Hospitals are peculiar workplaces, designed almost exclusively to the needs of users, giving their workers poor working conditions (Carvalhais, Santos, Lourenco, Teixeira, & Baptista, 2011). Inappropriate thermal comfort conditions in a building lead to lower work efficiency and higher possibility of personnel errors occurrence. The proper setting of thermal comfort parameters can provide suitable environment for the personnel (Pourshaghaghy & Omidvari, 2012). Some researchers have been undertaking the discussions of the desired thermal conditions for hospital occupants, including patients and healthcare professionals. Although, despite an increasing number of studies in thermal environment field, studying the indoor thermal comfort conditions for hospital occupants look to be a new matter (Khodakarami & Nasrollani, 2012).

In Portugal, the parameters specified in national legislation relating to the thermal environment, are restricted to the values of temperature and humidity, recommended by Decree Law No 243/86 of 20 August (Carvalhais et al, 2011). Other related legislated parameters, are more focused on energy consumption than the occupational comfort of occupants.

OBJECTIVES:
In addition to the legal compliance of legislated thermal parameters, this study aimed to determine levels of thermal comfort, sensations and preferences of healthcare workers.

MATERIALS AND METHODS:
This is a field study carried out in the sterilization service of a Hospital from Aveiro district, from March to July 2014 (spring and summer seasons). Structural and functional characterization of the sterilization service was made. This service centralizes the sterile processing activities, in which reusable medical devices, surgical instruments and equipment are processed and issued for diagnostic and surgical patient care procedures. The measurements were performed in three areas (Decontaminating area (A); Cleaning and Assembly area (B) and Sterilization area (C)). The analytical determination and interpretation of thermal comfort, was based on the assumptions of ISO 7726:1998 and ISO 7730:2005. For the measurement of the environmental parameters (air temperature, globe temperature, relative humidity and air velocity) two instruments were used: Area Heat Stress Monitor model HS-32 from *Quest* and a thermoanemometer model 8345-M-GB from *TSI*. The individual parameters (metabolic rate and clothing insulation) were estimated according ISO 7730:2005. Additionally it was applied a questionnaire to assess the subjective variables (thermal sensations and preferences), based on ISO 10551:1995.

The sample considered (n=11 (spring) and n=9 (summer)) was composed by all the professionals who were performing their tasks at the time of the measurements of environmental parameters. The service has two shifts (8 a.m-2p.m. and 2p.m-10p.m.).

RESULTS AND DISCUSSION:
Table 1, shows the calculated values of PMV and PPD indexes by day period and season.

Table 1 - PMV/PPD values according the day period and season.

Area	Day Period	PMV Spring	PMV Summer	PPD (%) Spring	PPD (%) Summer
A	Morning	0,81	1,10	18,9	30,3
B		0,86	1,18	20,6	34,4
C		0,61	1,08	12,6	29,6
Mean (day period)		0,76	1,12	17,4	31,4
A	Afternoon	0,98	1,17	25,1	33,6
B		1,03	1,16	27,5	33,3
C		0,76	1,00	17,1	26,1
Mean (day period)		0,92	1,11	23,2	31,0
A	Night	1,04	1,10	28,0	30,6
B		1,04	1,15	27,9	33,0
C		1,01	1,17	26,7	33,9
Mean (day period)		1,03	1,14	27,5	32,5
Mean (season)		0,90	1,12	22,7	31,3

By the analysis of table 1, it is clear that PMV and PPD indexes exceeded in all periods of the day the recommended range of -0.5 to +0.5 and <10%, respectively (Category B) (ISO 7730, 2005). By comparing with seven sensation scale, the predicted thermal sensation through the analytical approach, in both seasons was "Slightly warm" (0,90 Spring and 1,12 Summer). Regarding the subjective approach, Figure 1, shows the thermal sensations and thermal preferences, referred by participants in both seasons.

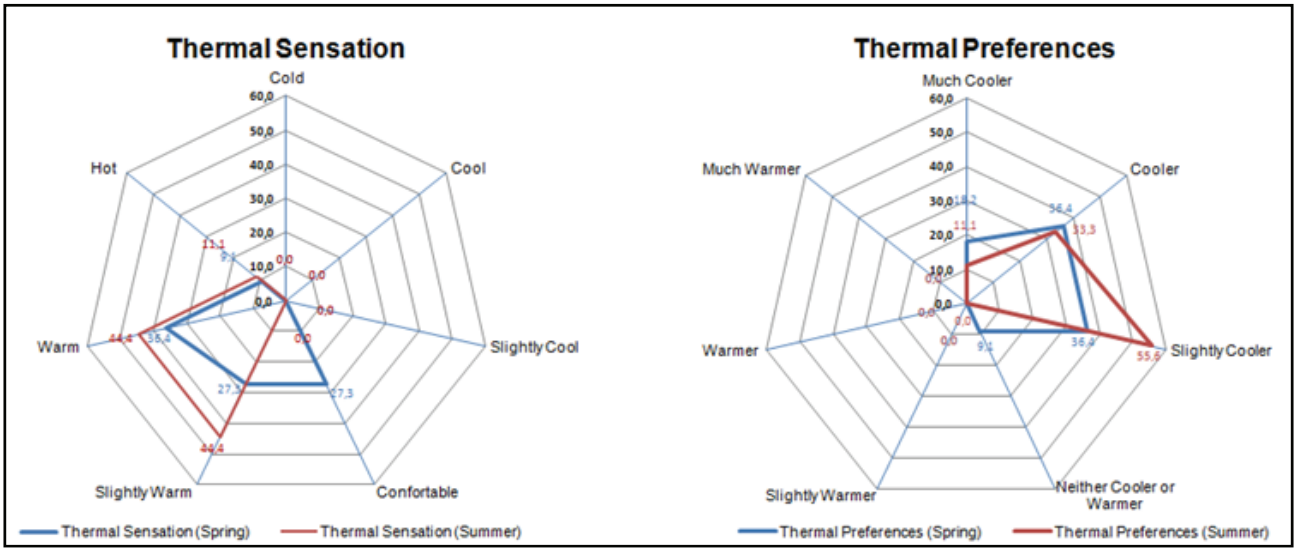


Figure 1 - Thermal sensations and preferences reported by workers.

Concerning to the thermal sensation, Figure 1 shows that workers tend to feel more comfortable in spring than in summer. In summer 88.8% of workers reported "Slightly warm" and "Warm" sensations (44,4% for each sensation), and 11.1% felt that the environment was "hot". In spring the mean for subjective thermal sensation was 1.27 and in summer was 1.67 ("Slightly warm" and "Warm", respectively). Other Portuguese study showed that the most frequent sensation felt in this kind of workplace was "comfortable" (Carvalhais et al, 2011). Probably structural conditions made the difference. Regarding to the thermal preferences during the same period the majority of workers questioned (36.4% at spring and 55.6% at summer) preferred to change the environment to "Slightly Cooler". The questionnaire analysis yielded other results, regarding thermal acceptability and tolerability. There was no significant difference between spring and summer

seasons concerning these two dimensions, where the majority of workers considered the environment "Acceptable" and "Slightly difficult to bear", respectively.

CONCLUSION:

This is part of an ongoing and larger study. The conclusions are limited and represent only the characterization of the thermal conditions on this particular service and seasons of the year (Spring and Summer). There is no significant difference between PMV/PPD spring/summer results. In both seasons the PMV index predicted the sensation as "slightly warm". However, the real sensations reported by workers showed that in summer they felt "warm". The analytical approach underestimated the real thermal sensation of workers. The assessment and interpretation of the thermal environment by analytical and subjective approaches revealed that in general the workers felt uncomfortable with the thermal conditions.

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Effect of an exercise program on musculoskeletal symptoms and productivity: an ongoing study in an automotive industry

Authors: Joana Santos[1,2], Carlos Carvalhais[1,2], Ana Sousa Costa[1], Betty Abreu[3], Manuela Vieira da Silva[1,2]

1 *Environmental Health Department, School of Allied Health Sciences of Polytechnic Institute of Porto (ESTSP-IPP), Portugal*
2 *Research Centre on Environment and Health (CISA), School of Allied Health Sciences of Polytechnic Institute of Porto (ESTSP-IPP), Portugal*
3 *Health and Safety Department, FicoCables, Lda, Maia, Portugal*

Presenting Author: Email: jds@estsp.ipp.pt

INTRODUCTION:

The ergonomic risk factors that can cause or aggravate musculoskeletal disorders include: repetitive motion, awkward posture, forceful exertions, pressure points, and static postures. There is no doubt that the new forms of work led to greater ease and comfort in the lives of workers, but the need for increase specialization of workers, improving product quality and reducing production costs, leads to the appearance of various health problems, including work related musculoskeletal disorders (WRMSDs). There are evidences that performing regular physical activity reduces the risk of chronic diseases (Bairati, Larouche, Meyer, Moore, & Fradet, 2000). The implementation of physical activity programs at worksites has been increasingly common. Some studies indicate that these programs allows the prevention/reduction of manifestation of the injury, the normalization of body functions, promote relaxation and socialization between workers, improving working conditions and preparing the participants for their daily activities. However, no evidence was found to indicate an effect of physical activity programs on productivity (Proper, Staal, Hildebrandt, van der Beek & van Mechelen, 2002).

OBJECTIVES:

The main objective of this research was to investigate the effectiveness of an Exercise Program (EP) on productivity. This paper will analyze the first six months of the EP.

MATERIALS AND METHODS:

This case study is being carried out in an automotive industry located in Portugal. In this type of industry, mainly due to the high manual materials handling and repetitive activities, workers are reporting a high number of MSDs. The implementation of the EP suitable to this type of tasks was developed according to the following steps:1) The selection of the sample came from previously conducted risk assessment trough OCRA method. Considering only the sections composed by assembly lines and taking into account the OCRA results, it was found that a specific section (composed by seven assembly lines with a total of61 workers (57femaleand4male)) had a great overload of the upper limbs and therefore with higher levels of risk;2) the Portuguese version of the Nordic questionnaire was applied to the workers of the section under study. This questionnaire was administered before the implementation of the EP to assess the perception of symptoms of MSDs;3) The development of the EP was based on some of the exercises proposed by a multidisciplinary team (doctors, physiotherapists and the OSH department of the company under study). The exercises focused in areas where injuries occur more often such lumbar, wrists / hands, arms and shoulders. The EP is being implemented since February in two shifts (morning (6a.m. to 2p.m.) and afternoon (2p.m. to 10p.m.)), with four weekly sessions, which had 7 to 10 minutes of duration. Alongside its implementation, productivity data were collected from daily production reports.

The data analysis was based on descriptive statistics.

RESULTS AND DISCUSSION:

After statistical analysis of the data collected, it was found that about 95% of workers had symptoms of discomfort / pain, the most affected areas were the neck (49.2%), the lumbarregion (54.1%), shoulders (45.9%), wrists / hands (54.1%) and ankles / feet (60.6%). On the other hand, the back area (32.8%), thighs (8.2%), the elbows (14.7%) and the knees (26.2%) were body areas which had less frequency of symptoms (see Figure 1). These results were similar to those found by Kjellberg & Wadman (2007), who found that the most frequent complaints of workers with assembly tasks focussed on the shoulders (45%), neck (43%) and lower back (37%). Fredrikson, Bildt, Hägg, & Kilbom (2001) also refer to areas most affected workers in the automotive industry, neck, shoulders, lower back and wrists / hands. Still Ghasemkhani, Aten, &Azam (2006) refer feet and the dorsal area as the most prevalent MSDs, which resulted in higher levels of absenteeism.

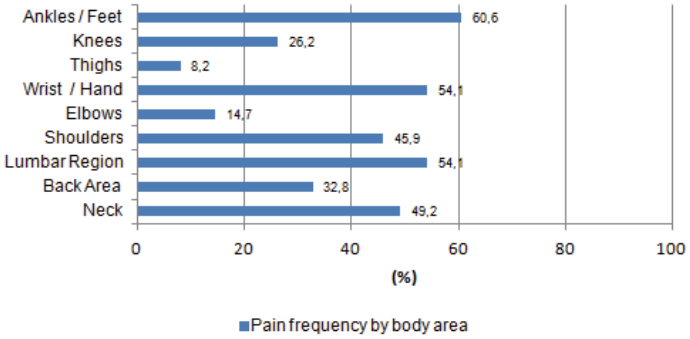
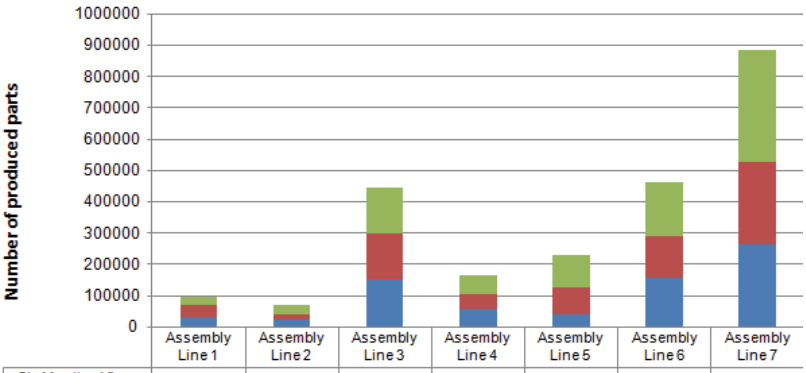


Figure 1 - Frequency of symptoms/pain by body area

An analysis of the productivity of different assembly lines of the section under study, showed no uniformity in the evolution of productivity. In some lines there was an increase in production, (i.e. increasing the number of parts produced), and in others it was found the opposite (see Figure 2).



	Assembly Line 1	Assembly Line 2	Assembly Line 3	Assembly Line 4	Assembly Line 5	Assembly Line 6	Assembly Line 7
Six Months After	24880	27540	144616	63507	103120	170947	356011
Three Months After	38460	20454	146940	46870	86378	137218	264139
Before	31139	20825	152340	56016	41620	154830	263583

Figure 2 - Productivity among the assembly lines before EP implementation, three months after EP implementation and six months after EP implementation.

No significant relation between productivity and the EP was found, at 6 months of implementation (p = 0.124). Some factors may be related to variations in the productivity namely: (1) technical problems of equipment, (2) failures in the raw material and (3) constant change in production volume.

CONCLUSION:

So far it seems that six months of EP implementation, is not enough to obtain significant results on the productivity. The program is still under implementation since this should be part of the routine of work. After full implementation it will be reassessed musculoskeletal symptoms as well as the impact on MSDs related to work. Given the trend towards increasing the framework of the MSDs, it is essential to characterize the

prevalence of pain and discomfort and different types of activity and determine the effect of intervention strategies.

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Is it enough training healthcare staff on noise reduction in Neonatal Intensive Care Units? A pilot study from NeoNoise Project

Authors: Carlos Carvalhais[1,2], Joana Santos[1,2], Manuela Vieira da Silva[1,2], Ana Xavier[1]

1 *Environmental Health Department, School of Allied Health Sciences of Polytechnic Institute of Porto (ESTSP-IPP), Portugal*
2 *Research Centre on Environment and Health (CISA), School of Allied Health Sciences of Polytechnic Institute of Porto (ESTSP-IPP), Portugal*

Presenting Author: Email: caa@estsp.ipp.pt

INTRODUCTION:

The noise in the neonatal intensive care units (NICU) is recognized by many researchers as an agent with negative implications on health and well-being of premature infants (Nicolau et al, 2005) and health professionals. Some sources of noise in hospitals include equipment, alarms, pneumatic tube systems, elevator buzzers, printers, ice machines, food carts, paging systems, call bells, telephones, televisions, and staff/visitors conversations. Several studies showed that "team conversation" was perceived by professionals as one of the main noise sources in NICU. Environmental modifications can effectively decrease noise levels, however the process of caring for hospitalized patients needs frequent and ongoing interpersonal dialogues. Minimizing the patient exposure to interpersonal communications between healthcare staff requires a behavior change. A well structured training program (TP) seems to be a low-cost measure to begin a noise reduction process in a hospital environment (Tsunemi, Kakehashi & Pinheiro, 2012).

OBJECTIVES:

The goal of this pilot study was to develop, implement and evaluate the effectiveness of a TP on noise reduction by comparing the noise levels before and six months after the implementation.

MATERIALS AND METHODS:

All measurements were performed in a NICU of a hospital located in Porto, Portugal between July 2011 (first phase) and July 2012 (second phase - six months after the implementation of the TP). The unit consists of two rooms (A and B), with physical separation between infrastructures. Both the preparation of the parenteral nutrition and medication are made in room A and, as a result, it had the greatest amount of staff activity. The measurements were carried out continuously during 24 hours in each measurement place (work station, traffic zone, inside incubator) and were performed using a sound level meter (01 dB, model Solo-Premium). The measurement protocol was based on the orientations of previous studies (Robertson, 1998). In the analysis and interpretation of the results were used reference values given by WHO (Berglund et al, 1999). The training program aimed to raise the professionals´ awareness about the acoustic reality at the unit along with the objective of reducing noise. This intervention was performed through a lecture of around 60 minutes and was conducted by the researchers. In order to ensure that all the staff of the NICU under study attend the lecture (n=79), were performed fourteen training sessions. The lecture included the results of the sound pressure levels obtained in the first phase, comparing them to the recommended values suggested by WHO and other regulatory agencies. It was also discussed the negative impact of noise on health, both for neonates and professionals, and some actions that needed to be implemented to ensure noise reduction.

RESULTS AND DISCUSSION:

Table 1 shows the results obtained for the mean, minimum and maximum values of L_{Aeq} (dB) and $L_{p,\,Cpeak}$ (dB) in the two rooms of NICU, before and after the implementation of the training program.

Table 1 - Values of mean LAeq (dB), before and after the implementation of the training program.

Room	Area	L_{Aeq} (dB) - Before Mean (min-max)	$L_{p, Cpeak}$ (dB) - Before	L_{Aeq} (dB) - After Mean (min-max)	$L_{p, Cpeak}$ (dB) - After
A	Work station	71.7 (47.8-114.6)	143.3	58.8 (47.6-76.4)	102.8
	Traffic Zone	60.4 (43.6-91.5)	115.8	59.5 (53.0-75.0)	101.5
	Inside Incubator	48.7 (42.2-68.1)	104.1	54.4 (53.3-65.7)	92.8
B	Work Station	59.9 (39.5-85.8)	106.3	60.3 (46.4-79.2)	99.7
	Traffic Zone	58.1 (43.8-82.0)	113.2	63.9 (47.0-82.2)	98.9

As the table 1 shows "Work Station" of Room A had a significant L_{Aeq} and $L_{p, Cpeak}$ decrease (71.7 to 58.8 dB and 143.3 to 102.8 dB, respectively). It was visible a greater care by professionals in carrying out their tasks regarding noise production. In the "Traffic Zone" of Room B, was found an increase of almost 6 dB after the TP. The L_{Aeq} values obtained in the "Work Station" and "Traffic Zone" before and after the implementation of the TP are above the recommended values given by WHO (45 dB). Regarding the values inside the incubator, despite the increase of L_{Aeq} values after the TP, the levels obtained are below 58.0-60.0 dB (maximum levels allowed given by regulatory agencies). The results can be justified by the number of newborns that were in NICU before (fourteen without specific care needs) and after TP (ten, which two of them were helped by an oximeter and ventilator, that may produce 60.0-78.0 and 60.0-80.0 dB respectively (Pugh, Jones & Griffiths, 2007)). Similar data was found by Tsunemi, Kakehashi & Pinheiro (2012) in their study. Despite the good adhesion by the healthcare staff to the TP there was no significant reduction of noise, in the NICU studied. Besides the fact that TP impact was lost over time, these results indicate that it is necessary to consider several factors that may ensure the effectiveness of the training programs.

CONCLUSION:

The results show that after six months of TP implementation, there was no significant noise reduction in the NICU and inside the incubator. While recognizing the importance of training programs in order to promote changes in the team´s attitudes, it must be recognized that the effects are not long lasting, then, it is emphasized to repeat them more often. Noise in all the rooms of a NICU can be reduced considerably by incorporating affordable behavioral and environmental modifications. Training the staff in order to implement quiet work behaviours is essential and must to be seen as a first step to implement a quiet time protocol in neonatal intensive units.

ACKNOWLEDGMENTS:

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Implementation of Risk Based Inspection (RBI) with the support of a web-based solution.

Authors: Flávio Morgado[1], Eleni Morgado Bortoletto[2], Mauro Duque Araujo[3], Mauricio NacibPontuschka[1]

1 *DepartmentofComputing, PUC-São Paulo, São Paulo, Brazil*
2 *Realide Tecnologia e Desenvolvimento de Software, São Paulo, Brazil*
3 *Araujo Engenharia, Vinhedo, Brazil*

Presenting Author: Email: fmorgado@pucsp.br | Tel.: +55 11 970 331 333

INTRODUCTION:
There, in Brazil, a standard of the Ministry of Labour aimed at inspection of boilers and pressure vessels, termed NR-13 (MTE, 2014). According HSE (2014), "statutory inspection under Health and Safety legislation has long been a requirement for boilers, pressure systems and other safety critical equipment". A breakthrough in the management of this class inspection equipment is the implementation of Risk Based Inspection (RBI), which, according API(2014), "has quickly become one of the industry's premiere instruments and preferred methods of inspection".

Risk based inspection involves "the planning of an inspection on the basis of the information obtained from a risk analysis of the equipment". The risk analysis is made to identify threats and potential degradation mechanisms to the integrity of the equipment and "to assess the consequences and risks of failure". The inspection plan can then mark the high risk equipment and "be designed to detect potential degradation before fitness-for-service could be threatened" (HSE, 2014). RBI "provides the means for quantifying the effect of inspections on the risk and thus for identifying cost optimal inspection strategies" (Straub, 2004).

OBJECTIVES:
This paper presents a methodology for the implementation of RBI, through the use of a specific software, developed by the authors, for managing inspections.

MATERIALS AND METHODS:
The software support the management of inspection programs using programming by NR13 for Boilers and Pressure Vessels; customize programs for other equipment such as tanks, pipes etc..; uses resources of standards such as API-510, API-573, API-570, in addition to custom suited to the kind of equipment standards, and enables autonomy in planning inspections and management costs through export inspection plan in spreadsheets. RBI combines the Bayesian decision analysis with structural reliability analisys, using the "available probabilistic models of the deterioration processes and the inspection performances to present a consistent decision basis" (Straub, 2004).

RESULTS AND DISCUSSION:
This is a software with the following features: Control equipment, components and elements; Calculating dates of inspections (according to NR-13 and RBI); Dashboard to schedule inspections and table RBI, with risk vs. consequences (Figure 1);

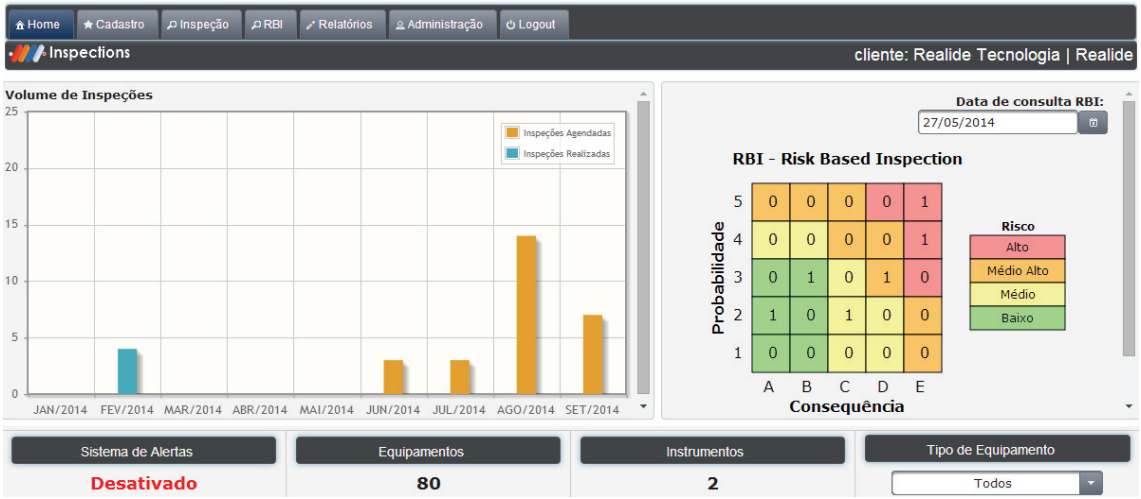


Figure 1 - Inspections Dashboard

The software have too Control recommendations; Damage Control and Failures; Export data for analysis tools; Integrity Control Program (Figure 2); Alert system with automatic sending of email and / or SMS messages to predefined recipients; Record of inspections and tests.

PROGRAMA DE CONTROLE DE INTEGRIDADE - RBI

TAG	Compo Nent	Conse Quency	Risk	Limit	Reference	2003								2012									2022								
E-1	CASCO	D	High	01/03/1995	01/01/1984																										
E-2	CASCO	B	Low	03/08/2027	03/08/2011																										
E-3	CASCO	B	medium high	01/01/2005	01/01/1973																										
E-4	CASCO	C	medium	25/04/2020	25/04/2008																										
E-5	CASCO	C	medium	01/08/2024	01/08/2012																										
E-6	FEIXE	B	Low	01/03/2024	01/03/2012																										
E-7	CASCO	D	High	10/01/2013	10/03/2005																										
E-8	CASCO	D	medium	03/08/2019	03/08/2011																										

Figure 2 - Integrity Control Program

Besides these features, the software uses the following technologies: Secure Hosting on Cloud Computing or local; Automatic backup journal; Fast Fault Recovery and Availability Monitoring in the Cloud option.

CONCLUSION:

The use of a specific software for risk based inspection can, as it has done with various chemical and petrochemical companies, accelerate the implementation of this methodology. An important step is the integration of inspection management modules, with the scope of a specific system with the systems of general maintenance.

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Hazards and effects of time-varying gradient fields in magnetic resonance imaging - a short review

Authors: Vítor Silva[1], Isabel Ramos[2], J. Agostinho Moreira[3], Manuel B. Marques[4]

1 *Doctoral Program on Occupational Safety and Health, Faculty of Engineering of University of Porto; Magnetic Resonance Imaging Department, Centro Hospitalar São João, EPE; Morphological Sciences Department, School of Allied Health Sciences, Polytechnic Institute of Porto, Portugal.*

2 *Faculty of Medicine, University of Porto.*

3 *IFIMUP and IN-Institute of Nanoscience and Nanotechnology, Department of Physics and Astronomy, Faculty of Sciences, University of Porto.*

4 *Department of Physics and Astronomy, Faculty of Sciences, University of Porto.*

Presenting Author: Email: vitorsoft@gmail.com | Tel.: +351 917 401 305

INTRODUCTION:

Magnetic resonance imaging (MRI) uses three types of fields for its operation: a static magnetic field (SMF), time-varying gradient fields (TVGF) and radiofrequency (RF) fields. The MRI advances on technology and medical demands, those magnetic fields are getting stronger, bringing hazards and effects that need to be well known. All of those electromagnetic fields have hazards and effects associated. In this document, only TVGF's hazards and effects will be shown.

TVGF have a variable intensity, depending on MRI equipment, but hazards and effects associated are similar in all MRI equipments. The fully understanding of those provides a more comprehensive handling of MRI equipment, with respect on MRI safety.

OBJECTIVES:

This document gives a short review of potential hazards and effects of TVGF used in MRI, as well as exposure limits of those gradient fields.

MATERIALS AND METHODS:

It was made a brief literature review on relevant databases, such as Scopus, MetaLib of Exlibris, PubMed / Medline and Springer Link, using some key-words, such as time-varying gradient fields, magnetic resonance imaging, hazards, effects, risks and exposure limits.

RESULTS AND DISCUSSION:

With the evolution on SMFs field strength used in MRI, TVGF have developed and become more sophisticated in the recent years. On MRI equipments, these gradient fields are used to produce resonance frequencies that encode the data spatially, which will produce diagnostic images from patients.

During MRI examination, TVGF are often switched on and off rapidly and for this reason, they will induce an electric field into patients' tissues (Crook, 2009). This can lead to depolarization of voltage sensitive ion-channels in cell membranes leading to nerve excitation and stimulation. As a result, biological effects associated to TVGF are peripheral nerve of skin or subcutaneous fat excitation, and muscle stimulation. Peripheral nerve excitation can lead to tingling feelings in the skin, some burning sensations and pain. One of example of this effect is the sensation of flashes of light, caused by induced electric currents stimulating the retina (phosphenes caused by electromagnetic fields - magnetophosphenes). However, this effect has not been shown to be harmful. It is temporary and ceases after few minutes after exposure. A more serious response to electric currents flowing through the body is that of ventricular fibrillation (Fuentes, 2008).

Other effects reported were muscle twitching and spasms, headaches, changes on heart activity and vertigo (Crook, 2009; Crozier, 2007; Fuentes, 2008). For example, in cardiac pacemakers' interaction with MRI, an

induction of ventricular fibrillation, a rapid atrial or ventricular pacing, a reed switch malfunction, an asynchronous pacing and an inhibition of pacing output can occur (Ferreira, 2013).

So, TVGF can induce biological effects, such as: headaches, peripheral nerve and muscle stimulation and excitation, burns, magnetophosphenes, dizziness and vertigo.

Both patients and MRI workers are exposed to TVGF. MRI workers are exposed when they are positioned or moving in some parts of MRI room, particularly at the end of the magnet, especially with some types of head movements (Crozier, 2007).

Another hazard from TVGF is acoustic noise. A variety of types of acoustic noise are produced during a MRI procedure. They are frequently described as loud tapping, chirping or knocking sounds and those are due to TVGF. As the gradient coils are rapidly switched on and off during image acquisition, a significant amount of acoustic noise will be generated in the core of magnet. This noise occurs during rapid alterations of currents within the gradient coils. These currents, in the presence of a strong magnetic field produce significant forces that act upon gradient coils and produce noise The development and demanding of high performance gradient systems for better image quality and ultra-fast imaging sequences has increased the acoustic noise levels during a MR scan (Hattori, 2007).

The forces produced are Lorentz forces and they are produced with the rapid switching of currents within the SMF deforming and vibrating the gradient coils. These coils will impact among their mountings, causing acoustic noise, which is different from a type of sequence to another, according to the selection of some parameters by the MRI staff. A second cause for acoustic noise comes from the RF pulses, used to produce images from patients. So, intrinsic acoustic noise of MR examinations creates a huge problem for both patients and MRI staff (Hattori, 2007). Depending on noise levels, hearing damage and psychological distress can occur, mainly if exposure to MRI examination is too long.

Echo planar imaging (EPI) sequences (a type of MR image) are those whose produce higher acoustic noise levels, as stated by Hattori *et al* (Hattori, 2007). These authors have recorded sound levels from MRI equipment that ranged from 126 to 131 dB(C) on a linear scale (peak value) and the equivalent noise level was from 110.0 dB(A) to 115.8 dB(A), which exceeded the legal requirements at a 3 Tesla (T) equipment. They also recommend the use of both earplugs and headphones during some type of sequences (Hattori, 2007). Earplugs systems provide more acoustic attenuation than headphones (Figure 1). Both can be used by patients simultaneously during a MRI examination.



Figure 1 - Noise cancelling headphones system which provides 14 dB of acoustic attenuation and a signal for using hearing protection used in MRI.

For TVGFs, the exposure limits are frequency dependent and they are based on the current induced in the body. The exposure limits for TVGFs are expressed in current densities, being difficult to measure and action values are often given in magnetic flux density.

According to European Commission (EC) Directive (in 2013) and ICNIRP (International Commission on Non-

Ionizing Radiation Protection), the current density averaged must not exceed a threshold of 10mA/m^2 for the range of 4-1000 Hz to head and trunk. EC Directive defines the action value (magnetic flux density) as 50 μT for 500 Hz (Crozier, 2007).

CONCLUSION:

TVGFs have variable intensity, but hazards and effects associated are similar in all MRI equipments. These fields can induce electric currents that could be sufficiently large in tissues to interfere with the normal function of nerve cells and muscle fibers, causing nerve and muscle excitation and stimulation. Other TVGF's effects are: headaches, vertigo, magnetophosphenes, dizziness and acoustic noise. These hazards and effects affect both patients and MRI workers. There are exposure limits to patients and workers and the latter have to be aware of that.

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INDOOR AIR QUALITY ABSTRACTS

Indoor Air Quality in Portuguese Buildings: New regulations

Authors: Susana Marta Almeida[1,*], Marina Almeida-Silva[1], Margarida Pinto[2]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Loures, Portugal*
2 *Agência para a Energia, Algés, Portugal*

Presenting Author: Email: smarta@ctn.ist.utl.pt | Tel.: +351 219 946 124

INTRODUCTION:

The residential and tertiary sector accounts for more than 40% of the final energy consumption in the European Community and is expanding, a trend which is bound to increase its energy consumption and hence its carbon dioxide emissions. This fact explain the priority that the EU has for reducing the energy consumption in the building sector, both for advancing in the compliance of international agreements, as well as for reducing its energy dependency, and therefore for leading its development path towards sustainability. The objective of the Directive 2002/91/CE is to promote the improvement of the energy performance of buildings within the Community. This Directive regards the energy certification that must provide a clear and detailed information about the building' s energy performance (energy labeling), allowing for the straight comparison between different buildings.

In Portugal, a step forward was given when the European Directive 2002/91/CE was transposed to the Portuguese law (Decreto-Lei 78/2006 and Decreto-Lei 79/2006) because it included Indoor Air Quality (IAQ) requirements such as comfort parameters, concentrations limits for indoor pollutants and minimum levels of air renovations in the energy certification mechanisms. Therefore, since 2009 until 2013 a significant fraction of the Portuguese buildings were obliged to make the control of their indoor chemical and biological pollutants in order to obtain a certificate. In 2013,a new national regulation was published (Decreto-Lei nº 118/2013) and the IAQ was excluded from the Energy Certification of the building. However, this diploma mention the importance of the IAQ and to ensure the conditions of well-being and health of the buildings' occupants, the members of the Government responsible for areas of energy, environment, health and social safety established in Portaria no. 353-A/2003 new protection thresholds for concentrations of indoor air pollutants.

OBJECTIVES:

The objective of this work is to evaluate the IAQ in1000 Portuguese buildings regarding the new reference values, identifying the main air quality problems and associated sources.

MATERIALS AND METHODS:

The IAQ audits were made under the scope of the Portuguese Energy and IAQ certification following a systematic approach defined by the national IAQ guidelines [1]. The IAQ audit started with the collection and analysis of the available architectural, mechanical and electrical drawings followed by a preliminary visit to the building. A walkthrough inspection was made in order to verify and update the information provided by the building owner; identify potential pollutant sources; assess the occupant's activities and complaints, perform an evaluation of the hygienic and maintenance conditions of the Heating, Ventilation and Air Conditioning (HVAC) systems and collect additional information which is deemed necessary for an adequate audit planning. All collected information about the building and its HVAC systems was considered to determine the quantity and locations of the sampling points, a crucial task for the suitable planning of the measuring campaign.

All occupied spaces of the building were grouped by zones with expected similar IAQ. The groups were made according to the ventilation system in each zone, the type of activity, thermal loads and emission sources. The minimum number of sampling points were estimated according the following equation:

$N = \sum_{i=1}^{n}(0.15 \times \sqrt{A_i})$, where N is the minimum number of sampling points in the building, n is the number of identified zones and A_i is the area of zone i in m^2.The following stage of the audit involved the measurement of the specified indicators including particles with aerodynamic diameter lower than 10 m (PM_{10}), carbon dioxide (CO_2), carbon monoxide (CO), formaldehyde (H_2CO), total volatile organic compounds (VOCs) and ozone (O_3). $PM_{2.5}$ was not measured because these audits were made before the new legislation that included by the first time this parameter. The measurement of the chemical parameters was made with portable equipments defined by NT-SCE-02.

RESULTS AND DISCUSSION:

The average PM_{10} concentrations measured in all buildings was 0.068 $mg.m^{-3}$. The PM_{10} limit level defined by the Portuguese legislation decreased from 0.15 $mg.m^{-3}$to 0.05 $mg.m^{-3}$and this value was exceeded in all typologies of buildings [2]. PM_{10} is usually associated with spaces that have lack of maintenance, high occupation and movement of the occupants [3].

The average CO_2 concentration measured in all buildings was 1368 $mg.m^{-3}$. The highest average concentrations were measured in restaurants and education institutions indicating lack of ventilation regarding the occupation [4].

The limit value established by the Portuguese legislation for H_2CO is 0.1 $mg.m^{-3}$. As expected the highest H_2CO concentrations were measured in health institutions because aqueous solutions of H_2CO is usefully used as a disinfectant as it kills most bacteria and fungi. In other building typologies as offices, education institutions, sport clubs, hotels, hypermarkets and restaurants, the maximum measured levels also exceeded de reference value of 0.1 $mg.m^{-3}$. This can be explain by the fact that H_2CO may also provide from combustion sources, cigarette smoke, gas ranges, open fireplaces and urea formaldehyde resins in furniture, parquet, insulators and personal computers.

The average of the VOC concentrations measured in all typologies was 0.55 $mg.m^{-3}$. The Portuguese limit value for VOC is 0.6 $mg.m^{-3}$ and this value was exceeded in offices, education institutions, health institutions, hotels, shoppings, hypermarkets and restaurants. VOC can provide information about the influence of indoor sources, such as aerosol sprays, solvents, cleaning agents, pesticides, paints, furniture and repellents.

CO did not exceed the limit values established by the legislation. The pollutant O_3 was removed from the recent Portuguese legislation but according the old reference value the buildings did not present any concern.

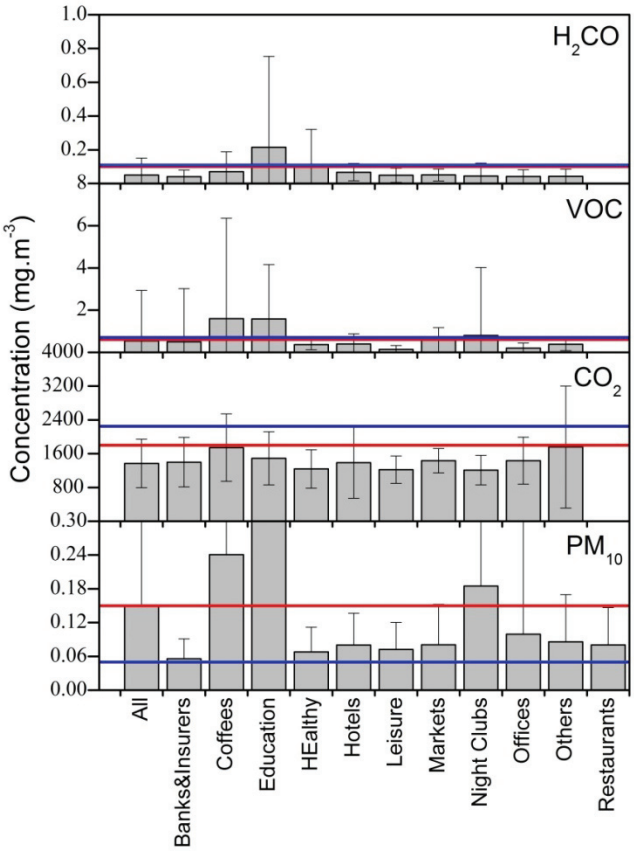


Figure 1 - PM_{10}, CO_2, VOC and H_2CO average concentrations measured in the audited buildings (red line - limit value based on Decreto-Lei 79/2006, blue line - new limit value based on Portaria no. 353-A/2003.

CONCLUSION:

This work analyzed the chemical parameters measured in Portuguese buildings. CO_2, VOCs and H_2CO were the chemical parameters that most exceeded the Portuguese limit concentrations. The highest CO_2 concentrations were measured in restaurants and education institutions. Associated with disinfectant products, H_2CO presented the highest concentrations in health institutions. The highest VOCs concentrations were measured in education and health institutions.

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Assessing workers' exposure to bioaerosols at large-scale organic waste treatment facilities - developing an evidence-base to support risk reduction strategies

Authors: P Sykes, PhD,[1]

1 *Cardiff Metropolitan University, Cardiff School of Health Sciences, Western Avenue, Cardiff, CF5 2YB. UK.*

Presenting Author: Email: psykes@cardiffmet.ac.uk | Tel.: 02920416831 | Fax: 0290416982

INTRODUCTION:

Commercial composting has become more prevalent in the UK as biodegradable municipal waste is being diverted from landfill. Furthermore, commercial composting activites are more frequently undertaken indoors to mitigate the potential environmental impacts such as noise, odour and bioaerosols. Composting indoors has however been shown to significantly increase worker exposure levels (Wouters et al,2006). Bacteria and fungi, actively grown during the composting of waste, are known to cause adverse effects on respiratory health when these organisms, or fragments of these organisms, become airborne forming a 'bioaerosol'. Workers' exposures to bioaerosols at composting sites is poorly characterised at this time and there is a lack of reliable dose-response data and accepted safe threshold levels of exposure.

OBJECTIVES:

The primary objective of this study is to characterise compost workers' exposure levels to bioaerosols by measuring their exposure to dust, endotoxin and β-(1-3) glucan and to use this exposure data to inform the development of evidence-based risk assessment and risk mitigation measures.

MATERIALS AND METHODS:

Personal exposure levels to inhalable dust and endotoxin was assessed at four large-scale composting facilities to determine task-related exposure data that would inform the development of evidence-based risk control measures. Furthermore, vehicle operators' exposure levels were investigated to determine the impact that cab cleaning and maintenance of the vehicle filtration system had on operator exposure levels.

Personal samples were collected and the inhalable dust fraction was calculated and the endotoxin concentrations were determined using a Limulus Amebocyte Lysate (LAL) assay. β-(1-3) glucan levels were estimated using a specific blocking agent to establish the contribution that these compounds gave to the original endotoxin assay (Wouters et al, 2006, Hryhorczuk et al., 2001). Geometric Mean (GM) and Geometric Standard Deviation (GSD) values were calculated following $\log_{10}$ transformation of the data.

RESULTS AND DISCUSSION:

Employees exposure to dust was generally low (GM 0.99 mg/m^3, GSD 2.99 mg/m^3, n=117), however endotoxin concentrations were elevated (GM 35.10 EU/m^3, GSD 9.97 EU/m^3, n=117). A third of all samples taken exceeded the 90 EU/m^3 occupational exposure limit recently adopted in the Netherlands (DECOS, 2010). Any activities involving the movement of waste gave rise to high peak exposure levels. Employees' exposure to β-(1-3) Glucan was low (GM 0.98 ng/m^3, GSD 13.39 ng/m^3).

Dust levels were elevated during manual sorting and screening of waste and high levels of endotoxin and β-(1-3) Glucan were observed during all practices involving the movement of waste. A significant correlation was observed between employee inhalable dust exposure and personal endotoxin concentration ($r = 0.726$, $p<0.05$)(Figure 1) demonstrating that inhalable dust concentration may be a valuable predictor for endotoxin concentration in the sites studied (Sykes et al, 2011).

Figure 3.13: Linear Regression of Inhalable Endotoxin versus Inhalable Dust

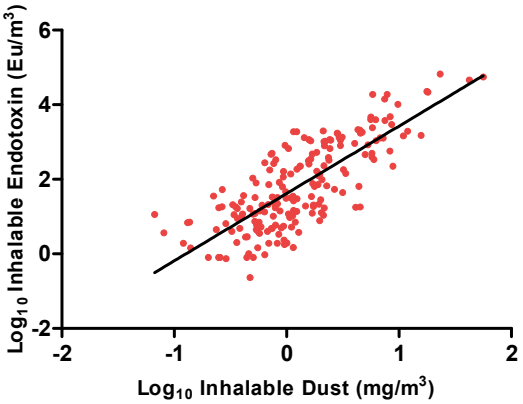


Figure 1 - Linear Regression of Inhalable Endotoxin versus Inhalable Dust

Vehicle operator exposures to endotoxin were consistently high despite working in air conditioned cabs with vehicle filtration systems fitted. The cleaning and maintenance of vehicle cabs had a significant reduction on operators' exposure ($p<0.05$, 2 sample t-test) but these improvements were not maintained.

CONCLUSION:

The findings of this study support previous published data in that employees of composting sites are exposed to high levels of bacterial endotoxin consistent with the occurrence of inflammatory outcomes (Wouters et al., 2006) even though in most cases, their personal dust exposures are low. Consequently, effective risk mitigation measures are needed to protect compost workers.

Site operators need to comprehensively assess the risk to site operatives from exposure to bioaerosols and implement procedures and site rules that minimise employees' exposures. Multifaceted controls will be needed to reduce employee exposures 'as low as reasonably practicable' in accordance with the legal framework. Consideration should be given to vehicle cab cleaning and maintenance, zoning of high exposure areas and high risk activities, health surveillance and provision of appropriate Respiratory Protective Equipment (RPE). Education and training is also required to ensure that employers and employees understand the potential occupational risks from exposure to bioaerosols.

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Estimating the inhaled dose of pollutants during indoor physical activity

Authors: Carla A. Ramos[1,3], Joana F. Reis[2], Tiago Almeida[2], Francisco Alves[2], Hubert Th Wolterbeek[3], Susana M. Almeida[1]

1 *C2TN, Instituto Superior Técnico, Universidade de Lisboa, Loures, Portugal*
2 *Faculdade de Motricidade Humana, Universidade de Lisboa, Lisboa, Portugal*
3 *Technical University of Delft, Delft, The Netherlands.*

Presenting Author: Email: carla.ramos@ctn.ist.utl.pt | Tel.: +351 219 946 156 | Fax: +351 219 941 039

INTRODUCTION:

Athletes and the common individual can be at risk when they are practicing exercise in polluted environments due the fact that 1) the increasing of the minute ventilation increase proportionally the quantity of inhaled pollutants; 2) most of the air is inhaled through the mouth, bypassing the normal nasal mechanisms for filtration of large particles and 3) the increased airflow velocity carries pollutants deeper in to the respiratory tract (Carlisle and Sharp, 2001).

OBJECTIVES:

The main goal of this work is to estimate the inhalation dose of pollutants during fitness classes in fitness centers.

MATERIALS AND METHODS:

Ten men and ten women from 18 to 39 years-old (the most representative age group in fitness centers) were invited to participate in this study. A questionnaire to the participants was applied in order to identify the exercise patterns, health status and respiratory problems. This evaluation was made in two steps. Firstly participants were submitted to a incremental test to volitional exhaustion in a treadmill and their tidal volume (L), minute ventilation (V$\dot{E}$ - L/min) and O_2 uptake were recorded using a breath-by-breath analyser (Metamax 3B, Cosmed, Leipzig, Germany). Heart rate was recorded telemetric using a Polar Team2 (Polar eletrco Oy, Kempele, Finland). The regression equations between V$\dot{E}$ and HR were calculated for each subject. Within a two week period of the incremental test the subjects participate together in two fitness classes with different intensities, where their heart rate during was recorded. Air pollutants (CO_2, tVOC, PM_{10}, O_3, and CO) were measured with direct reading equipments in the same fitness classes. To estimate the exposure dose, the following equation was applied:

Dose per class (µg) = C_a x V$\dot{E}$ x t

Ca - Pollutant concentration in the air (mg.m^{-3})
t - time of exposure (min)
V$\dot{E}$ - Minute ventilation (L/min)

RESULTS AND DISCUSSION:

Table 1 presents the average concentrations of pollutants measured in the studios of the fitness center and the estimation of inhaled dose of pollutants. The obtained averaged V$\dot{E}$ was different in the assessed classes and between genders. In the holistic class that involves stages of localized force, stability, balance and breathe control we obtained an average V$\dot{E}$ of 19.31L/min for women and 20.93 L/min for men, while in the more active class, characterized by intense cardio and aerobic movements, the average V$\dot{E}$ was 38.20L/min for women and 37.90 L/min for men. It was expected that men had a greater VE than women, especially in the aerobic fitness class where the cardiovascular component is more required, because they have a bigger chest and lungs than women. However it doesn't happened: women presented a higher HR in the aerobic

class which causes the increase in VĖ. This situation may be related with better adaptations to exercise for men (who use to practice exercise more often) than women. Regarding inhalation doses, the higher values were obtained for the aerobic class. For men, the inhaled dose during the aerobic class was 1.8x higher than in the holistic class and for women this discrepancy on dose was 0.5x. This results show that men have a greater intake than women. CO_2 is the pollutant with more expression, mostly because is the one with the highest concentrations, but because it result from human metabolism it toxicity presents no problems at these concentrations (Persily, 1997).

Table 1 - Estimated inhaled doses of pollutants in two fitness classes

Pollutants	C_a ($mg.m^{-3}$)	T (min)	VĖ Holistic Class (L/min)		Inhaled Dose (µg)		T (min)	VĖ Aerobic Class (L/min)		Inhaled Dose (µg)	
			M	W	M	W		M	W	M	W
CO_2	2768 ± 692	48	20.93	19.31	2781468	2566180	49	37.90	38.20	5141607	5182306
tVOC	0.297 ± 0.019				300	275				553	557
PM_{10}	0.045 ± 0.018				45	42				84	84
PM2.5	0.029 ± 0.012				29	27				54	54
PM1	0.027 ± 0.012				27	25				50	51
O_3	0.1 ±1.47				101	93				187	188
CO	0.05				50	46				93	94

In Figure 1 are plotted the data for VĖ, $PM_{2.5}$ concentrations and their product, the inhalation rate. The gray shadow is the integral of the inhalation rate, resulting in the inhaled dose of $PM_{2.5}$ in that class.

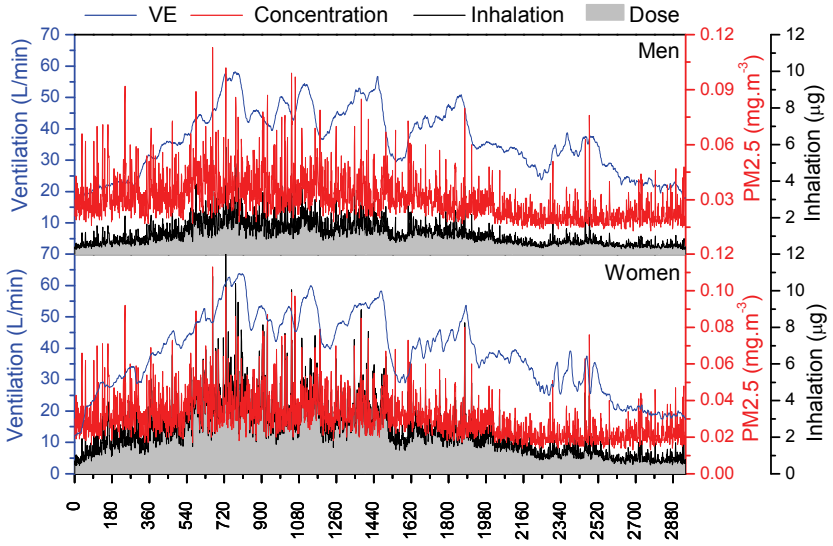


Table 1 - Estimated inhaled doses of $PM_{2.5}$ in two fitness classes

The high CO_2 levels registered indicate that in general the fitness centers have inefficient ventilation, considering the type of activity that is preconized indoors. The levels of particles were highly influenced by the intense indoor activities and by the type of ventilation. The IAQ results showed that the localization of the air intakes and the efficiency of the air filtration are essential for the maintenance of a good IAQ. Taking into account that VOC spikes were observed during cleaning activities and that cleaning products are recognized as risk factors for respiratory health, low emitting agents and “green” practices should be adopted.

CONCLUSION:

With this study we can verify that are inhaled high doses of pollutants while practice indoor physical activity. The results of this study will provide a better comprehension of the exposure risk to air pollutants during physical activity and then balance the risks and the benefits of practicing exercise.

ACKNOWLEDGMENTS:

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Health and Indoor Environment in Elderly Care Centers

Authors: Ana Mendes[1], Lívia Aquiar[1], Cristiana Pereira[1], Paula Neves[1], Susana Silva[1], Diana Mendes[1], Teresa Palmeiro[2], Iolanda Caires[2], Amália Botelho[2], Pedro Carreiro-Martins[2], Nuno Neuparth[2], Stefano Bonassi[3], João Paulo Teixeira[1]

1 *Environmental Health Department, Portuguese National Health Institute Doutor Ricardo Jorge, Porto, Portugal*

2 *CEDOC, Departamento de Fisiopatologia, FCM-UNL, Lisbon, Portugal*

3 *IRCCS San Raffaele S.p.A, Rome, Italy*

Presenting Author: Email: asestevao@gmail.com | Tel.: +351 223 401 140

INTRODUCTION:

According to the United Nations estimates, the total number of people aged 65 years and older was 506 million in 2008 and is anticipated to double to 1.3 billion by 2040. Furthermore, it is estimated that people spend 80 to 90% of their day indoors in developed countries, and elderly are likely to spend even more time indoors. Thus, indoor air pollutants may have special significance for this age group, even at low concentrations due to long exposure periods. Also, aging is associated with a decline in immune defenses and respiratory function, and predisposition to respiratory infections (Bentayeb et al., 2013).

OBJECTIVES:

The aim of this paper was to evaluate 1) the indoor air quality (IAQ) and thermal comfort (TC) in a representative sample of elderly care centers (ECC) in Porto, Portugal, and compared with national and international standards, 2) to study the variability among different spaces within single ECC, 3) how buildings characteristics may affect the extent of indoor air pollution or thermal regulation, and 4) explore the impact of IAQ variables on respiratory health of ECC residents.

MATERIALS AND METHODS:

Out of a total of 58 ECC located in Porto urban area, 38% (n=22) accepted to participate in this study. Indoor environmental parameters were measured twice, during winter and summer, in 141 ECC rooms within dining rooms, drawing rooms, medical offices and bedrooms. These areas were assessed for IAQ chemical (CO_2, CO, Formaldehyde, TVOC, PM_{10}, $PM_{2.5}$) and biological contaminants (total bacteria and fungi). TC parameters were measured following ISO 7730:2005 (PMV and PPD indexes). A walk-through building questionnaire was performed prior the monitoring and outdoor samples were also collected for comparison. From September 2012 to April 2013, the standardized and validated Portuguese version of BOLD (Burden of Obstructive Lung Disease) questionnaire was applied by an interviewer to the elderly who gave their informed consent and were able to participate (n=143). All the participants should had ≥ 65 years old and live in the ECC for more than two weeks. A descriptive analysis of the responses was performed. Classical statistical methods were used to estimate means, medians and frequencies (percentages) in order to obtain insight into the ECC characteristics and environmental monitoring results within and between buildings. The variables were tested for normality with Shapiro-Wilk test. Mann-Whitney *(U)* test and Kruskal-Wallis *(H)* for independent samples were conducted for seasonal effects assessment, indoor/outdoor and within buildings location differences. It was also performed a student t-test for the variable 'air temperature'. A 0.05 level of significance was used for all analyses. All data were analyzed using IBM SPSS 21.0.

RESULTS AND DISCUSSION:

The 22 ECC were located in the urban area of Porto city, most of them (n=17) in heavy traffic areas. A total of 716 elderly lived in these centers with a range of 7 to 136 occupants per building. As regards construction characteristic, 66% were an adaptation to ECC of an existing residential building, and 40% were also developing activities of day care centers for elderly. Most of them were built in stone masonry construction (49%) with single pane windows (87%). Only 30% had roof and walls insulation, while 61% of the sampled presented condensations and infiltrations along walls and roofs inside the buildings. All ECC were smoke-

free. Regarding the ventilation type, 87% had mixed ventilation (natural ventilation in the rooms along with exhaustion systems in the kitchen and bathrooms) while 13% had only natural ventilation in all the indoor areas. The overall $PM_{2.5}$ mean concentration of the 22 ECC was above national (25 g/m^3) and international reference levels (35 g/m^3) in both seasons. These findings showed as these parameters are critical for air quality and could influence on human health. Other recent study (Bentayeb et al., 2013) also found, high levels of $PM_{2.5}$ in similar indoor environments, and the link with lung function and respiratory diseases such as COPD (Osman et al., 2007) has been quite demonstrated. Although all the other indoor air pollutants were within the reference levels peak values of PM_{10}, TVOC, CO_2, bacteria and fungi exceeded the reference levels, compromising indoor air comfort and worsening the already existent respiratory chronic diseases. TVOC, Bacteria, CO and CO_2 showed significantly higher indoor levels compared to outdoor, in both seasons. Indoor PM_{10}, TVOC, Bacteria and CO_2 present significant differences between seasons ($p < 0.01$). TVOC, bacteria and CO_2 show significant variation between ECC rooms ($p < 0.01$) and 4% of fungi samples were positive for pathogenic *Aspergillus species*. The winter PMV index is between the 'slightly cool' (-1) and 'cool' (-2) points in the thermal sensation scale, which may potentiate respiratory tract infections. PPD and PMV indexes show significant differences by room and by season ($p < 0.01$). The building variables 'Insulation', 'Heating Ventilation' and 'Windows frames' were significantly associated to chemical, biological and TC parameters. 'Bacteria', 'Fungi', 'Temperature', Relative Humidity', and 'PPD index' are the mostly affected by building characteristics (Table 1). In elderly respondents, breathlessness (27.5%) and cough (23.1%) were the major respiratory symptoms, and allergic rhinitis (21.7%) the main self-reported illness. Heart troubles were reported by 36.6% residents. Symptoms of wheezing (10.5%) in the last 12 months and asthma diagnosis (8.4%) were more common in females, as opposed to symptoms breathlessness (4.9%) and sputum (3.5%), more frequent in males. Smoking habits, both past and present, were more frequent in men (11.9%).

Table 1 - Building characteristics in the indoor environmental evaluation

(significant differences by building variable and environmental evaluation: $p < 0.001$; $p < 0.01$; $p < 0.05$)

Buildings Variables	Parameters											
	PM_{10}	$PM_{2.5}$	TVOC	HCHO	Bacteria	Fungi	CO	CO_2	Temp.	RH	PPD	PMV
Adapted to ECC												
Building occupation												
Sources of pollution												
Walls												
Insulation												
Roof lining												
Ventilation type												
Heating ventilation												
Building pathologies												
Windows type												
Sealants												
Type sealants												
Windows frames												
Glass type												
Flooring												

CONCLUSION:

Our study suggested that the IAQ in the ECC of Porto area is acceptable and no immediate intervention is required. Attention is needed to peak concentrations and fungi species that might compromised IAQ comfort. To prevent low indoor temperatures and discomfort, especially on winter season, simple measures could provide health benefits to ECC residents and workers, such as insulating ceilings, walls, and windows, maintaining natural and passive ventilation, solutions that are common in Portugal due to the advantage of the country's generally mild weather. Investigations are still needed to better understand the links between IAQ and respiratory health impairment in elderly. In this sense, logistic regression analysis is ongoing, thus focusing on the impact of IAQ and respiratory health symptoms on ECC residents.

ACKNOWLEDGMENTS:

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Assessment of Indoor Air Contamination by Fungi and Bacteria in Fitness Centers

Authors: Carla A. Ramos[1], Carla Viegas[2], Sandra Cabo Verde[1], Humbert T. Wolterbeek[3], S. M. Almeida[1]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa*
2 *Environmental Health RG - Lisbon School of Health Technology - Polytechnique Institute of Lisbon*
3 *Faculty of Applied Sciences, Department of Radiation, Radionuclides and Reactors, Technical University of Delft, The Netherlands.*

Presenting Author: Email: carla.ramos@ctn.ist.utl.pt | Tel.: +351 219 946 156 | Fax: +351 219 941 039

INTRODUCTION:

Fitness centers are special places which gather conditions for microbiological proliferation. Moisture due to perspiration and water condensation, marked human presence, elevated physical activity and contact between the occupants and surfaces are circumstances that meet in this type of buildings. Exposure to microbial contaminants is clinically associated with respiratory disorders and people who work out in polluted environments are more susceptible to contaminants.

OBJECTIVES:

This work studied the indoor air contamination in three gymnasiums in the city of Lisbon.

MATERIALS AND METHODS:

Three gymnasiums in the city of Lisbon were selected and termed as follows: gymnasium 1 (G1), gymnasium 2 (G2) and gymnasium 3 (G3). Inside the fitness centers, two sampling sites were evaluated: the studios and the bodybuilding rooms. The sampling campaigns were performed between October and December of 2012 with a Merck MAS-100 air sampler (Merck, Germany) that collected by impaction 250 L of air in each plate, with a flow rate of 100 L/min. Samples were collected in two periods of the day - in the morning (at the opening of the gymnasium) and at night (at the closure) - in order to recognize the differences before and after the occupation. Air samples were collected from ground level of central positions of the sampling sites. A Greywolf (WolfSense Solutions) was used to control continuously the comfort parameters (temperature, relative humidity and CO_2). Fungal colonies were grouped by macroscopic colonies characteristics (e.g. color, shape and elevation). Morphological identification was achieved through macro and microscopic characteristics as noted by Hoog et al. (2002). The obtained bacterial isolates were characterized based on their macroscopic traits (e.g. pigmentation, texture, and shape), microscopic morphology (cellular morphology, and presence/ absence of endospores) and biochemical characteristics (gram staining, catalase and oxidase activities). The definition of the morphological types was based on the Bergey's Manual of Determinative Bacteriology (Holt, 1994).

RESULTS AND DISCUSSION:

Bacteria concentrations exceeded in more than 350 CFU.m^{-3} the outdoor concentrations during the night period in the studio of G2 in the second day of sampling and in the bodybuilding of G3 in the first day of sampling. In the previous situations the critical limit of 500 CFU.m^{-3} was also exceeded. Results showed that at the end of the day the bacterial load was significantly higher indoors than outdoors indicating the importance of the occupation for the bacteria development (Figure 1). For fungi, indoor concentrations were higher than in the outdoor in G2 in six measurements and in G1 in the bodybuilding room at night. Results show that the highest concentrations were registered in G2 that uses natural ventilation (Figure 2). A total of 22 genera and 11 fungal species of fungal colonies were identified in this study. *Chrysosporium* sp. presented 56% of the fungal genera in the morning, decreasing it presence to 10% in the night, while *Cladosporium* sp. clearly emerged in the night time with about 51% of prevalence. *Chrysonillia* sp. decreased it prevalence, changing from 27% in the morning to 5.8 % in the closing time; the same behavior was registered outdoors,

shifting from 35% to 18%. *Penicillium* sp. showed an increase in indoors between the two studied periods (1.9% to 10%), although this increase was also found in outdoor (10% to 16%). *Acremonium* sp. was only identified indoors at the end of the day (7.5%). Our results indicated that the Gram-negative, catalase-positive cocci was the most prevalent airborne bacterial morphological type indoors in all fitness centers (25% in G1, 30% in G2 and 30% in G3) as well as in outdoor environment. The second most prevalent bacterial phenotype was the gram-positive, catalase positive cocci, which appeared indoors in the three studied gymnasiums. This phenotype includes species such as *Staphylococcus* and *Micrococcus*, which are abundant on human skin and mucous membranes (Fox et al., 2005; Aydogdu et al., 2010).

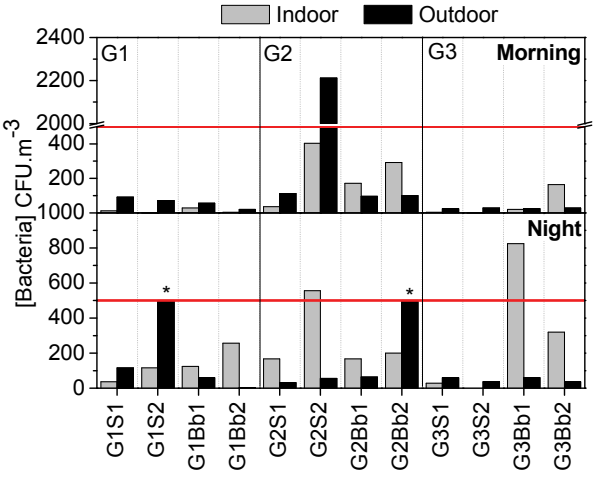


Figure 1 - Concentrations of airborne bacteria in the indoor and outdoor of the analyzed fitness centers for each sampling site and period. The red line establishes the critical limit of 500 CFU.m^{-3}. The * indicates that the number of colonies were countless and therefore a concentration above 500 CFU.m^{-3} was assumed.

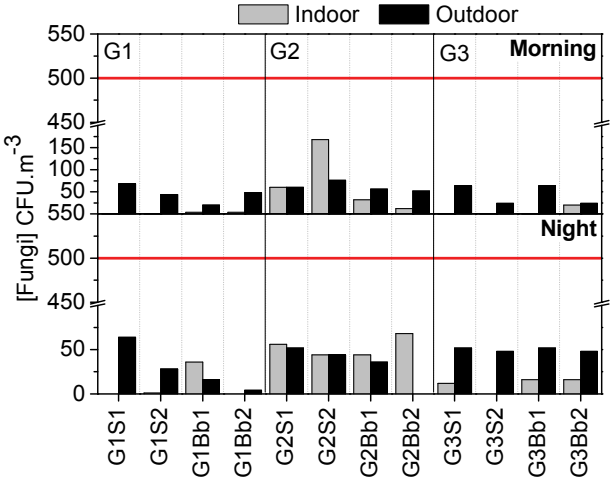


Figure 2 - Concentrations of airborne fungi in the indoors and outdoors of the analyzed fitness centers for each sampling site and period. The red line establishes the critical limit of 500 CFU.m-3.

CONCLUSION:

The indoor microflora is a complex mixture that varies according to the activity undertaken, human occupation, ventilation system and physical parameters such as temperature and humidity. It was found that natural ventilation used in the G2 had an influence on the fungal concentrations indoor as no physical barrier exists to filter the outdoor air. This work provides an understanding about the microbiological exposure during physical activity.

ACKNOWLEDGMENTS:

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ENVIRONMENTAL TOXICOLOGY ABSTRACTS

Chromium and Nickel in human hair of an orally exposed population.

Authors: Sazakli Eleni[1], Villanueva Cristina M.[2,3,4,5], Kogevinas Manolis[2,3,4,5,6], Maltezis Kyriakos[7], Leotsinidis Michalis[1]

1 *Lab of Public Health, Medical School, University of Patras, GR-26504, University campus, Patras, Greece.*
2 *Centre for Research in Environmental Epidemiology (CREAL), Doctor Aiguader 88, 08003-Barcelona, Spain.*
3 *IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain*
4 *Universitat Pompeu Fabra (UPF), Barcelona, Spain*
5 *CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain*
6 *National School of Public Health, Athens, Greece.*
7 *Health Centre of Aliartos, Aliartos, GR-32001,Greece*

Presenting Author: Email: elsazak@upatras.gr | Tel.: +30 261 096 9112 | Fax: +30 261 096 9112

INTRODUCTION:

Health effects due to exposure to chromium and nickel via consumption of water and foods with relative elevated concentrations have not been investigated. The two dominant forms of chromium, trivalent and hexavalent, show totally different metabolic pathways and toxicity when ingested. Nickel can be harmful to health if its intake is in large quantities.

OBJECTIVES:

An epidemiological cross-sectional study was conducted in an area in Greece to i) evaluate chromium and nickel levels in the hair of orally exposed subjects to these metals and ii) to examine associations of chromium and nickel in hair with hematological and biochemical parameters of the study subjects.

MATERIALS AND METHODS:

The entire study area is characterized by both agricultural and industrial activities. Chromium and nickel were determined in drinking water and local crops samples. The results were combined with previously published data (Dermatas 2012; EFET 2012) to construct a database of 676 Cr records in water samples, 274 Cr records and 325 Ni records in crop samples. The study population was randomly selected from three sub-regions based on exposure to chromium via drinking water: one currently exposed (A1), another with past exposure (A2) and a reference area (A0). Overall, 304 subjects participated. Total chromium and nickel concentrations were determined in hair samples of the study subjects. Hematological, biochemical and inflammatory parameters were determined in blood samples. Associations between chromium or nickel body burden and biomarkers were assessed through linear regression models, after log-transformations if needed. Non-parametric Kruskal-Wallis H test was employed to compare groups, followed by Mann-Whitney U test. Multivariate logistic regression models were employed to investigate associations between chromium and nickel hair levels and groups of subjects with normal and “out of normal” values in certain hematological/ biochemical parameters. The statistical significance level was set at a=0.050.

RESULTS AND DISCUSSION:

Chromium concentrations in drinking water range from <0.5 $\mu g \cdot L^{-1}$ up to 90 $\mu g \cdot L^{-1}$. Chromium in six different fresh local vegetable groups ranges from 0.002 to 1.200 $\mu g \bullet g^{-1}$ wet weight, while the corresponding nickel concentrations range from 0.016 to 2.449 $\mu g \bullet g^{-1}$.

The median concentration of chromium in hair samples is 0.22 $\mu g \bullet g^{-1}$, with a range from 0.03 to 1.26 $\mu g \bullet g^{-1}$. Chromium in hair differs between the three areas (Figure 1). Nickel concentration in hair has a median of 0.46 $\mu g \bullet g^{-1}$ and a range 0.01 - 6.13 $\mu g \bullet g^{-1}$. Nickel hair levels do not differ across the three areas (Kruskal Wallis test, p=0.359). Chromium and nickel concentrations in hair are moderately correlated (Spearman’s rho 0.520, p<0.001).

Reported reference values for chromium in hair in the majority of studies are 0.08 - 2.50 μg•g^{-1}, while the corresponding ones for nickel are 0.002 - 4.05 μg•g^{-1} (Caroli et al., 1994; Chojnacka et al., 2005; Leotsinidis and Kondakis, 1990). Chromium concentrations in our population fall within the above ranges, while for nickel a percentage of 1.4% exceeds reported "normal values".

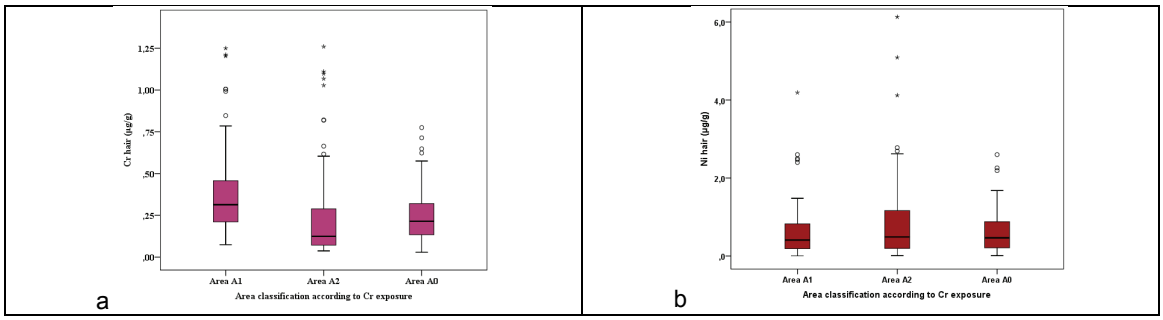


Figure 1 - Chromium (a) and Nickel concentrations (b) in hair samples of the subjects in the three areas

Certain hematological and biochemical parameters are weakly associated with chromium and nickel hair levels. Both the highest standardized coefficient (SRC=0.341) and the highest R^2 (0.188) are found in the regression model between ln(Cr) in hair and total proteins. All models are adjusted for sex and age.

In order to further investigate if the associations revealed by the regression models between hematological/ biochemical parameters and Cr and Ni hair levels reflect alterations in certain parameters in the population, subjects were classified into groups according to normal or "out of normal" values per each parameter. Multivariate logistic regression models that keep Ni in hair ($p<0.050$) are only those regarding albumin and calcium, Cr in hair is kept in the cholesterol model while both Cr and Ni hair levels are kept in the model concerning Low-Density Lipoprotein (LDL) values. The percentages of "out of the normal" subjects are 16.2% for albumin, 15.8% for calcium, 55.0% for cholesterol and 42.5% for LDL.

CONCLUSION:

In our population certain biochemical alterations are associated with chromium and nickel hair levels, even though chromium was found to be within the "normal reported values" for the entire population and nickel for a percentage of 98.6%.

ACKNOWLEDGMENTS:

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Effects of interaction and bioaccessibility of the cyanotoxins microcystin and cylindrospermopsin in aquatic and terrestrial species

Authors: Marisa Freitas[1,2,3], Joana Azevedo[1], António Paulo Carvalho[1,2], Sébastien Planchon[4], Jenny Renaut[4], Vera Mendes[5], Bruno Manadas[5], Edgar Pinto[6], Aldo Barreiro[1], Joana Neves[1], Alexandre Campos[1], Vitor Vasconcelos[1,2]

1 *CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, P 4050-123 Porto, Portugal*

2 *Faculty of Sciences, Porto University, Rua do Campo Alegre, 4169-007 Porto, Portugal*

3 *Polytechnic Institute of Porto.Department of Environmental Health, Escola Superior de Tecnologia da Saúdedo Porto. CISA/Research Center in Environment and Health, Rua de Valente Perfeito, 322, 440-330 Gaia, Portugal*

4 *Department of Environment and Agro-biotechnologies (EVA), Centre de Recherche Public-Gabriel Lippmann, 41, rue du Brill, 4422 Belvaux, Luxembourg*

5 *Center for Neuroscience and Cell Biology, University of Coimbra, Portugal*

6 *REQUIMTE/ Departamento de Ciências Químicas, Laboratório de Bromatologia e Hidrologia da Faculdade de Farmácia da Universidade do Porto*

Presenting Author: Email: marisaalexandrafreitas@gmail.com | Tel.: +351 222 061 000 | Fax: +351 222 061 001

INTRODUCTION:

The occurrence and proliferation of toxic cyanobacteria blooms as a potential consequence of eutrophication and climate change are an emergent environmental concern worldwide. Microcystin-LR (MC-LR), mainly produced by *Microcystisaeruginosa* is the most documented and studied cyanotoxin. Cylindrospermopsin (CYN) has been recognized of increased concern due to the invasive nature of its main producer, *Cylindrospermopsisraciborskii*. Recent studies support the hypothesis that MC-LR and CYN exert harmful effects on crop plants. Lettuce, *Lactuca sativa*, is an important commercial leafy vegetable, which supplies important components for a healthy diet (e.g., fibers, minerals and antioxidants). Therefore, it is of particular interest the knowledge of lettuce sensitivity to ecologically relevant concentrations of cyanotoxins, inclusively mixtures. Proteomic technologies seem to be suitable to investigate the effects of MC-LR and CYN and may allow the identification of early stress responses, which are not perceptible by traditional endpoints. Proteomics may also provide new insights of protein biomarkers of exposure and the identification of allergenic proteins, which may be of interest for human health risk assessment. However, human health problems due to MC-LR and CYN are most likely associated to its chronic exposure by drinking water and contaminated food. Previous studies have shown that aquatic organisms, especially bivalves (filter-feeding organisms), can accumulate high levels of cyanotoxins without lethal effect. Based on the potential for human health risks, a provisional tolerable daily intake (TDI) of 0.04 and 0.03µg/kg-body weight, has been established for MC-LR and CYN, respectively. However, the risks associated to the consumption of contaminated food may increase if the consumers use storage and processing practices that enhance the concentration of cyanotoxins and their bioaccessibility. It has been reported that MCs are stable at high temperatures (above 300 °C) and they can with stand several hours boiling. Likewise, CYN is highly water-soluble and stable to extreme temperatures and pHs, thus the knowledge of the influence of storage and cooking practices as well as human digestion on MC-LR and CYN concentration in food is required to a more accurate human risk assessment.

OBJECTIVES:

This study aimed: (1) to assess biochemical and physiological effects in lettuce after single and simultaneous subchronic exposure to the cyanotoxins MC-LR and CYN, using traditional endpoints and a proteomic-based approach;(2) to assess the changes on MC-LR and CYN concentration in edible bivalves after applying different storage and cooking practices, and (3) to assess MC-LR and CYN bioaccessibility.

MATERIALS AND METHODS:

Lettuce plants (*Lactuca sativa*) (four to five weeks' maturity) were exposed to the concentrations of 1, 10 and 100 µg/L of MC-LR and CYN (single cyanotoxins and in mixture) for 5 and 10 days, and the effects were assessed by parameters such as fresh weight, activity of antioxidant enzymes (GST and GPx) and mineral content in edible parts (Ca, Mg, K, P, Mn, Fe, Cu, Zn, Mo). The lettuce leaves were also studied by a differential-abundance proteomics approach. Proteins were separated by 2-DE, and those that were differentially abundant were then identified by MALDI-TOF/TOF MS.

To assess the changes on MC-LR and CYN concentration in bivalves after common food storage and processing practices, clams of *Corbiculafluminea* fed microcystin-producing *M.aeruginosa,* and mussels of *Mytilusgalloprovincialis* fed cylindrospermopsin-producing *C.raciborskii*, for 4 days, were refrigerated, frozen, boiled, steamed and subjected to microwave radiation over different periods of time (Table 1) and then analyzed by LC-MS/MS. Bioaccessibility of MC-LR and CYN were assessed in uncooked clams and in uncooked and steamed mussels, respectively, which were *in vitro* digested with salivary, gastric and duodenal juices.

Table 1 - Storage and processing practices applied in the experiments.

	Storage process			Cooking process		
Conditions	**25º C**	**4º C**	**- 20º C**	**Microwaving (550W)**	**Boiling (100°C)**	**Steaming**
Period of time	0h	24h	48h	0.5 min	5 min	10 min
	24h	48h	1 week	1min	10 min	15 min
			1 month		15 min	

RESULTS AND DISCUSSION:

Overall, in almost all treatments, an increase in root fresh weight was obtained, however, leaf-fresh weight was significantly reduced in lettuce plants exposed to 100 µg/L. Overall, GST activity was significantly increased in roots, contrary to GPx activity, which decreased in roots and leaves. The mineral content in lettuce leaves changed due to its exposure to cyanotoxins; in general, the mineral content decreased with MC-LR and increased with CYN, and apparently these effects are time and concentration-dependent. The effects of the MC-LR/CYN mixture were almost always similar to the single cyanotoxins, although MC-LR seems to be more toxic than CYN. Although there is scarce information on the lettuce genome, a high rate of proteins was successfully identified (>73%). The biological functions of the proteins that were most represented in both experiments were photosynthesis and carbon metabolism and stress/defense response. Proteins involved in protein synthesis and signal transduction were also highly observed in the MC-LR/CYN experiment. Although distinct protein abundance patterns were observed in both experiments, the effects appear to be concentration-dependent, and the effects of the mixture were clearly stronger than those of CYN alone. The results of our study highlight the putative tolerance of lettuce plants to CYN at concentrations up to 100 µg/L and the potential profit of lettuce plants when exposed to 1 µg/L of MC-LR/CYN, however, some presumed allergens were also identified in these edible tissues.

Overall, cooking for short periods of time resulted in a significantly higher concentration ($P< 0.05$) of free MC-LR in clams; specifically microwave (MW) radiation treatment for 0.5 (57.5%) and 1 min (59%) and boiling treatment for 5 (163.4%) and 15 min (213.4%). The bioaccessibility of MC-LR after proteolytic digestion was reduced to 83%, potentially because of MC-LR degradation by pancreatic enzymes. Mussels stored frozen allowed a significantly higher recovery of CYN (52.5% to48 h and 57.7% to one week). The cooking treatments did not produce significant differences in CYN concentration in mussel matrices (flesh), however, CYN was found in the cooking water, suggesting that heat processing can be used to reduce the availability of CYN in this food item. The *in vitro* digestion with salivary and gastrointestinal juices considerably decreased the CYN availability in uncooked and steamed mussels, highlighting the importance in integrating the bioaccessibility in the human health risk assessment.

CONCLUSION:

In conclusion, these findings provide new insights of the biochemical and physiological mechanisms of lettuce response to MC-LR and CYN and may contribute to the identification of potential protein markers of exposure and novel proteins that may confer tolerance to CYN and MC-LR/CYN mixture, although these need to be functionally characterized and validated. Furthermore, because lettuce is an important crop worldwide, this study may improve our understanding of the potential impact of environmental stress conditions in the fields of food safety (e.g., presence of allergens) and security (e.g., mineral content).This study also provides an enhancement of knowledge on the MC-LR and CYN concentration available in food after employing techniques commonly used for their preservation and processing. This might be of particular interest in setting acceptable thresholds, because the TDI can be exceeded in undercooked food. Treatments applied in this study can also be considered for the definition of critical control limits, considering the HACCP approach as a promising tool for risk management. Our results also suggest that risk assessment based on direct comparison between MC-LR and CYN concentrations determined in raw food products and the TDI values set for the MC-LR and CYN might not be representative of human exposure.

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Toxicological assessment of ibuprofen, paracetamol and metformin in terrestrial ecosystems

Authors: Inês Moreira, Cláudia Silva, Piedade Barros, Mafalda Nunes

Research Centre on Health and Environment (CISA), School of Allied Health Sciences of Polytechnic Institute of Porto, Portugal

Presenting Author: Email: ines.ca.moreira@gmail.com | Tel.: +351 934 196 915

INTRODUCTION:

Pharmaceuticals (PhACs) are a class of emerging contaminants that have, in recent years, been associated with potential risks for the environment (Fent, Weston & Caminada, 2006). Pharmaceutical substances are often engineered for persistence, so they remain unaltered as they pass through the organism. This leads to the persistence of these substances when they are excreted, leading to potential environmental problems (European Environment Agency, 2010). Residues of pharmaceuticals have been detected at trace quantities in aquatic systems, with studies finding over 80 PhACs from various prescription classes in sewage, surface and groundwater, in several countries all over the world (Heberer, 2002). These facts make the evaluations of potential effects of pharmaceuticals on ecosystems relevant and necessary. Acute aquatic toxicity has been more extensively studied since aquatic organisms are more easily identifiable as important targets, as they might be exposed to wastewater over their whole life. However, only little is known about the ecotoxicological effects of pharmaceuticals on terrestrial organisms (Fent et al., 2006). Residues of PhACs may reach the terrestrial environment through several sources/routes (Heberer, 2002) but, since removal of PhACs in Wastewater Treatment Plants (WWTPs) is many times not efficient, contamination comes mostly from applying contaminated sludge in agriculture.

While single substances are mostly found in the environment in modest quantities, it's important to note that in the environment, we find complex mixtures of pharmaceuticals and metabolites that may interact (Escher et al., 2011). Assessments of individual substances may therefore be misleading (European Environment Agency, 2010).

Ibuprofen, Paracetamol and Metformin are among the PhACs most consumed in Portugal and their metabolization by the organism is low, therefore it is important to assess their ecotoxicity.

OBJECTIVES:

Assess if Ibuprofen, Paracetamol and Metformin and their combination effect (Metformin + Ibuprofen, Ibuprofen + Paracetamol and Paracetamol + Metformin) represent a potential risk to terrestrial ecosystems, by studying their effects on earthworms (*Eiseniafetida*) and seeds of lettuce (*Lactucasativa)*, garden cress (*Lepidiumsativum*) and dwarf bean (*Phaseolus vulgaris*).

MATERIALS AND METHODS:

A reference toxicity test using boric acid was conducted to assess, under standardized conditions, the sensitivity of a portion of the population of adult earthworms (Environment Canada, 2004).

Acute avoidance tests were conducted using test units that consist of a circular container with an outer diameter of 23 cm, partitioned into a central cylinder with an inner diameter of ˜5cm, and six pie-shaped interconnecting compartments each with a capacity for ˜350 mL of soil. A series of ˜1cm holes enable the free movement of earthworms from the central cylinder (devoid of substrate) to the test compartments and between the compartments. A total of five test units were used, one for each concentration [0.10; 0.32; 1.00; 3.20; 10.00 mg/kg]. An identical weight of negative control soil was transferred to every second test compartment (three/test unit) within each test unit. The remaining compartments were filled with the same

weight of contaminated test soil. Test units were placed in a darkened facility for 48 hours at room temperature (22ºC±1ºC). At that time a side partition was quickly inserted adjacent to the side of each test compartment and the number of worms in each compartment was counted and recorded.

Toxicity tests of PhACs to terrestrial seed plants were conducted by placing a filter paper circle in Petri dishes, adding 2 mL of the contaminant solutions at different concentrations [0.00; 0.10; 0.18; 0.32; 0.56; 1.00; 1.80; 3.20; 5.60; 10.00mg/L] and placing 5 seeds (dwarf bean) or 10 seeds (garden cress and lettuce) in each Petri dish, equally distanced, so they wouldn't interfere with each other's growth (three replicates for each concentration). Petri dishes were covered with lids, sealed and incubated in the dark at room temperature (22ºC±1ºC) for 7 days. After incubation, root lengths were measured (from transition point between hypocotyl and root to root tip) and the seed germination was evaluated using root length of seedlings as endpoint (primary root > 2 mm).

RESULTS AND DISCUSSION:

Seven-day LC50 was determined to be 4858.7 mg of boric acid/kg soil (dry wt). Expected seven-day LC50 has endpoint values ranging from 3295-4915 mg of boric acid/kg soil (dry wt), meaning results fit within the expected range.

In Acute Avoidance Tests, all worms survived all five concentrations of the three different test contaminants and their mixtures. Results show that the avoidance response of worms to soil contaminated with the PhACs doesn't change with increasing concentrations between 0.10 and 10.00 mg/kg. Earthworms don't avoid soils contaminated with these PhACs, having no preference for clean soils. At the chosen concentrations, acute exposure to these contaminants poses no risk to *Eiseniafetida*. Question stands if higher concentrations could have an effect on earthworms. Tests with higher concentrations would, however, cease to have environmental relevance.

Results indicate that while lettuce seeds exposed to Ibuprofen and to the mixture solution of Ibuprofen + Paracetamol showed a slight raise in root lengths as concentrations increased. Inlettuce seeds exposed to Paracetamol, a relation between concentration and root length is not visible. Lettuce seeds exposed to Metformin and to the mixture solution of Metformin + Ibuprofen showed a small decrease in root lengths. Root lengths for garden cress and dwarf bean seedlings exposed to the three contaminants oscillated several times as concentrations increases, and no relation between the concentration of contaminant and the average root growth is visible. Variability between seeds of garden cress and dwarf bean roots length inside each group of seeds may be responsible for the differences between tests with different concentrations of the same contaminant. In lettuce, the variance between groups was not as high. Still, Ibuprofen and Paracetamol didn't appear to have any effect on the growth of seeds, with root lengths being equally arbitrary among concentrations. Even though, there isn't an apparent effect of the three pharmaceuticals in garden cress and dwarf bean, disparity between the length of roots inside and among concentrations groups and contaminants make it impossible to know, for sure, if the contaminants influence the development of the root or not. The only contaminant for which it seemed there was an effect on lettuce root growth was Metformin. However, statistical significant differences between the control group and seeds exposed to Metformin were not detected. Furthermore, variances in root length among groups are similar to those found in lettuce exposed to Paracetamol, where no apparent effect or pattern can be detected, and Ibuprofen, where root lengths increased slightly with increasing test concentrations. Therefore, we can't be sure that the apparent decrease in root lengths of lettuce seedlings is caused by Metformin, as there is a chance that it might be simply a result of the variability existent among the lettuce seeds used in the test.

Chronic toxicity studies with environmentally relevant concentrations might show results in both worms and seeds, seeing as both can take up and accumulate pollutants present in the soil (Wu, Wu, Qiu, Zhong, & Chen, 2011).

CONCLUSION:

Avoidance results for acute exposure to single PhACs, namely Ibuprofen, Paracetamol and Metformin and their mixtures don't indicate a toxicity of these compounds to *E. fetida*, at environmentally relevant concentrations, with no added toxicity being found for pharmaceutical mixtures. The same conclusion applies to tests using seeds, with no acute toxicity noted for these pharmaceuticals, either for single or combined pharmaceuticals. Acute exposure to Ibuprofen, Paracetamol and Metformin at environmentally relevant concentrations did not prove to have an impact on terrestrial ecosystems. The future realization of chronic studies for the same concentrations of these pharmaceuticals is, however, crucial for the determination of the environmental impact of these contaminants in terrestrial environments. Since several PhACs have been detected in the environment, and to fill the gap existing in studies of toxicity of pharmaceuticals in terrestrial ecosystems, future testing is needed on other PhACs and their interactions in the environment.

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Flow cytometry analysis of γH2AX levels in fresh and cryopreserved human peripheral blood lymphocytes

Authors: Vanessa Valdiglesias[1], María Sánchez-Flores[1,2], Eduardo Pásaro[1], Blanca Laffon[1]

1 *DICOMOSA Group, Department of Psychology, Area of Psychobiology, Universidade da Coruña, Spain*
2 *Department of Cell and Molecular Biology, Universidade da Coruña, Spain*

Presenting Author: Email: vvaldiglesias@udc.es | Tel.: +34 981 167 000 | Fax: +34 981 167 172

INTRODUCTION:
DNA double strand breaks (DSB) are the gravest form of DNA damage in eukaryotic cells. Both environmental factors, such as UV light and a great variety of chemicals, and normal metabolic activities can cause DNA damage, resulting in as many as one million individual molecular lesions per cell per day. Failure to detect DSB and activate appropriate DNA damage responses can cause genomic instability, leading to tumourigenesis and possibly accelerated ageing. Phosphorylated histone H2AX (γH2AX) has been proposed as a biomarker of cellular response to DSB due to its potential for monitoring DNA damage and repair in human populations. However, the lack of experimental standardization of the assay leads to a high heterogeneity in the results obtained and their interpretations, which affect the reliability of the assay and make its establishment as regular biomarker difficult.

OBJECTIVES:
On this basis, the aim of the present study was to validate the most advantageous methodological conditions for the γH2AX assay to be employed as a biomarker of DNA damage response in human peripheral blood lymphocytes. We evaluated the differences between fresh and cryopreserved cells, and the influence of phytohaemagglutinin stimulation prior the γH2AX analysis in the final results.

MATERIALS AND METHODS:
Peripheral blood samples were obtained from three healthy donors (women, aged 27-40) and mononuclear leucocytes were separated by gradient centrifugation. A variety of known genotoxic agents (i.e. bleomycin, camptothecin, methyl-methanesulfonate and actinomycin D) were employed to induce DNA strand breaks by different mechanisms, and γH2AX levels were determined after treatments by flow cytometry analysis.

RESULTS AND DISCUSSION:
Results showed that either fresh or frozen stimulated cells can be employed to evaluate γH2AX levels, but there are important outcome-related differences to consider when the analysis is made on resting or proliferating cells. Moreover, the approach resulted not reliable when using frozen unstimulated lymphocytes.

CONCLUSION:
These findings confirm the use of flow cytometry analysis of γH2AX levels as a rapid screening tool for genotoxicity evaluation, as well as define the optimum protocol conditions, particularly referring to cell culture settings, to be properly employed as DNA damage biomarker in biomonitoring studies.

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Presence of the drug carbamazepine in the clam *V.decussata*: comparison of acute and chronic toxicity tests

Authors: Ângela Almeida[1], Rosa Freitas[2], Vânia Calisto[3], Valdemar Esteves[3], Amadeu M.V.M Soares[2], Etelvina Figueira[2]

1 *Department of Biology, University of Aveiro, Portugal*
2 *Department of Biology & CESAM, University of Aveiro, Portugal*
3 *Department of Chemistry & CESAM, University of Aveiro, Portugal*

Presenting Author: Email: aaalmeida@ua.pt | Tel.: +351 234 370 350 | Fax: +351 234 372 587

INTRODUCTION:

Pharmaceuticals are used in increasingly high amounts throughout the world. After being taken up, some drugs are not completely degraded, being excreted as a mixture of the parent compound, metabolites or conjugates. In wastewater treatment plants (WWTPs) elimination rates of drugs are often incomplete and thus the release of final effluents of WWTPs into aquatic systems is considered as the primary source of pharmaceutical drugs in the environment(Zhang et al., 2008). This is the case of carbamazepine (CBZ), an antiepileptic drug increasingly found in the environment. It has been commonly detected in WWTPs influents and effluents, surface waters and groundwater, with concentrations ranging from 0.03 to 6.3 µg/L (Martin-Diaz et al., 2009).

One of the main concerns about the presence of CBZ in the aquatic environment resides in its persistence, with a half-time life of approximately 100 days. Therefore, the potential for bioaccumulation and development of biological effects on aquatic organisms, even at low concentrations, is high. For instance, filter feeding organisms, such as bivalves, are known to accumulate some organic contaminants at high concentrations as they filter large quantities of water for feeding and respiration. In this way, the CBZ accumulated can be not only detrimental to these organisms, but can also be transferred along the food chain, being potentially harmful to humans. Some studies have investigated the effects of CBZ on aquatic organisms, reporting the development of a stress response. Induction of lipid peroxidation (LPO) is one of the most frequently determined responses of organisms exposed to CBZ (Martin-Diaz et al., 2009). In this sense, the assessment of CBZ ecotoxicological risks for aquatic biota is of upmost importance, being also necessary to quantify its levels in this environment. The most common analytical techniques used for CBZ quantification in aquatic samples are liquid or gas chromatography coupled with mass spectrometry. However, these techniques are time-consuming, use expensive equipment and require sample pre-treatment procedures, such as pre-concentration. To overcome these problems, the immunoassay techniques have proven to be a valuable tool for sensitive detection of various pollutants in water (Calisto et al., 2011).

OBJECTIVES:

This work aimed to study, through the use of a battery of biomarkers,how the clam species *V.decussata* is affected by environmental CBZ concentrations, both in acute and chronic exposures. Moreover, the application of an enzyme-linked immunosorbent assay (ELISA) to quantify CBZ in the tissues of this organisms was attempted for the first time.

MATERIALS AND METHODS:

Acute and chronic tests lasted 96 h and 28 days, respectively. These tests were conducted with 25 organisms (for each test), distributed by five concentrations of CBZ (control=0; 0.03; 0.3, 3.0 and 9.0 µg/L) with five replicates per concentration (one organism/replicate). Organisms were placed in plastic containers filled with 300 mL of medium (artificial seawater, at a salinity of 25 g/L), with aeration, at a temperature of 18 ± 1ºC and a photoperiod of 12:12 h (light/dark). A blank test for each condition with no organisms was also performed to study the losses of CBZ during the assay. Water was renewed every 48 h for acute test and

twice a week for chronic test, and the CBZ concentrations were re-established. At the end of the exposure periods, the surviving organisms were frozen at -20ºC for biochemical analysis and CBZ quantification. For this, clams soft tissues were homogenized with liquid nitrogen and extraction (1:2 w/v) was done with the specific buffer for the subsequent analysis. The following parameters were determined as described previously by Figueira et al. (2012): lipid peroxidation (LPO), superoxide dismutase (SOD) activity, catalase (CAT) activity, total (GSH_t), reduced (GSH) and oxidized (GSSG) glutathione content. The procedure for the determination of CBZ concentrations in clams' tissues was adapted from Calisto et al. (2011).

RESULTS AND DISCUSSION:

In order to verify the presence of oxidative imbalance induced by CBZ, LPO levels were measured. After 28 days of exposure, oxidative damage significantly increased at 9.0 µg/L of CBZ compared to the other conditions. On the other hand, the results for the acute test showed an opposite response: the oxidative damage was significantly lower at the highest CBZ concentration compared to control and lower concentrations. In both toxicity tests, the SOD activity revealed a significant increase in clams exposed to higher CBZ concentrations. The increase in SOD activity may be explained as an adaptive response to neutralize the impact of ROS generated, as superoxide anion. However, CAT activity, in both toxicity tests, decreased at all exposure concentrations compared to control. If hydrogen peroxide is not enzymatically decomposed, it can be converted to the hydroxyl radical, inducing the LPO observed in clams exposed chronically to CBZ(Yoon et al., 2010). In the case of the acute test, the CAT activity seemed to be enough to degrade hydrogen peroxide. In this perspective, the ROS formed could be neutralized by the joint action of SOD and CAT, which with the increase in antioxidant molecules (eg. GSH), could have decreased the LPO at the highest concentration of CBZ. Indeed, the GSH content increased at the highest CBZ concentration, compared to 0.3 and 3µg/L.GSH_t increase was more pronounced in the chronic test. As GSH had small variations between conditions, the increase in GSSG was responsible for the increase in GSH_t content, indicating that little GSH was available to participate in detoxification reactions, and clams became less capable to handle with oxidative stress. Figure 1 illustrates the biochemical responses of the clam *V. decussate* to CBZ. The quantification of CBZ in clams revealed an increase along the conditions tested for both toxicity tests. *V. decussata* exposed to CBZ during 28 days presented a higher rate of accumulation for CBZ of 0.3 and 3.0 µg/L, when compared with clams exposed during 96 h. In the two toxicity tests, clams concentrated similar levels of CBZ at the higher exposure level (0.010±0.004 µg/g in the acute test and 0.011±0.001 µg/g in the chronic test). Overall, the results obtained are in agreement with previous studies that demonstrated a toxic effect of CBZ in aquatic organisms (e.g. Martin-Diaz et al., 2009).

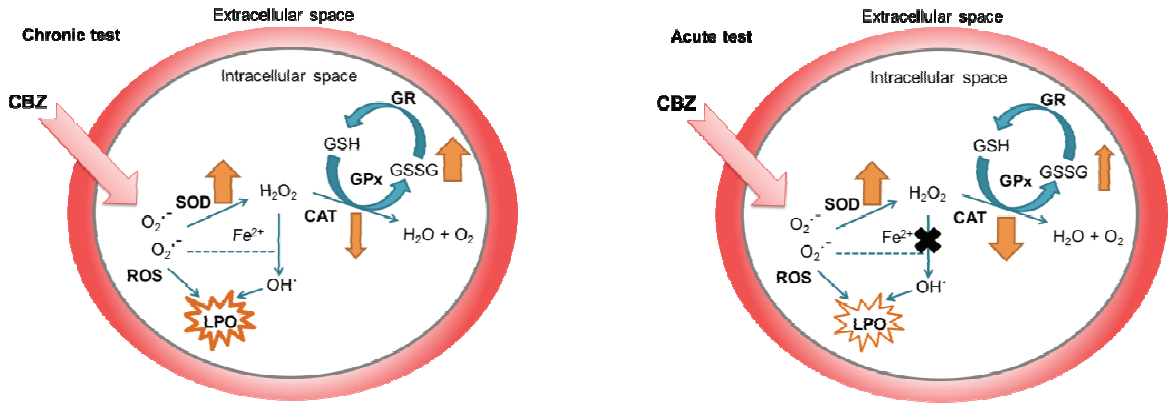


Figure 1 - Biochemical responses of *V. decussata* exposed to CBZ for 28 days (chronic test) and 96 hours (acute test). The arrows orientation and thickness indicate the increased or decreased enzymatic activity.

CONCLUSION:

The results of this work showed for the first time the application of the ELISA immunoassay technique to quantify CBZ not only in water but also in biological samples. Furthermore, changes in oxidative stress markers and antioxidant defenses in *V. decussata* exposed for 96 hours and 28 days to CBZ, were demonstrated. Although clams accumulated lower levels of CBZ than the concentrations present in water, these were enough to disturb their biochemical equilibrium. Moreover the evidenced bioaccumulation of CBZ in clams point to its possible transference along the food chain, and to potentially affect non-target organisms as humans.

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Fluoride intoxication affects (3H) glucose uptake in the brain and some peripheral tissue of adult Wistar rats

Authors: Anna Rogalska[1], Jolanta Malinowska-Borowska[1], Elżbieta Świętochowska[2], Marta Buczkowska[3], Joanna Gadomska[3], Anna Głogowska-Gruszka[3], Szubert Katarzyna[3], Przemysław Nowak[1]

1 *Department of Toxicology and Occupational Health, Public Health Faculty, Medical University of Silesia, Medykow 18, 40-752 Katowice, Poland*

2 *Department of Clinical Biochemistry, Silesian Medical University, Jordana 19, 41-800 Zabrze, Poland*

3 *Department of Communal Hygiene and Sanitary Surveillance, Public Health Faculty, Medical University of Silesia, Medykow 18, 40-752 Katowice, Poland*

Presenting Author: Email: pnowak@sum.edu.pl | Tel.: +48 32 088 743 | Fax: +48 32 088 743

INTRODUCTION:

Excessive fluoride intake in mammals is found to cause harmful effects to teeth, bones and other organs including brain. Neurological symptoms as reduced intelligence quotient in children, cognition and memory impairment and decreased learning ability have been reported in fluoride polluted areas [1]. From animal studies we have learned that chronic exposure to fluoride causes alterations in activity of the neurotransmitters and their metabolism, and results in a marked increase in oxidative stress, lipid peroxidation and a decrease in the activity of antioxidant enzyme in discrete brain regions. Furthermore, exposure to high level of fluoride through drinking water impairs glucose tolerance by decreasing insulin secretion resulting in hyperglycemia [2]. Conversely, glucose is the obligate energetic fuel for the central nervous system (CNS) and it is the only substrate able to completely sustain neural activity. Its levels represent a net balance among glucose uptake (from the circulation), glucose metabolism to lactate and CO_2, and glucose transport back to the circulation [3].

OBJECTIVES:

The aim of the present work was to determine the effect of chronic fluoride exposure on the (^{3}H)glucose uptake in the brain of adult Wistar rats.

MATERIALS AND METHODS:

For 4 weeks fluoride (NaF) 10 and 50 ppm was being added to drinking water of adult 8-weeks- old Wistar rats. Control rats was consuming tap water. On the last day of exposure, rats were injected ipwith6-[3H]-D-glucose (Amersham Radiochemicals, Pittsburgh, PA, USA; 41 Ci/mmol; 0.5 μCi/g BW). After 15 min, the rats were scarified and trunk blood was taken for plasma glucose (estimated using the glucose oxidase-peroxidase method with automated clinical chemistry analyzer - Optium Omega; Abbott, Alameda, USA) and insulin assay (estimated with the use of ELISA with commercially available kit; Rat Insulin ELISA EIA+ - 2048; DRG Instruments GmbH, Germany). Simultaneously brain specimens (cerebral cortex, hippocampus, striatum and thalamus with hypothalamus) and some peripheral tissues were taken for determination of radioactivity in a liquid scintillation counter. Each tissue sample was weighed and placed in a 20 ml scintillation vial containing 1 ml of Soluene-350 (Packard Inc., Downers Grove, Ill., USA). Each vial was then tightly sealed and incubated at 37°C for 48 h, to solubilize tissue. Later scintillation cocktail (10 ml, Dimilume-30, Packard Inc., Downers Grove, Ill., USA) was added, and the vials were briefly vortexed before being counted with a scintillation counter (Liquid Scintillation Counter, DSA 1409, Wallac, Finland). Results are presented as disintegrations permin (DPM) per 100 mg of wet tissue (mean SEM) for each group [4].

RESULTS AND DISCUSSION:

It was found that in rats intoxicated with a fluoride at lower concentration (10 ppm), [(3)H]glucose uptake was unaltered in all tested parts of the brain. However in rats exposed to higher levels of fluoride(50 ppm), [(3)H]glucose uptake in cerebral cortex, hippocampus and thalamus with hypothalamus was reduced in

comparison to saline group ($p < 0.05$) (Fig. 1). Changes in peripheral tissues (heart muscle, liver, kidney, lung, skin and bones) were negligible (data are not presented in graphical form). We also demonstrated that insulin plasma level was significantly elevated in rats exposed to fluoride (50 ppm) in comparison to control group, at the same time no changes in glucose plasma concentration were found (Fig. 2). It is well known that glucose metabolism not only meets the energy requirements of the brain, but also provides ribose precursors for the synthesis of nucleosides and NADPH, which is required for the synthesis of lipids and neurotransmitters as well as for the removal of free radicals [5]. Furthermore if we assume that increased glucose uptake (by neural tissue) more or less reflects neuronal activity, we can conclude that the damage produced by fluoride results in a compensatory increase in glucose uptake and utilization that enhances the regenerative ability of neurons and/or glia. The above, at least in part, explains our results.

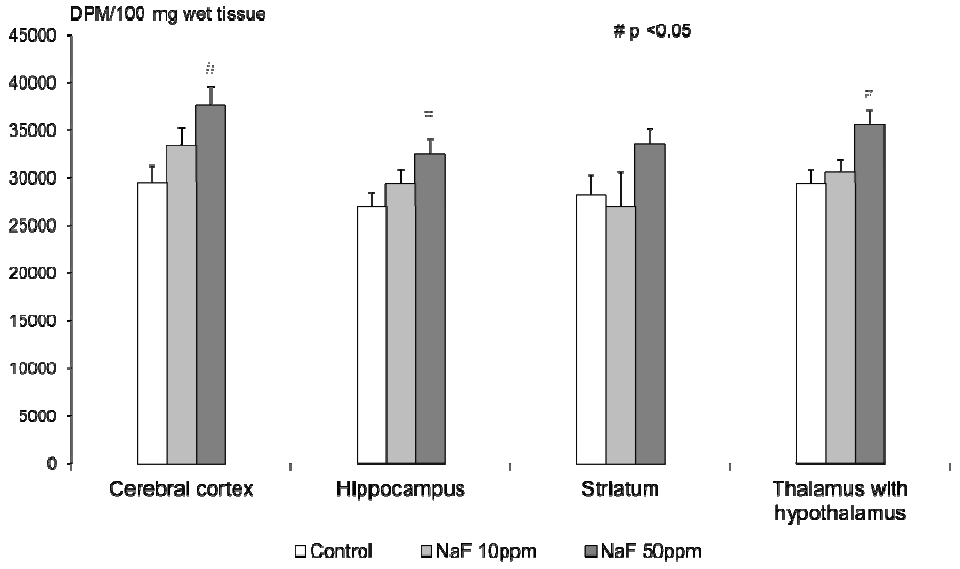


Figure 1 - Effect of fluoride intoxication on (3H)glucose uptake in the brain in adult rats (n=6)

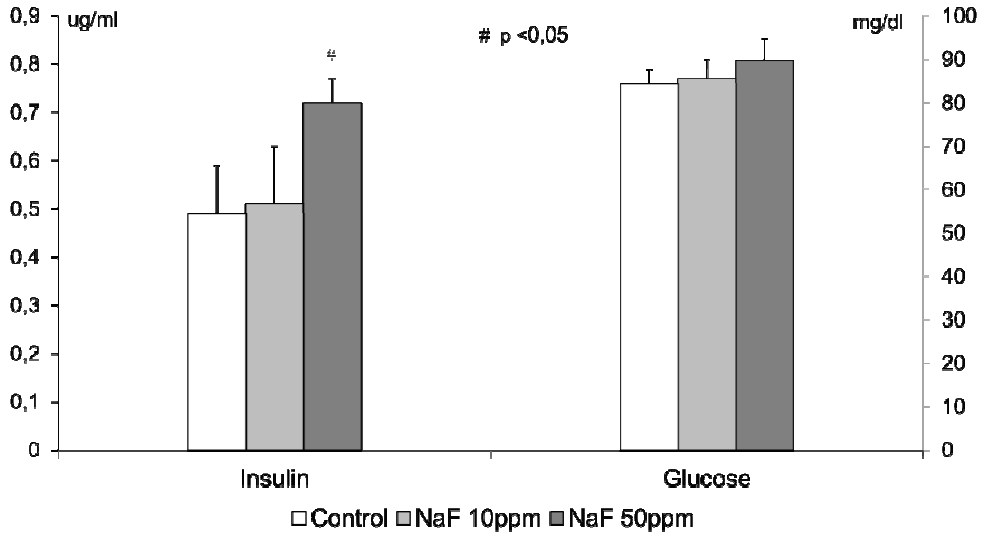


Figure 2 - Effect of fluoride intoxication on plasma glucose and insulin level in adult rats (n=8)

CONCLUSION:

The results of the present study suggest that the increase of glucose brain uptake may be a compensatory mechanism that supports the regenerative ability of neurons and/or glia damaged by fluoride ions.

ACKNOWLEDGMENTS:

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Perinatal manganese exposure and hydroxyl radical formation in rat brain

Authors: Michał Bałasz[1], Ryszard Szkilnik[3], Ryszard Brus[2], Jolanta Malinowska-Borowska[1], Damian Nowak[3], Richard M. Kostrzewa[4], Przemysław Nowak[1]

1 *Department of Toxicology and Occupational Health, Public Health Faculty, Medical University of Silesia, Medykow 18, 40-752 Katowice, Poland*

2 *Department of Nurse, High School of Strategic Planning, Koscielna 6, 41-303 Dabrowa Gornicza, Poland*

3 *Department of Basic Medical Sciences, Medical University of Silesia, Piekarska 18, 41-902 Bytom, Poland*

4 *Department of Biomedical Sciences, Quillen College of Medicine, East Tennessee State University, P.O. Box 70577, Johnson City, TN 37614, USA*

Presenting Author: Email: pnowak@sum.edu.pl | Tel.: +48 32 088 743 | Fax: +48 32 088 743

INTRODUCTION:

Manganese (Mn) is an enzymatic cofactor that plays an important role in a number of physiologic processes. However, when present at high tissue concentration, Mn produces cellular toxicity, including neurotoxicity in brain [1]. Occupational exposure (miners, smelters, welders, workers in dry-cell battery factories; also inhalation of Mn in aerosols/dusts) accounts for the major source of Mn intoxication in humans. An important but non-occupational source of Mn is methylcyclopentadienyl manganese tricarbonyl (MMT), the antiknock agent in gasoline. Another source is potassium permanganate, a powerful oxidizing agent for purifying drinking water, treating wastewater, and as an agricultural fungicidal and bactericidal agent. Occupational Mn exposure results in an imbalance between dopamine (DA) and γ-aminobutyric acid (GABA) in the basal ganglia, eliciting the syndrome called manganism, which shares multiple features with Parkinson's disease. Manganism has been observed recently in intravenous methcathinone abusers because this substance is illicitly produced by a potassium permanganate oxidation process. Additionally, Mn is a natural component of many foods, particularly of nuts, grains, and tea, and is an essential trace element used by humans in enzymatic processes. There are numerous reports of Mn intoxication related to long-term total parenteral nutrition [2]. The strongest correlation between any type of environmental exposure and increased susceptibility to Parkinsonism is observed in the Mn-exposed population [3].

OBJECTIVES:

The current study was conducted in order to assess the effects of paired gestational Mn exposure with overt dopaminergic neurotoxicity (i.e., 6-hydroxydopamine, 6-OHDA) on hydroxyl radical (HO•) production in rat brain. A better understanding of the causes of HO• production in brain and the effects on neurodegenerative processes is especially important because the brain is at high risk for oxidative injury because of (1) high levels of oxygen consumption combined with (2) low catalase, superoxide dismutase and glutathione peroxidase activity, (3) high iron concentration, and (4) elevated polysaturated fatty acid content of neuronal membranes.

MATERIALS AND METHODS:

Wistar rats were given tap water containing 10,000 ppm manganese chloride during the duration of pregnancy and until the time of weaning. Control rat dams consumed tap water without added Mn. Three days after birth, rats of both groups were treated with 6-OHDA at one of three doses (15 g, 30 g or 67 g, intraventricular on each side), or saline vehicle. Mn was assayed in control and Mn-exposed dams immediately following parturition. Rats were sacrificed, and the frontal cortex, neostriatum and hippocampus were dissected. The kidney, liver, femoral bone, femoral muscle and heart muscle also were removed. Mn levels were estimated in both control and Mn-exposed rats at P14 and P56. Approximately 100 mg of each tissue was dissolved in 1.0 ml of ultra-pure nitric acid (Merck). Next, Mn2+ content was assayed using SP-2900 PyeUnicam AA (Cambridge, UK) atomic absorption spectrometer and handled according to the Company's brochure. Results were presented in mg/g wet tissue [4].The indirect method for assessment

of HO• generation was applied. This method is based on quantitative, chromatographic measurement of 2.3-dihydroxybenzoic acid (2.3-DHBA), 2.5-dihydroxybenzoic acid (2.5-DHBA) and salicylic acid in the examined part of the brain. Control and Mn-intoxicated rats were injected with salicylic acid (100 mg/kg, i.p.). Thirty minutes later animals were decapitated. In 14-day-old offspring, the right hemisphere was taken for analysis; in 8-week-old rats, the frontal cortex, hippocampus, neostriatum, thalamus, cerebellum and pons were dissected and frozen on dry ice and stored at -80oC until HPLC/ED analysis. Microdialysis was performed in urethane-anesthetized rats. The microdialys is probe was implanted into the right neostriatum (A +0.7, L +3.0, V -7.0). The free ends of the probe were connected with Teflon tubes and continuously perfused with artificial cerebrospinal fluid containing 5 mM salicylic acid. The dialysate were injected into DHBA column and analyzed by HPLC/ED method [5].

RESULTS AND DISCUSSION:

We found that Mn content in the brain, kidney, liver and bone was significantly elevated in dams exposed to Mn during pregnancy. In neonates, the major organs that accumulated Mn were the femoral bone and liver. However, Mn was not elevated in tissues in adulthood. To determine the possible effect on HO• generation in Mn-induced neurotoxicity, we analyzed the contents of 2.3- and 2.5-dihydroxybenzoic acid (DHBA) (spin trap products of salicylate; HO• being an index of in vivo HO• generation). 6-OHDA-depletion of DA produced enhanced HO• formation in the brain tissue of newborn and adulthood rats that had been exposed to Mn; and the latter effect did not depend on the extent of DA denervation. Additionally, the extraneuronal, microdialysate, content of HO• in neostriatum was likewise elevated in 6-OHDA-lesioned rats. Interestingly, there was no difference in extraneuronal HO• formation in the neostriatum of Mn-exposed versus control rats. In summary, findings in this study indicate that Mn crosses the placenta but in contrast to other heavy metals, Mn is not deposited long-term in tissues. Also, damage to the dopaminergic system acts as a "trigger mechanism," initiating a cascade of adverse events leading to a protracted increase in HO• generation. And the effects of Mn and 6-OHDA are compounded.

CONCLUSION:

Ontogenetic Mn exposure, resulting in reactive oxygen species, HO• formation, represents a risk factor for dopaminergic neurotoxicity and development of neurodegenerative disorders.

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Flow cytometry assessment of cell death in haemocytes from *Mytilusgalloprovincialis* exposed to okadaic acid.

Authors: M.Veronica Prego-Faraldo[1], Vanessa Valdiglesias[2], Blanca Laffon[2], José M. Eirín-López[3], Josefina Mendez[1]

1 *XENOMAR Group Department of Cell and Molecular Biology, Universidade da Coruña, Spain.*
2 *DICOMOSA Group, Department of Psychology, Area of Psychobiology, Universidade da Coruña, Spain.*
3 *Chromatin Structure and Evolution (CHROMEVOL) Group, Department of Biological Sciences, Florida International University, North Miami, FL 33181, USA*

Presenting Author: Email: veronica.prego@udc.es | Tel.: +34 981 167 000 | Fax: +34 981 167 065

INTRODUCTION:

Within Harmful Algal Blooms (HABs), those commonly known as "red tides", constitute one of the most relevant sources of pollution in aquatic ecosystems. These blooms can end up producing very high concentrations of harmful biotoxins susceptible from being accumulated across the food chains, producing harmful effects on marine organisms and human populations, as well as causing significant economic losses. One of the most frequent and abundant biotoxins in the European coast is Okadaic Acid (OA). This compound is the main responsible for the Diarrhetic Shellfish Poisoning (DSP) syndrome, a gastrointestinal illness characterized by nausea, vomits, diarrhoea, and abdominal ache. Given its harmful impact on humans, the effects of OA acid on mammalian cell lines have been extensively studied. Yet, OA effects on bivalve molluscs have received little attention.

OBJECTIVES:

In the present work we determined the percentage of cell death on haemocytes of mussels Mytilus galloprovincialis in vitro exposed to okadaic acid.

MATERIALS AND METHODS:

Haemolymph was extracted from the adductor muscle of several mussels and cell viability was evaluated by trypan blue dye exclusion. Pooled haemolymph from 20 individuals was subsequenlty treated in vitro with OA and the percentages of early apoptotic and necrotic cells were evaluated by means of flow cytometry analysis of Annexin V- 7AAD double staining.

RESULTS AND DISCUSSION:

The cell viability determined in the starting sample was above 80%. After exposure, our results showed that none of the OA concentrations used (10, 50, 100, 200 and 500 nM) or exposure times (1 and 2 h) resulted in a significant increase in apoptosis levels. Such an apparent lack of OA-induced damage is in agreement with previous reports describing a decrease in the number of micronucleus after exposure to high concentrations of this toxin in the mussel Perna perna (Carvalho Pinto-Silva et al., 2005). Similarly, in vitro and in vivo studies have provided evidence suggesting low levels of apoptotic cells in samples with higher content of OA (Ferraz-Mello et al., 2010)(Prado-Alvarez et al., 2012).

CONCLUSION:

The present results consolidate the notion suggesting the existence of a protective mechanism against the harmful effects of OA in bivalve molluscs. Nevertheless, further studies are needed in order to fully elucidate the precise effects of OA on these organisms and the elements involved in their protection against this biotoxin.

ACKNOWLEDGMENTS:

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Evaluation of in vitro cytotoxic effects of oleic acid-coated magnetite nanoparticles on human neuronal cells

Authors: M.Verónica Prego-Faraldo[1], Carla Costa[2], Solange Costa[2], Gözde Kiliç[1,3], Aida Castelo[1,3], João Paulo Teixeira[2], Josefina Méndez[1], Vanessa Valdiglesias[3], Blanca Laffon[3], Eduardo Pásaro[3]

1 *Department of Cell and Molecular Biology, Universidade da Coruña, Spain*
2 *Department of Environmental Health, National Institute of Health, Portugal*
3 *DICOMOSA Group, Department of Psychology, Area of Psychobiology, Universidade da Coruña, Spain*

Presenting Author: Email: veronica.prego@udc.es | Tel.: +34 981 167 000 | Fax: +34 981 167 172

INTRODUCTION:

The specific properties of nanoscale particles, large surface-to-mass ratios and highly reactive surfaces, have increased their commercial application in many fields. However, the same properties are also important for the interaction and bioaccumulation of the non-biodegradable nanoscale particles in a biological system and are a cause for concern. Magnetite (Fe_3O_4) is one of the most frequently used forms of iron oxide nanoparticles. Magnetite nanoparticles (MNP) have great potential as magnetic resonance imaging contrast agents, heating mediators in hyperthermia-based cancer therapy and nanocarriers in targeted drug/gene delivery. Moreover, the superparamagnetic properties of MNP allow the delivery and trapping of drug-loaded MNP in the target site via an external magnetic field. The coating of MNP with synthetic and natural chemical moieties minimizes hydrophobic interactions, thus enhancing their desirable properties and biocompatibility. Due to the increasing biomedical applications, both diagnostic and therapeutic, of MNP a thorough investigation of their impact on human cells and of MNP-cell interactions is necessary.

OBJECTIVES:

The aim of this study was to evaluate the possible effects induced by oleic acid-coated MNP on SHSY5Y human neuroblastoma cell viability and functionality.

MATERIALS AND METHODS:

Dynamic light scattering (DLS) was used to characterize the size and zeta potential of oleic acid-coated MNP in water, complete cell culture medium and serum-free (incomplete) medium. A combination of cell viability assays was applied to evaluate MNP-induced cytotoxicity: MTT and Alamar Blue assays, based on mitochondrial functionality, and lactate dehydrogenase (LDH) assay, which determines cell membrane integrity. Three different times of exposure (3, 6 and 24 h) and ten doses in the range 5-300 µg/ml were tested.

RESULTS AND DISCUSSION:

The results data from DLS showed a similar hydrodynamic size for the MNP tested in water and in complete medium, but it was quite higher in incomplete medium, likely to be related to the instability of the nanoparticles in the absence of serum proteins. Besides, values of zeta potential were similar when the MNP were dispersed in complete and incomplete culture media, but they were around 3-fold lower than the value obtained in water. The results obtained from cytotoxicity evaluation showed negative results for the three tests applied at all treatment times up to 150 µg/ml. Higher concentrations induced some positive responses, especially for cell membrane integrity at 24 h exposure.

CONCLUSION:

Results obtained in this *in vitro* study demonstrate that oleic acid-coated MNP generally exhibit a low-toxicity profile and good biocompatibility, although neuronal cell viability may be affected at high concentrations.

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Impact of microcystin contaminated water on quality of carrots (*Daucuscarota*)

Authors: J. Machado[1,2], J. Azevedo[1], M. Freitas[1,5], E. Pinto[3,5], V. Vasconcelos[1,4], A. Campos[1]

1 *Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Rua dos Bragas 289, P 4050-123 Porto, Portugal*

2 *Institute of BiomedicalSciences Abel Salazar, Porto University, Rua de Jorge Viterbo Ferreira 228, P 4050-313 Porto, Portugal*

3 *REQUIMTE, Department of Chemical Sciences, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira 228, P 4050-313 Porto, Portugal*

4 *Department of Biology, Faculty of Sciences, University of Porto,Rua do Campo Alegre, P 4069-007 Porto, Portugal.*

5 *Polytechnic Institute of Porto. Superior School of Health Technology of Porto. CISA/Research Center in Environment and Health, Rua de Valente Perfeito, 322, P 440-330 Gaia, Portugal*

Presenting Author: Email: joana.ffmachado@gmail.com

INTRODUCTION:

Cyanobacteria blooms are often found in freshwaters and may reflect the increased eutrophication of these environments and because of this many water resources worldwide may have a limited utilization. The presence of these microorganisms may pose a serious threat to water quality because many of them produce a large diversity of toxins that can be harmful to human health (Figueiredo *et a*l., 2004). On the other hand contaminated waters used to agricultural purposes may pose additional concerns to food safety. The absorption of toxins by plants may induce morphological and physiological changes that can lead to a loss of productivity as well as toxin bioaccumulation in edible tissues. However, the impact of cyanotoxins on plants and its ability to enter the food chain via this pathway is not fully understood (Kitler*et al.*, 2012).

Microcystin (MC) is the cyanotoxin most frequently present in eutrophic freshwaters causing serious problems to human health, being the liver the main target. These toxins are potent and specific inhibitors of protein phosphatases PP1 and PP2Ain both animals and higher plants (Figueiredo et al., 2004). It is known that MC affect a number of physiological processes in plants (Corbel *et al.*, 2013).

The plant root system is usually more exposed, via soil irrigation, and therefore root-vegetables more prone to contamination. Carrots (*Daucuscarota*) are root-vegetables with great importance for human nourishment and economy, with extensive use all over the world (Singh *et al.*, 2012).

This work aims to evaluate the effects of the use of water containing MC in the growth and production of this specific horticultural crop as well as toxin accumulation.

MATERIALS AND METHODS:

Young carrots (˜ 1 month old) were grown in soil during 1 month in greenhouse conditions. Three groups were performed with plants being irrigated twice a week with non-contaminated water (control group) or with a crude *Microcystis aeruginosa* extract containing respectively 10 and 50 µg/L MC.

Fresh weight of plants was determined 3 times (0, 15 and 30 days), weighing leaves and roots (carrot) and dry weight was estimated in lyophilized leaves and roots.

Chlorophyll fluorescence was accessed through pulse amplitude modulation (PAM) fluorometry following the method described by Maxwell and Johnson (noninvasive method)(Maxwell & Johnson., 2000). Plants were adapted to darkness for at least 30 minutes and then leaves were illuminated with a pulse of saturating light and the fluorescence emitted was measured using PAM 2000 (Walz, Effeltrich, Germany).

The accumulation of toxin on soil and in carrots was determined using the MicroCystest which is based on the phosphatase activity inhibition by MC.

RESULTS AND DISCUSSION:

No significant variations (P<0.05) in fresh and dry weight of leaves and carrots were observed at any concentration of toxin, suggesting that the plant exposure to a *M. aeruginosa* toxic extract containing concentrations of MC between 10 and 50 µg/L does not impair plant growth. The results showed that both short and prolonged exposure to MC concentrations does not seem to be a stress factor for plants leading, inclusively, to a stimulation of photosynthesis. The results of MicroCystest showed a significant accumulation in the soil (13,225µg MC /g soil), however accumulation of MC in carrots is below the limit of detection of the method.

CONCLUSION:

According to our knowledge this is the first work reporting the effects of MC on carrots, in which growth, photosynthesis and accumulation were investigated. Exposure of carrots to these concentrations of MC did not seems to have adversely affected any of the parameters evaluated. However we hypothesize that other physiological and metabolic processes (synthesis of vitamins and nutrient accumulation) should be also studied in the future.

Our future work will focus on the evaluation of the toxin accumulation with more sensitive techniques (liquid chromatography and mass spectrometry)and the nutritional value of carrots (nutrients and vitamins).

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Cytostatics occupational exposure - genotoxic effects assessment

Authors: Carina Ladeira[1,2], Susana Viegas[1,2], Elisabete Carolino[1], Manuel C. Gomes[3], Miguel Brito[1]

1 *Escola Superior de Tecnologia da Saúde de Lisboa, Av. D. João II, lote 4.01.69, 1990-096 Lisboa, Portugal*
2 *CMDT - PublicHealth and Policy, Escola Nacional de Saúde Pública, ENSP, Universidade Nova de Lisboa,1600-560 Lisboa, Portugal*
3 *Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisboa, Portugal*

Presenting Author: Email: carina.ladeira@estesl.ipl.pt | Tel.: +351 218 980 445 | Fax: +351 218 980 460

INTRODUCTION:
Although the potential therapeutic benefits of hazardous drugs outweigh the risks of side effects for ill patients, exposed health care workers can have the same side effects with no therapeutic benefit. Workers may be exposed to a drug at different stages of its life cycle, being in the hospital settings the main focus dwelled upon the pharmacy, and nursing personnel. The exposure to these substances is epidemiologically linked to cancer and nuclear changes detected by the cytokinesis-block micronucleus test (CBMN). This method is extensively used in molecular epidemiology, since it determines several biomarkers of genotoxicity, such as micronuclei (MN), which are biomarkers of chromosomes breakage or loss, nucleoplasmic bridges (NPB), common biomarkers of chromosome rearrangement, poor repair and/or telomeres fusion, and nuclear buds (NBUD), biomarkers of elimination of amplified DNA.

OBJECTIVES:
The aim of this study is to compare the frequency of genotoxicity biomarkers, provided by CBMN assay in peripheral lymphocytes between pharmacists, pharmacy technicians, and nurses occupationally exposed to cytostatic drugs and a control group.

MATERIALS AND METHODS:
The group of cases was constituted by 46 workers exposed occupationally to cytostatics and 46 unexposed individuals (controls), from whom both peripheral blood was collected in order to measure the genetic endpoints proposed in this study.

RESULTS AND DISCUSSION:
All the genotoxicity biomarkers studied presented statistical significant differences between exposed and non-exposed subjects to cytostatics (Mann-Whitney test), namely MN (9.83±1.28 *vs*5.09±0.89, $p \leq 0.0001$), NPB (0.65±0.140 vs 0.11±0.05, $p=0.001$) and NBUDs (2.43±0.37 vs 1.37±0.32, $p=0.006$), MN in mononucleated cells (1.35±0.32 vs 0.41±0.11, $p=0.027$), and MN in multinucleated cells (4.09±0.78 vs 1.46±0.22, $p=0.044$) respectively. Using multiple linear regression all the variables under study; gender, tobacco and alcohol consumption did not show significant results for all the genotoxicity biomarkers, only age had a significant result in the frequency of MN ($p \leq 0.002$) in both groups.

CONCLUSION:
Occupational exposure to cytostatics drugs is associated with genotoxic effects that can be evaluated by biomarkers. In this study, the results suggest that pharmacy and nursing personnel who prepare and/or administer cytostatics drugs in the studied Portuguese units showed increase frequency of genotoxicity biomarkers in comparison with non-exposed.

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Neurotoxicity induced by silica-coated iron oxide nanoparticles

Authors: Carla Costa[1,2], Gözde Kiliç[3,4], Solange Costa[1,2], Eduardo Pásaro[3], Vanessa Valdiglesias[3], Blanca Laffon[3], João Paulo Teixeira[1,2]

1 *Department of Environmental Health, Portuguese National Institute of Health, Porto, Portugal*
2 *Institute of Public Health, University of Porto, Porto, Portugal*
3 *DICOMOSA Group, Department of Psychology, Area of Psychobiology, Universidade da Coruña, Spain*
4 *Department of Cell and Molecular Biology, Universidade da Coruña, Spain*

Presenting Author: Email: jpft12@gmail.com | Tel.: +351 223 401 147 | Fax: +351 223 401 149

INTRODUCTION:
Superparamagnetic iron oxide nanoparticles smaller than 30 nm constitute an efficient contrast agent used to enhance relaxation differences between healthy and pathological tissues due to their high saturation magnetization, high magnetic susceptibility and apparent low toxicity. The dramatic growth of possible applications that these nanoparticles (NPs) have to offer accompanies the risks associated with exposure. Recent findings reported that some engineered NPs are able to pass the blood-brain barrier and reach the brain of small animals raising concern on the neurotoxic potential of nanomaterials. Therefore, there is a considerable need to address biocompatibility and biosafety concerns associated with their usage, namely in human *in vivo* imaging.

OBJECTIVES:
Given the unknown possible effects of these particles in the human body, the main objective of this work was to examine the possible cytotoxic effects of silica-coated iron oxide (Fe_3O_4, magnetite) NPs in human neuroblastoma (SHSY5Y) cell line.

MATERIALS AND METHODS:
NPs were characterized for their size and zeta potential by DLS (dynamic light scattering). Human neuroblastoma SHSY5Y cells were exposed to different concentrations of NPs, prepared in either complete cell culture medium or incomplete (serum-free) cell culture medium for three exposure times (3, 6, 24h). Cell viability was evaluated after exposure to NPs by means of the MTT assay, NRU assay and Alamar Blue assay. Membrane integrity was also evaluated through lactate dehydrogenase (LDH) assay in cell culture medium. For each experimental condition three independent experiments were performed.

RESULTS AND DISCUSSION:
The mean hydrodynamic diameter of NPs (0.3 mg/ml) in water, complete medium, and incomplete medium was 93.3 nm, 111.1 nm and 141.6 nm, respectively. Zeta potential was -32 mV in water, -10 mV in complete medium and -22 mV in incomplete medium. Silica-coated magnetite NPs prepared in incomplete medium induced a dose-dependent decrease in viability of SHSY5Y cells after 24 h treatments, assessed by MTT, NRU and LDH assays (significant only at high concentrations). The differences observed in viability after exposure to NPs prepared in complete and serum-free medium are probably due to the fact that NPs suspended in supplemented medium can be covered by proteins reducing their ability to be taken up by the cells and induce toxicity.

CONCLUSION:
Even though results suggest that these NPs are biocompatible at low concentrations, further studies on this matter are necessary to understand the underlying mechanism for the cytotoxicity observed at high concentrations and to examine possible genotoxic effects even at low concentrations. These results contribute to the knowledge of the effects of magnetite NPs on the human nervous system. Understanding the potential harmful effects

that these NPs may pose to humans is essential to establish satisfactory regulatory policies on the safe use of magnetite NPs in human *in vivo* imaging.

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Transplacental exposure to tobacco. Observed genotoxicity and epigenetic alterations.

Authors: Carla Costa[1,2], João Paulo Teixeira[1,2]

1 *Department of Environmental Health, Portuguese National Institute of Health, Porto, Portugal*
2 *Institute of Public Health, University of Porto, Porto, Portugal*

Presenting Author: Email: jpft12@gmail.com | Tel.: +351 223 401 140 | Fax: +351 223 401 149

INTRODUCTION:
For the last 200 years, there is evidence of the harmful effects of tobacco, initially in relation to oral cancer, and then associated with vascular disease and lung cancer. Since then, our knowledge has expanded significantly and scientific evidence was published documenting the implications not only of tobacco use, but also of exposure to tobacco in morbidity and mortality.

Research on diet and cancer-related effects has so far focused mainly on adult populations; however, increased vulnerability towards *in utero* carcinogenic exposures has been proposed due to a high rate of cell proliferation, relatively high numbers of target cells at risk, altered ability to repair DNA damage, immaturity of metabolism, endocrine immunological systems and longer life span ahead in which to develop chronic disease as compared with adults. Thus, studies based on cancer-related biomarkers of exposure and effects in umbilical cord blood have been initiated to investigate the effects of *in utero* exposures related to maternal exposures.

OBJECTIVES:
The present study aims to review available data on DNA damage and changes in patterns of DNA methylation in newborns resulting from *in utero* exposure to tobacco.

MATERIALS AND METHODS:
The identification of studies to be included in this review was carried out through literature search using the PubMed database. Eligible studies were those conducted in humans, written in English, reporting DNA damage or DNA methylation in newborns and/or placental tissue and with well-characterized *in utero* exposure to tobacco.

RESULTS AND DISCUSSION:
Results showed several knowledge gaps relating, for example, to the impact of genotype, nutritional and life-style factors, methodological concerns and prospective association of DNA damage and DNA methylation with disease risk, to mention a few. On this basis, further investigations are required to provide scientific support for the possible implementation of coherent and effective health promotion and disease prevention in early stages of life.

CONCLUSION:
Data here obtained identifies knowledge gaps in research related to risk of exposure and maternal consumption of tobacco for the health of the newborn (specifically, the genotoxic effects). Understanding the harmful effects of tobacco on the health of a developing fetus is a compelling reason to promote cessation of tobacco use during pregnancy and optimize the current and future health not only of women but also their children.

ACKNOWLEDGMENTS:
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Cr(VI)-induced cytotoxic and genotoxic effects in human bone cells *in vitro* at concentrations found in patients with metal-on-metal(MoM) prostheses

Authors: Cristina Monteiro[1], Helena Oliveira[1], Conceição Santos[1]

1 *Laboratory of Biotechnology and Cytomics, CESAM and Department of Biology, University of Aveiro, Portugal*

Presenting Author: Email: cristinamonteiro@ua.pt | Tel.: +351 234 370 350 | Fax: +351 234 372 587

INTRODUCTION:

Chromium (Cr) has been extensively used in numerous industries, as chromate production, chromate pigment production, chrome plating, etc. and the hexavalent state [Cr(VI)] is considered human carcinogen (Chiu et al., 2010). Metal-on-metal (MoM) prostheses are often made of stainless steel or cobalt (Co)-Cr alloys. As metals in contact with biological systems undergo corrosion, these implants are associated with high levels of Co and Cr ions in the serum and blood of patients (Keegan, Learmonth & Case, 2008), raising the urge of a comprehensive evaluation of these metals toxicity in a broad range of human cell types.

OBJECTIVES:

To evaluate cytotoxic and genotoxic effects of clinically relevant Cr(VI) concentrations on the human osteoblast-like cell line MG-63. The lowest doses of Cr(VI) in the assays, 0.1 and 0.5 μM, are concentrations previously found in patients with MoM prostheses (Figgitt et al., 2010). Higher Cr(VI) concentrations were tested to better understand the cellular response depending on the concentrations applied.

MATERIALS AND METHODS:

Human osteoblast-like cell line MG-63 was *in vitro* cultured in minimum essential medium (MEM)-α medium without nucleosides, supplemented with 10% (v/v) fetal bovine serum, penicillin, streptomycin and fungizone at 37 °C, 5% CO_2, in humidified atmosphere. For metal exposure, cells were left 24 h for adhesion. Then, the medium was replaced by medium containing potassium dichromate at concentrations ranging from 0.1 to 5 μM. Culture medium without Cr(VI) served as control in each experiment. For the assessment of cell viability by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, MG-63 cells were cultured in the referred conditions for 24, 48, and 72 h. The level of DNA damage was assessed using comet and cytokinesis-block micronucleus (CBMN) assays, showing from low levels of DNA damage to formation of micronuclei (MN) (a biomarker of chromosome breakage and/or whole chromosome loss), and nucleoplasmic bridges (NPBs) (a biomarker of DNA misrepair and/or telomere end-fusions) (Fenech, 2007). Cell cycle alterations induced by Cr(VI) were analyzed by flow cytometry and CBMN assay.

RESULTS AND DISCUSSION:

The results pointed out 20% of decrease in cell viability after 24 h of Cr(VI) exposure at the highest dose (5 μM) and no cytotoxic effects at lower doses. With 48 and 72 h of exposure, the decrease of cell viability showed a dose- and time-dependent relationship, where the inhibitory concentration 50% (IC_{50}) is 3.076 ± 0.419 μM at 48 h and 1.342 ± 0.053 μM at 72 h. At 48 and 72 h of metal exposure only the lowest tested Cr(VI) concentrations were non-cytotoxic, i.e. 0.1-1 μM Cr(VI) at 48 h and 0.1 μM Cr(VI) at 72 h. Due to the highly cytotoxic effects of Cr(VI) after 72 h, the evaluation of genotoxic effects was done to the cells exposed for 24 h and 48 h. After visual scoring analysis of nucleoids observed in the comet assay, it was detected DNA damage at non-cytotoxic doses, i.e. 1-4 μM Cr(VI) at 24 h and 0.1-1 μM Cr(VI) at 48 h. Cr(VI) induced an increasing number of MN at 24 h of exposure from the lowest Cr(VI) dose (0.1 μM) to 1 μM, and formation of NPBs at the concentration of 1 μM. At 48 h an increase of MN frequency was observed with the Cr(VI) dose of 1 μM. Flow cytometric analysis confirmed the clastogenic effects of Cr(VI). Regarding cell cycle changes, there were significant effects on the cell cycle in all groups of Cr(VI)-exposed cells after 24 h of

exposure and for concentrations above 0.1 μM Cr(VI) after the 48 h, with accumulation of cells at G_2 phase and also at S phase when cells were subjected to 5 μM Cr(VI). Despite the cell cycle arrest at G_2 phase, the nuclear division index (NDI) was not affected at the doses up to 1 μM at both times of exposure, 24 and 48 h.

CONCLUSION:

Cr(VI) decreases cell viability of the human osteoblast-like cell line MG-63 in a time- and dose-dependent manner, however at Cr(VI) concentrations found in patients with MoM prostheses, namely 0.5 μM, the cell viability was only reduced after 72 h of Cr(VI) exposure. DNA damage was found at non-cytotoxic doses of Cr(VI), including at Cr(VI) concentrations found in patients with MoM prostheses (0.1 and 0.5 μM) after 48 h of exposure. Regarding the formation of MN and NPBs, only MN were detected at concentrations found in patients (0.1 and 0.5 μM) after 24 h of Cr(VI) exposure. Cr(VI) has induced cell cycle arrest at G_2 and/or cell cycle delay at S phase on exposed cells at both times of exposure. With the clinically relevant Cr(VI) doses found in patients with MoM prostheses (0.1 and 0.5 μM) there was a cell cycle arrest at G_2 phase, however the NDI was not affected. Overall, Cr(VI) is cytotoxic and genotoxic to human bone cells at Cr(VI) concentrations found in patients with MoM prostheses and higher doses after short time periods under the conditions tested.

ACKNOWLEDGMENTS:

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Toxicity testing of simvastatin, sertraline, 4-MBC, propylparaben and triclocarban using zebrafish and sea urchin embryos bioassays

Authors: Tiago Torres[1], Sílvia Ribeiro, Isabel Cunha[1], Rosário Martins[1,3], Miguel Machado Santos[1,2]

1 *CIMAR/CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal*
2 *FCUP -Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal*
3 *CISA - Research Centre on Health and Environment, Superior School of Health Technology of Porto, Polytechnic Institute of Porto, Porto, Portugal*

Presenting Author: Email: mrm@estsp.ipp.pt | Tel.: +351 222 061 000 | Fax: +351 222 061 001

INTRODUCTION:

In the past decade, many emergent compounds, including some active substances and ingredients of Pharmaceuticals and Personal Care Products (PPCPs) have been detected in water at levels that can negatively impact aquatic ecosystems (Lapworth et al., 2012; Jiang et al., 2013). The recent knowledge of their occurrence has raised concerns about human health effects and ecosystem risks. Although these compounds are frequently detected at concentrations that are not likely to induce adverse effects in humans and may be too low to cause acute effects in other organisms, there is still a serious lack of information about the effects in non-target species, particularly considering chronic exposure or effects resulting from interactions between them (Lapworth et al., 2012).

Pharmaceuticals and Personal Care Products enter the aquatic environment from different point and non-point sources and wastewater treatments plants cannot ensure complete removal of many compounds, and therefore they may be present at significant concentrations in effluents (Jiang et al., 2013). Hence, it is essential to understand the effects of these substances on aquatic organisms. Owing to the large number of new chemicals that must go through toxicity testing, short-term early-life-stages have been frequently used as an alternative to long-term exposures due to its high sensitivity and logistic advantages.

OBJECTIVES:

The main aim of the present work was to assess the toxicity of five emerging pollutants: simvastatin, sertraline, triclocarban, propylparaben and 4-methylbenzylidene camphor (4-MBC) during the embryonic development of zebrafish (*Daniorerio*) and the sea urchin (*Paracentrotuslividus*).

MATERIALS AND METHODS:

After fertilization, zebrafish and sea urchin eggs were randomly allocated to 24 wells plate. The experimental solutions were obtained by diluting the stock solutions of the five selected compounds in artificial seawater (sea urchin assays) or in freshwater (zebrafish assays). All solutions were prepared in order to have a final DMSO concentration of 0.01%. The 24-wells plates of zebrafish assay were incubated at 26.5ºC during 80h and under the same photoperiod conditions as the zebrafish stock. The medium was renewed daily in order to maintain oxygen and toxic nominal concentrations constant during the assay and to remove fungi or other organisms that could develop in the well. Sea urchin embryos were incubated at 20ºC in dark for 48h and were fixed by adding three drops of 37% formaldehyde at the end of exposure. Zebrafish embryos observations were performed at 8, 32 and 80 hours post fertilization and different parameters were recorded (Table 1), while sea urchin observations were only performed at the end of the assay and larvae length of fixed organisms was measured.

Table 1-Endpoints recorded at 8, 32 and 80hours post fertilization (hpf) in zebrafish assay

Endpoint	8hpf	32hpf	80hpf
Mortality rate	*	*	*
75% of epiboly stage	*		
Abnormal cell growth	*		
Head abnormalities		*	*
Tail abnormalities		*	*
Eyes abnormalities		*	*
Yolk-sac abnormalities		*	*
Pericardial edema		*	*
Heart rate		*	*
Hatching rate			*
Muscular involuntary contractions[(1)]			*

[(1)]Only for 4-MBC exposure

RESULTS AND DISCUSSION:

All selected compounds induced significant effects on the embryonic development of both test species after individual exposure (Table 2). These effects were compound and concentration dependent. However our results show that sea urchin embryos were more sensitive than zebrafish embryos. Regarding the relative toxicity, simvastatin showed the highest toxicity in zebrafish bioassay, while triclocarban was the most toxic compound in sea urchin bioassay. Furthermore, triclocarban and 4-MBC induced significant effects in sea urchin embryos at concentrations close to reported concentrations in surface water.

Table 2 - Overview of NOEC and LOEC values of selected PPCPs reported in this study. Maximal concentrations of selected PPCPs reported in the literature in surface water and in WWTPs influents and effluents. (µg/L)

	Zebrafish		Sea urchin		Maximal concentrations		
PPCP	NOEC	LOEC	NOEC	LOEC	WWTPs Influents	WWTPs Efluents	Surface water
SIMV	5.0	50.0	2.0	5.0	0.09	0.001	0.0001
SER	1000.0	10000.0	4.0	10.0	0.02	0.015	0.57
4-MBC	500.0	5000.0	0.8	2.0	6.5	2.7	*0.799
PP	1000.0	10000.0	160.0	400.0	2.8	0.021	0.207
TCC	100.0	1000.0	0.256	0.64	50.0	> 10.0	6.75

CONCLUSION:

This study highlights the risk of these compounds to aquatic ecosystems. Hence, it is important to conduct more comprehensive studies on possible chemical interactions in the environment and the mechanisms involved in order to perform more reliable risk assessment and to implement guidelines for the protection of the aquatic environment.

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Evaluation of safety of synthetic trinuclearchromium(III) glycinatecomplex in rat

Authors: Zbigniew Krejpcio[1], Halina Staniek[1], Rafal W. Wojciak[1], Ewelina Król[1], Daria Wieczorek[2], Henryk Szymusiak[2]

1 *Department of Human Nutrition and Hygiene, The Poznan University of Life Sciences, Poznan, Poland*

2 *Department of Technology and Instrumental Analysis, The Poznan University of Economics, Poznan, Poland*

Presenting Author: Email: zkre@up.poznan.pl | Tel.: +48 618 487 336 | Fax: +48 618 487 332

INTRODUCTION:

Chromium(III) has been considered as an essential element playing a role in carbohydrate and lipid metabolism [4]. Among various Cr compounds proposed for dietary supplements and therapeutics, of particular interest is the Cr(III) propionate cation (CrProp), $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]$+, known as Cr3 [1, 2]. This compound belongs to the trinuclearoxo-centered metal carboxylate assemblies of the general formula $[Me_3O(O_2CR)_6L_3]^+$ (where: Me = trivalent 3d metal, R = aliphatic or aromatic group, L = monodentate ligand). CrProp was found to mimic the ability of low-molecular-weight chromium-binding substance or chromodulin to stimulate tyrosine kinase activity of the insulin receptor. Clodfelder et al. [1] reported that $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ is absorbed with very high efficiency of 40-60%, while popular Cr supplements such as: $CrCl_3$, Cr(III) nicotinate or Cr picolinate are absorbed at only 0.5-1.3% of the gavaged dose.

In this study, a new form of trinuclear chromium(III) complex with glycine $[Cr_3O(NH_2CH_2CO_2)_6(H_2O)_3]^+NO_3$ (CrGly)was synthesized as a potential source of supplementary Cr, and evaluated concerning its safety.

OBJECTIVES:

This study aimed at assessing the safety of CrGly in rat (subchronic toxicity assay)

MATERIALS AND METHODS:

Chromium(III) glycinatecomplex (CrGly), in the form of nitrate salt $[Cr_3O(NH_2CH_2CO_2)_6(H_2O)_3]^+NO_3 \bullet H_2O$ (25.7%Cr) (**Please see Figure 1)**, was synthesized in our laboratory according to the method described previously [5].

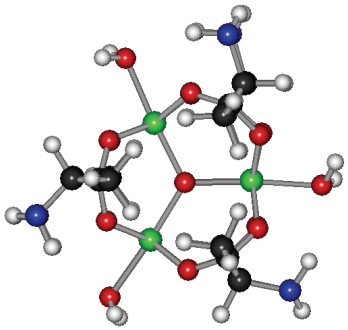


Figure 1 - Chemical structure of CrGly $[Cr_3O(NH_2CH_2CO_2)_6(H_2O)_3]^+$cation

The safety of CrGly was evaluated using subchronic toxicity assay (90-day test) performed on 64Wistarrats (32 males and 32 females) fed *ad libitum*standard *Labofeed B* diets, supplemented with CrGly, at dosages of 1 (control), 10, 50 and 500 mg Cr/kg diet (approx. 0.1; 1; 5 and 50 mg Cr/kg b.w./day) for 12 weeks. The effects of supplementary Cr on feed intake, body mas gain, FER, internal organs weights, blood morphology and haematology indices, blood/serum biochemistry indices, blood lymphocyte genotoxicity (alkaline comet assay), as well as Fe and Cr levels were evaluated.

RESULTS AND DISCUSSION:

The acute toxicity class of CrGly was assessed in our previous paper [3]. The results demonstrated that LD_{50} of CrGly is greater than 2,000 mg/kg when administrated orally to rat; thus, this compound appears to belong to the fifth category in the GHS system or the fourth class ("unclassified") in the EU classification system.

In this subchronic toxicity assay, supplementary CrGly given with diet at dosages of 1-50 mg Cr/kg b.w./day, to male and female rats did not affect overall nutritional and growth indices, blood morphology and haematology indices, blood/ serum biochemistry indices (lipid profile, glucose, insulin, TAG, urea, creatinine, protein, ALT, AST, ALP), Fe status indices, did not produce genotoxic changes in blood lymphocytes (*alkaline comet assay*), however higher dosages of CrGly (5 and 50 mg Cr/kg b.w./day) increased Cr levels in liver and kidneys of rat **(Please see Table 1).**

Table 1 - Tissular Cr content in rats

Group	Supplementary Cr (mg/kg b.w./day)	Male rats		Female rats	
		Liver Cr (µg/g d.m.)	Kidney Cr (µg/g d.m.)	Liver Cr (µg/g d.m.)	Kidney Cr (µg/g d.m.)
Control	0.1	0.835 a	0.846 a	1.354 a	1.240 a
10	1	1.950 b	1.099 ab	1.306 a	1.387a
50	5	2.204 c	1.869 ab	1.585 a	2.727 b
500	50	3.578 d	11.152 c	7.250 b	15.797 c
p		P < 0.001	P < 0.001	P < 0.001	P < 0.001

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CONCLUSION:

Trinuclear Cr complex with glycine (CrGly) given by gavage at dosages of 1-50 mg Cr/kg b.w. (approx. 4-200 mg/kg b.w./day for 90 days) does not affect major physiological indices, thus may be considered as safe for rat. However, prolonged exposure to this compound may lead to accumulation of Cr in liver and kidneys.

ACKNOWLEDGMENTS:

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Changes in the proportions of leucocytes and oxidative stress markers in lymph nodes

Authors: Ana Mojsović Ćuić[1], Irena Landeka Jurčević[2], Vesna Benković[3], Duje Lisičić[3], Anica Horvat-Knežević[3], Lana Feher-Turković[1], Nada Oršolić[3], Domagoj Đikić[3]

1 *University of Applied Health Studies, Mlinarska cesta 38, Zagreb, Croatia*
2 *Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia*
3 *Faculty of Science, Department of Animal Physiology, University of Zagreb, Zagreb, Croatia*

Presenting Author: Email: ana.mojsovic.cuic@gmail.com | Tel.: +385 1 5495981 | Fax: +385 1 5495900

INTRODUCTION:

Most of the toxicological and ecotoxicological studies of pesticides have explored pesticide individually. However, the concentration of pesticide residues detected in food, entries daily through the diet in the human and is often a mixture of various residual pesticides absorbed simultaneously (Hughes and Woods, 2002; Groten, 1999). It is known, though insufficiently explored, that exposure to combinations of pesticides can result in an additive, synergistic, antagonistic or potentiation effects on the cells, mainly because the metabolism of one pesticide may affect the metabolism of the other (Plavšić,1999; Norgaard & Cedergreen, 2010). Pesticides imazalil, cypermethrin and carbendazim are the most common pesticides residual detected in the fruit and vegetables on market in European countries. Combined actions and interactions of these three pesticides in food are still unexplored.

OBJECTIVES:

The aim of our research was to explore the effects of low doses of pesticides imazalil, cypermethrin and carbendazim in combinations, on the leukocytes counts and oxidative stress markers in lymph nodes of Swiss mice.

MATERIALS AND METHODS:

The design of the experiment was consistent with the written standard OECD 407. The animals were distributed into 7 groups (14 animals per group). Each group was exposed to the NOAEL doses of the each individual pesticide and their combinations: imazalil+cypermethrine, cypermethrine+carbendazime, carbendazime+imazalil (NOAEL, 1:1). Pesticides were applied orally during 28 days on a daily basis. After the 28-day period the animals were anesthetized and blood samples were taken by cardiac puncture. Standard haematological methods were used to measure leukocyte count (WBC) and differential blood count was expressed as relative percentage of each type of white blood cell. Oxidative stress in the lymph nodes was determined in two ways; measuring the formation of antioxidant enzymes in the tissues-superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) as well as by the occurrence of products of lipid peroxidation caused by oxidative stress, such as malondialdehyde (MDA) and 8-iso-prostaglandinF2α.

RESULTS AND DISCUSSION:

The group that was exposed to imazalil and carbendazim and to a combination cypermethrin+carbendazim showed a statistically significant ($p \leq 0.05$) increase in the number of leukocytes in comparison to the control group, while the group that was exposed to the combination of imazalil+carbendazim showed a statistically significant ($p \leq 0.05$) decrease in the number of white blood cells in comparison to the control group (Figure 1). The number of lymphocytes was the most statistically ($p \leq 0.05$) increased after the treatment with imazalil+carbendazim, while completely opposite happened in the group treated with the combination of imazalil+carbendazim in which there was statistically significant ($p \leq 0.05$) decrease in the number of lymphocytes compared with the control group. A statistically significant change ($p \leq 0.05$) in the number of monocytes and neutrophil occurred in all treatment groups compared to the control group. While all pesticides and their combination caused a statistically significant ($p \leq 0.05$) increase in the number of

neutrophils, at the same time, the combination of imazalil+carbendazim showed a statistically significant ($p \leq 0.05$) decrease in the number of monocytes compared with the control group. Catalase activity in the popliteal lymph nodes was significantly ($p \leq 0.05$) increased compared to the control group, in the group treated with carbendazim and in the group treated with cypermethrin, as well as in groups treated with the combination cypermethrin+carbendazim and imazalil+carbendazim (Table 1).

Furthermore, in the case of treating animals with imazalil there was statistically significant ($p \leq 0.05$) decrease in the concentration of reduced glutathione (GSH) in the popliteal lymph nodes, while the activity of superoxide dismutase (SOD) was statistically significant ($p \leq 0.05$) decreased in two treatment groups, with cypermethrin and cypermethrin+carbendazim compared to the control group (Table 1). Concentration of 8-iso-prostaglandin F2α in the popliteal lymph nodes was significantly ($p \leq 0.05$) increased compared to the control group of animals by pesticide exposure to the carbendazim while in any other experimental group there were not statistically significant ($p \leq 0.05$) changes compared to the control (Table 1).

Pesticide exposure of animals, either separately or in combination, did not cause statistically significant ($p \leq 0.05$) increase or decrease the concentration of MDA in the popliteal lymph node in any group (Table 1).

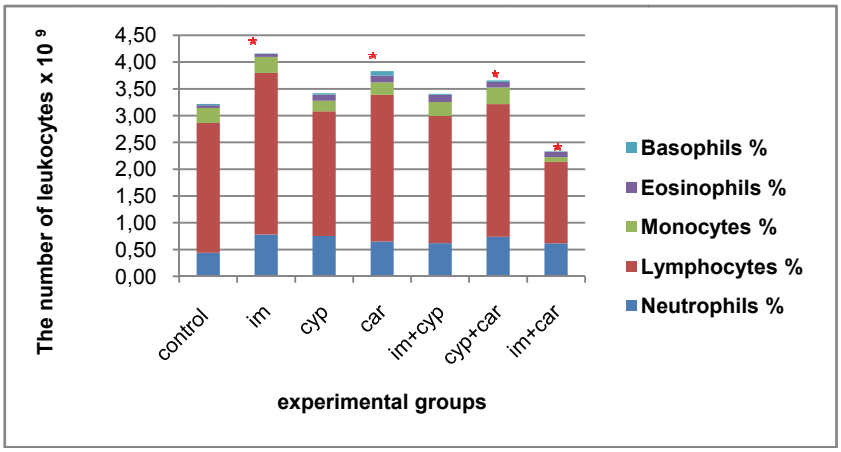


Figure 1 - The number of leukocytes and percentages ratios of individual cells in the experimental groups of Swiss mice treated with Im (imazalil); cyp (cypermethrin); car (carbendazim); im+cyp (imazalil+cypermethrin); cyp+car (cypermethrin+carbendazim); im+car (imazalil+carbendazim). The asterisk marks the experimental groups that are significantly different ($p \leq 0.05$) from the control group.

Table 2 - Changes in oxidative stress markers in the popliteal lymph nodes of Swiss mice treated with Im (imazalil); cyp (cypermethrin); car (carbendazim); im+cyp (imazalil+cypermethrin); cyp+car (cypermethrin+carbendazim); im+car (imazalil+carbendazim).

	experimental groups					
oxidative stress markers	im	cyp	car	im+cyp	cyp+car	im+car
CAT	↔	↑	↑↑	↔	↑	↑
GSH	↓	↔	↔	↔	↔	↔
SOD	↔	↓	↔	↔	↓	↔
8-iso-PG F2α	↔	↔	↑	↔	↔	↔
MDA	↔	↔	↔	↔	↔	↔

The arrow marks the experimental groups that are significantly different ($p \leq 0.05$) from the control group; the direction of the arrow point whether there was increase (↑) a decrease (↓) or no change (↔) in the activity or concentration of respectable marker compared to the control.

CONCLUSION:

This study represents a significant contribution to the toxicity testing of imazalil, carbendazim and cypermethrin and their combinations at low doses, and study results represent a practical contribution to the daily risk assessment of the interactive effects of these three pesticides in the human body. From this research, we conclude that the exposure of animals to low doses of pesticides has led to the change in the proportions of leukocytes as well as stimulated oxidative stress in the lymph nodes. The biggest change was caused a combination of imazalil and carbendazim. We can assume that although the legal basis for the MRL in food are based on the NOAEL doses of certain animal models, these limits should be lower because it is defined for each pesticide in particular, and through this study we conclude that the combination of low doses over a long period of exposure can achieve significant physiological and Immuno effects.

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The effect of perinatal lead (Pb) exposure on purine receptor $P2X_4$ expression and astrogliosis in morphine dependent rats

Authors: Irena Baranowska-Bosiacka[1], Izabela Gutowska[2], Anna Falkowska[1], Karolina Dec[2], Agnieszka Kolasa[3], Maciej Tarnowski[4], Dariusz Chlubek[1]

1 *Department of Biochemistry and Medical Chemistry, Pomeranian Medical University in Szczecin, Poland*
2 *Department of Biochemistry and Human Nutrition, Pomeranian Medical University, Poland*
3 *Department of Histology and Embriology, Pomeranian Medical University in Szczecin, Poland*
4 *Department of Physiology, Pomeranian Medical University in Szczecin, Poland*

Presenting Author: Email: ka@pum.edu.pl | Tel.: +48 466 1515 | Fax: +48 466 1516

INTRODUCTION:
Lead (Pb) toxicity is still a major health problem, associated with both environmental and occupational exposure. Developmental Pb exposure causes behavioral and cognitive deficits in children. Impairments in executive functions, are one of the hallmark effects, even at low levels of lead in whole blood, and suggest the involvement of cholinergic, glutamatergic and dopaminergic neurotransmission. Perinatal Pb-exposure can affect several aspects of neurotransmission in a dose-related and brain region-specific manner. The effects include changes in neurotransmiters metabolism, release, and reuptake. Receptors and transporters gene polymorphisms have also been associated with behavioral manifestations observed in children exposed to Pb. Several experiments confirm that early (gestational/lactational) exposure to Pb may affect the matters relating to drug addiction. For example, perinatal Pb-exposure indicated an increase in acquisition and maintenance of cocaine self-administration when subjects were tested again as adults. The correlation between early Pb-exposure and cocaine-induced seeking behavior has also been demonstrated in animals. Some data report the relationship between perinatal Pb-exposure and morphine dependence.

OBJECTIVES:
The aim of the present study was to investigate the pre- and neonatal Pb-exposure on purine receptor P2X4 expression and astrogliosis in morphine dependent adult rats. To recognize the mechanisms underlying morphine dependence the mRNA (RT PCR) and protein expression (Western Blotting) of purine receptors P2X4, astrocytes gliosis symptoms (glial fibrillary acidic protein (GFAP) expression) were investigated in several structures of the mesolimbic system (hippocampus, prefrontal cortex and striatum), while the immunohistochemical changes in purine receptors expression and gliosis were examined in hippocampus.

MATERIALS AND METHODS:
Rats were orally treated with 0.1% lead acetate from conception, through gestation, up to postnatal day (PND) 28; subsequently molecular studies were conducted on adult (PND 60) male rats.

RESULTS AND DISCUSSION:
It has been shown in the present study that morphine withdrawal was more expressed in morphine administered and Pb-exposed animals than in morphine administered rats. The significant molecular changes has also been developed in purine receptor P2X4 as well as astrocytosis in hippocampus and prefrontal cortex, which are able to induce the intensification of morphine withdrawal signs in morphine dependent rats. It is important to note that all changes are developed when the Pb concentration is similar to that found in children from contaminated urban areas. The finding that perinatal Pb exposure may augment morphine dependence should be of central interest with respect to public health concerns. As Pb exposure during pregnancy and nursing results in a long-term increase in morphine sensitivity, the increased risk of drug addiction becomes more likely in those environments where Pb contamination is widespread. As indicated, the inner-city sectors of major metropolitan areas continue to be burdened by elevated Pb-levels. Accordingly, it must be acknowledged that environmental contamination may be one of the factors contributing to the commonly reported high levels of drug abuse in inner-urban environments.

The effect of perinatal lead (Pb) exposure on purine receptor P2X7 expression in rats' brain in tolerance to morphine analgesia

Authors: Izabela Gutowska[1], Irena Baranowska-Bosiacka[2], Andrzej Sobieniecki[2], Agnieszka Kolasa[3], Maciej Tarnowski[4], Agnieszka Łukomska[1], Dariusz Chlubek[2]

1 *Department of Biochemistry and Human Nutrition, Pomeranian Medical University, Poland*
2 *Department of Biochemistry and Medical Chemistry, Pomeranian Medical University in Szczecin, Poland*
3 *Department of Histology and Embriology, Pomeranian Medical University in Szczecin, Poland*
4 *Department of Physiology, Pomeranian Medical University in Szczecin, Poland*

Presenting Author: Email: izagut@poczta.onet.pl | Tel.: +48 914 414 806 | Fax: +48 914 414 806

INTRODUCTION:

Environmental exposure to lead (Pb) remains a problem in many countries and the health risks are especially of concern for populations living in contaminated urban areas. Young children are particularly susceptible to Pb exposure from behavioral factors, such as frequent hand-to-mouth activities, greater gastrointestinal absorption and an immature blood/brain barrier. The developing central nervous system is a primary target for lead. Acute Pb contamination in children (lead concentration in whole blood - PbB > 70 μg/dL), which is currently very rare, can have a dramatic effect on the central nervous system, i.e. brain edema, convulsions, coma, and lead encephalopathy.

Exposure to lower doses of Pb can lead to subtle non-specific disorders in brain functions - reduced perception; impaired cognition, impaired hearing and sight; even disorders in neurobehavioral functioning, including aggression. It has also been shown that Pb-B, even below 10 μg/dL, may be one of the factors that induces lower IQ in schoolchildren. Moreover, chelation therapy, which is recommended for children with Pb-B above 45 μg/dL, may reduce the amount of Pb in the organism but will not compensate for cognitive and behavioral problems resulting from Pb exposure earlier in childhood. Opioids (including morphine) are used in the treatment of acute pain and pain associated with cancer. Recently, they have become increasingly common in dealing with chronic pain, although their long-term use results in serious health consequences and a risk of addiction. Long-term administration of opioids is also associated with the rise of antinociceptive tolerance, resulting in the need for administration of increasingly high doses to achieve the same analgesic effect. The mechanism of tolerance to morphine is not fully understood, although it has been shown that chronic exposure to morphine may result in increased expression of purine receptors in spinal microglia. In addition, other factors such as stress, poisons or environmental factors like heavy metals may affect the morphine tolerance. Purinergic receptor P2X7 belongs to ionotropic P2X class. Under pathological conditions of prolonged activation with high levels of ATP (agonist), it forms non-selective transmembrane pore that leads to cell death via necrosis or apoptosis releasing of glutamate and ATP and inflammatory response.

OBJECTIVES:

The aim of the present study was to investigate the molecular effects of pre- and neonatal Pb exposure on the expression of purine receptor P2X7 mRNA and protein and apoptosis in several structures of the mesolimbic system - striatum (S), hippocampus (H), prefrontal cortex (FC) of rats expressing morphine tolerance to antinociceptive effect.

MATERIALS AND METHODS:

Rats were orally treated with 0.1% lead acetate from conception, through gestation, up to postnatal day (PND) 28; subsequently molecular studies were conducted on adult (PND 60) male rats. To assessment morphine tolerance antinociceptive effect in the tail-immersion test was used.

RESULTS AND DISCUSSION:

The analysis of protein and mRNA expression revealed significant upregulation of P2X7 protein and mRNA expression in S and FC, but not in hippocampus, in Pb-exposed and morphine administered rats in comparison with morphine administered rats. These findings suggest that in morphine tolerant rats the perinatal Pb-exposure can affect P2X7 receptor expression in brain region-specific manner what can influence on morphine tolerance.

PUBLIC HEALTH ABSTRACTS

In vitro analysis of neuronal DNA damage induced by magnetite nanoparticles

Authors: Blanca Laffon[1], Gözde Kiliç[1,2], Aida Castelo[1,2], Carla Costa[3], Solange Costa[3], João Paulo Teixeira[3], Eduardo Pásaro[1], Vanessa Valdiglesias[1]

1 *DICOMOSA Group, Department of Psychology, Area of Psychobiology, Universidade da Coruña, Spain*
2 *Department of Cell and Molecular Biology, Universidade da Coruña, Spain*
3 *Department of Environmental Health, National Institute of Health, Portugal*

Presenting Author: Email: blaffon@udc.es | Tel.: +34 981167000 | Fax: +34 981 167 172

INTRODUCTION:
Magnetic iron oxide nanoparticles (ION) are available with various functionalizations and have a wide range of potential applications. Among these, the most important applications for biology and medicine are the utilization of ION as contrast agents in magnetic resonance imaging, as carrier for drug delivery or transfection and as therapeutic agents in cancer therapy by magnetic field-mediated hyperthermia. Although in general ION show a good biocompatibility, little is known about their neurotoxicity so far.

OBJECTIVES:
The main objective of this study was to evaluate the genotoxicity induced by two types of ION (magnetite coated with silica or oleic acid) on human SHSY5Y neuronal cells.

MATERIALS AND METHODS:
ION suspensions were prepared in complete and incomplete (serum-free) cell culture media. The comet assay in its standard alkaline version was used to determine primary DNA damage. Besides, oxidative DNA damage was assessed by means of comet assay with an additional step of incubation with OGG1, enzyme which specifically breaks the DNA strand where oxidized guanines are present. The possible interference of the ION used with the comet assay methodology and OGG1 enzyme was previously tested, since ION might associate with nucleoid DNA and affect its behaviour during the comet assay procedure, or might interfere with OGG1 activity in converting oxidized guanines into strand breaks. Four different ION concentrations in the range 10-200 µg/ml, and two different treatment times (3 and 24 h) were tested.

RESULTS AND DISCUSSION:
Silica-coated magnetite NP did not show interference with the comet assay procedure or the OGG1 enzyme, either in complete or in incomplete medium. They induced primary DNA damage in SHSY5Y cells treated in complete medium, and oxidative DNA damage in both complete and incomplete media. Increases in DNA damage, primary and oxidative, were also observed after treatment with oleic acid coated-ION, even though interference with OGG1 enzyme activity was observed in serum-free cell culture medium.

CONCLUSION:
In conclusion, our results showed that surface coating of ION and dispersion media used are important factors in determining their genotoxic potential. Further investigations are necessary to figure out the specific mechanisms underlying DNA damage induced by these ION.

ACKNOWLEDGMENTS:
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Cadmium Exposure and Chronic Kidney Disease in Canada: results of the Canadian Health Measures Survey (2007-2011)

Authors: Patrick Levallois[1,2], Elhadji Anassour-Laouan[1] , Julie St-Laurent[1] , Pierre Ayotte[1,2], Jasmin Levallois[3], Jean-Philippe Lafrance[4]

1 *Axe Santé des populationset pratiques optimales en santé, Centre de recherche du CHU de Québec et Université Laval, Québec, QC, Canada*

2 *Direction de santé environnementale et de toxicologie, Institut national de santé publique du Québec, QC, Québec, Canada*

3 *Centre de Néphrologie, Hôpital Georges-Dumont, Moncton, NB, Canada*

4 *Service de Néphrologie, Hôpital Maisonneuve-Rosemont et Université de Montréal, Montréal, QC, Canada*

Presenting Author: Email: patrick.levallois@msp.ulaval.ca | Tel.: +1 (418) 650-5115 #5216 | Fax: +1 (418) 654-3144

INTRODUCTION:

Chronic kidney disease (CKD) is a major public health issue, leading to important morbidity and mortality. Diabetes, hypertension and obesity are well-recognized risk factors for this disease, however environmental exposure might also increase the risk of CKD. Cadmium is a nephrotoxic agent at high doses, which was well evaluated in occupational settings but its effect in the general population exposed to low levels is still uncertain.

OBJECTIVES:

This study was conducted to evaluate the association between the biological exposure to cadmium and the prevalence of CKD among 40-79 years Canadians.

MATERIALS AND METHODS:

Data were collected during cycles 1 and 2 of the Canadian Health Measures Survey (CHMS), which was conducted in a representative sample of about 12,000 Canadians recruited between March 2007 and February 2009 (Cycle 1) and August 2009 to November 2011 (cycle 2). Overall response rates for the biological part of the study were 51.7% and 55.5% for cycle 1 and cycle 2 respectively. Cadmium concentration was measured in whole blood and urine samples by inductively coupled plasma mass spectrometry (ICP-MS).Blood lead level was also measured by ICP-MS and considered as a potential confounding factor. Albumin and creatinine levels were determined in spot urine samples. CKD was considered present if glomerular filtration rate (GFR) estimated from serum creatinine using the CKD-EPI equation was <60ml/min/1.73m^2 or urinary albumin ≥30mg/g of creatinine. Data were available for 4,523 subjects aged between 40-79 years. Logistic regression was used to estimate the relationship between reduced GFR or albuminuria and cadmium exposure measures categorized in quartiles. The following co-variables were adjusted for in the analyses: age, sex, smoking status, alcohol intake, hypertension, diabetes, body mass index (BMI) and blood lead. Potential interaction with age, sex and smoking was also evaluated.

RESULTS AND DISCUSSION:

The prevalence of CKD in the adult Canadian population was estimated at 9.84% (95% CI: 8.85-10.83). In univariate analysis, there was a link between CKD and blood and urinary cadmium. However in multivariate analysis, the only association was between CKD and blood cadmium, with the maximum OR found for the highest quartile of blood cadmium levels compared to the lowest: adjusted OR=2.46 (95% Confidence Intervals: 1.31-4.60).Detailed results are presented on Table 1.

Table 1 - Association between Cadmium biological exposure and prevalence of CKD in 40-79 years Canadians (CHMS, 2007-2011)

	Crude OR(95% CI)	Adjusted OR*(95% CI)
Blood Cadmium(µg/L)		
< 0.23	1	1
0.23 - < 0.37	1.59 (0.98 - 2.56)	1.36 (0.81 - 2.87)
0.37 - < 0.78	2.57 (1.80 - 3.68)	2.01 (1.34 - 3.01)
≥ 0.78	2.45 (1.52 - 3.94)	2.46 (1.31 - 4.60)
Urinary Cadmium(µg/g creat)		
< 0.34	1	1
0.34 - < 0.54	1.60 (1.05 - 2.42)	1.17 (0.72 - 1.92)
0.54 - < 0.91	1.62 (1.15 - 2.29)	0.91 (0.61 - 1.35)
≥ 0.91	2.13 (1.95 - 3.12)	1.13 (0.72 - 1.76)

*Adjusted for the following variables: age, sex, smoking, hypertension, diabetes, BMI and blood lead

The relationship was somewhat stronger when only participants with reduced GFR were considered (comparing quartile 4 of blood cadmium to quartile 1): Adjusted OR=3.35 (95% CI: 1.66-6.75). No modification of age, sex or smoking status was found.

Our results are in agreement with those of Ferraro and al (2010) who only found an association of blood cadmium with CKD in Americans aged ≥20 years participating to NHANES (1999-2006). Theoretically, urinary cadmium would be a better marker of chronic exposure to cadmium than blood cadmium, which is supposed to reflect recent exposure. However, the use of urinary cadmium level as a biomarker of low-level exposure was recently questioned (Akerstrom et al 2013).

Despite a large sample size, a very good quality control of biological measures and consideration of several potential confounders, this study has some limitations that should be considered in the interpretation of the results. First, because of the cross-sectional design of the study, the possibility of reverse causality cannot be excluded. Also, participation rate was limited, leading to possible selection bias.

CONCLUSION:

In the adult Canadian population, there is a positive relationship between low dose exposure to cadmium measured in blood and the prevalence of CKD. However, no such relationship was found when urinary cadmium was considered as a marker of past exposure. The reason for the discrepancy of the results between different markers of cadmium exposure should be clarified.

ACKNOWLEDGMENTS:

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Why should we focus in cold extremes health negative outcomes in Mediterranean climate like Porto, Portugal?

Authors: Ana Monteiro[1,2], Luís Fonseca[1]

1 *Institute of Public Health, University of Porto, Project ERA/URBAN/0001/2009, Porto. Portugal*
2 *Faculty of Arts, University of Porto, Department of Geography; CITTA, Porto. Portugal*

Presenting Author: Email: anamonteirosousa@gmail.com | Tel.: +351 919 698 402

INTRODUCTION:
Scientific research and media refer persistently the fatal effects of heat waves due to global climate change and diminish the importance of the cold in Mediterranean areas. However, the climate adaptation of human beings, their lifestyles and the architectural features gathered with the greater economic differences in southern Europe, show a high vulnerability of the poorer, older and less informed to sporadic and unexpected episodes of low temperatures during winter (Monteiro, 2012a), (Monteiro et al., 2012b), (Monteiro et al., 2012c), (Monteiro et al., 2011). Eurowinter Group (1997) or Healy (2003) are some examples of important research done that suggest the inadequacy of the existent warning systems(Almeida, 2012).

OBJECTIVES:
Our goal is to understand the importance of cold episodes in a mild winter climate like Porto through the comparison of Physiological Equivalent Temperature (PET) and morbidity and mortality in Porto (2000-2007).

MATERIALS AND METHODS:
First we collect the daily minimum and maximum temperature of Porto (1900-2007) to define the 10th and 5th percentiles and define the extreme cold events (duration and intensity) considering the importance of adaptation to climate context. Then, using Solweig software, we calculate the PET of Porto to differentiate the climate data recorded and the perceived one. Afterwards, we gather the daily admissions at the 4 main hospitals (2002-2007) with the corresponding cause. We assemble also the daily mortality (2002-2007). Finally, we estimate the excess of morbidity and mortality and compare the observed values during the predefined extreme cold events with the homologous periods.

RESULTS AND DISCUSSION:
A good way to identify the relation between health and cold stress is looking to periods with extreme cold. From 2002 to 2007, the same period with mortality, morbidity and climatic data, emerge 9 extreme cold events. During the majority of these extreme cold events we observe an increase of mortality from 12% to 34%. The excess of morbidity showed a considerably increase either in the respiratory or in the circulatory diseases (all causes), or in some diseases like for instance bronchitis and asthma admissions reach an increase of 76%, COPD above 40%, pneumonia and pleurisies above 50%, myocardial infarction had more than 50% and stroke overpass in some events 100% (Table 1).

EXTREME COLD EVENT	Observed (O)	Expected (E)	(O-E)	(O-E) / E *100		Tmrt	PET
MORTALITY							
20 january - 20 february 2005	1316	1171	145	12%		[-6ºC-4ºC]	[-6ºC-1ºC]
23 february -11 march 2005	751	559	192	34%		[-6ºC-3ºC]	[-9ºC-(-1)ºC]
19 - 27 december 2006	358	297	61	21%		[-2ºC-3ºC]	[-5ºC-(-1)ºC]
MORBIDITY							
RESPIRATORY DISEASES (ALL CAUSES)							
20 january - 20 february 2005	1319	1045	274	26%		[-6ºC-4ºC]	[-6ºC-1ºC]
23 february - 11 march 2005	598	455	143	31%		[-6ºC-3ºC]	[-9ºC-(-1)ºC]
19 - 27 december 2006	304	209	95	46%		[-2ºC-3ºC]	[-5ºC-(-1)ºC]
CIRCULATORY DISEASES (ALL CAUSES)							
9 - 17 january 2003	295	284	11	4%		[-6ºC-(-1)ºC]	[-8ºC-(-4)ºC]
23 february -11 march 2005	530	498	32	6%		[-6ºC-3ºC]	[-9ºC-(-1)ºC]
19 - 27 december 2006	253	194	59	30%		[-2ºC-3ºC]	[-5ºC-(-1)ºC]
BRONCHITIS AND ASTHMA							
20 january - 20 february 2005	125	71	54	76%		[-6ºC-4ºC]	[-6ºC-1ºC]
23 february -11 march 2005	36	28	8	27%		[-6ºC-3ºC]	[-9ºC-(-1)ºC]
19 - 27 december 2006	15	12	3	29%		[-2ºC-3ºC]	[-5ºC-(-1)ºC]
CHRONIC DISEASE OBSTRUCTIVE							
20 january - 20 february 2005	151	107	44	41%		[-6ºC-4ºC]	[-6ºC-1ºC]
23 february - 11 march 2005	64	44	20	44%		[-6ºC-3ºC]	[-9ºC-(-1)ºC]
19 - 27 december 2006	34	24	10	43%		[-2ºC-3ºC]	[-5ºC-(-1)ºC]
PNEUMONIA AND PLEURISY							
20 january - 20 february 2005	276	185	91	49%		[-6ºC-4ºC]	[-6ºC-1ºC]
23 february -11 march 2005	137	83	54	65%		[-6ºC-3ºC]	[-9ºC-(-1)ºC]
19 - 27 december 2006	82	42	40	97%		[-2ºC-3ºC]	[-5ºC-(-1)ºC]
MYOCARDIAL INFARCT							
9 - 17 january 2003	39	33	7	20%		[-6ºC-(-1)ºC]	[-8ºC-(-4)ºC]
20 january - 20 february 2005	121	109	12	11%		[-6ºC-4ºC]	[-6ºC-1ºC]
23 february -11 march 2005	55	54	1	1%		[-6ºC-3ºC]	[-9ºC-(-1)ºC]
19 - 27 december 2006	39	26	13	51%		[-2ºC-3ºC]	[-5ºC-(-1)ºC]
STROKE							
9 - 17 january 2003	35	32	3	9%		[-6ºC-(-1)ºC]	[-8ºC-(-4)ºC]
20 january - 20 february 2005	136	109	27	25%		[-6ºC-4ºC]	[-6ºC-1ºC]
23 february -11 march 2005	86	58	28	49%		[-6ºC-3ºC]	[-9ºC-(-1)ºC]
3 - 17 january 2006	65	61	4	6%		[-1ºC-5ºC]	[-4ºC-1ºC]
19 - 27 december 2006	56	27	29	107%		[-2ºC-3ºC]	[-5ºC-(-1)ºC]
HEART FAILURE							
20 january - 20 february 2005	107	104	3	3%		[-6ºC-4ºC]	[-6ºC-1ºC]
23 february -11 march 2005	59	56	3	5%		[-6ºC-3ºC]	[-9ºC-(-1)ºC]
3 - 17 january 2006	62	55	7	12%		[-1ºC-5ºC]	[-4ºC-1ºC]
19 - 27 december 2006	40	26	14	57%		[-2ºC-3ºC]	[-5ºC-(-1)ºC]

Table 1 - Observed positive mortality and morbidity surplus during the selected extreme cold events in Porto's climate (2002-2007).

CONCLUSION:

In climates with mild winters, the low temperature thresholds considered as a risk to human beings health should be redesigned and adapted to a complex equation where climate and socioeconomic vulnerability need to be included with equal weight. The impulsive behaviour of climate system maintains anomalies of either extreme cold or heat even during warmer times. Moreover, the seasonality of Mediterranean climates added with the greatest socioeconomic differences among society, create a lack of comfort efficiency of housing to protect people from the cold and damp particularly acute among the poorer social classes who, in

most cases, do not have the economic resources to bear the costs of artificial heating in their homes. The elderly, who in many cases live alone, and the increased number of homeless people, comprise the more vulnerable groups during these cold episodes. Since they are less supervised, aware and prepared, they take much longer to follow the necessary steps to protect themselves.

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Environmental contamination by dog feces with zoonotic helminthes and human infection risk in Ponte de Lima Portugal

Authors: Teresa Letra Mateus[1,2,3], Maria Madalena Vieira-Pinto[2,4], João Niza Ribeiro[3], António Castro[5]

1 *Departamento de Medicina Veterinária, Escola Universitária Vasco da Gama, Coimbra, Portugal*
2 *CECAV, Centro de Ciência Animal e Veterinária, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*
3 *Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal*
4 *Dept. Ciências Veterinárias, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*
5 *Instituto Nacional de Saúde Dr. Ricardo Jorge, Porto, Portugal*

Presenting Author: Email: tlmateus@gmail.com | Tel.: +351 239 444 444 | Fax: +351 239 437 627

INTRODUCTION:
In human society, dogs play many roles such as pets, guarding, hunting and shepherding, and they are also used in therapeutic programmes, life saving actions, transport, and last but not least for fun and research. There is evidence of the role of dogs in physical and psychological human well being (Arhant-Sudhir, Arhant-Sudhir, & Sudhir, 2011). As communities become more urban, the presence within the housing of pets has increased in popularity. However, they are not only in our daily life and environment as they also share with us potential risks - the transmission of zoonoses (Chomel & Ben, 2011). The number of domestic animals in urban areas has progressively increased and dog feces are not systematically removed from the streets, representing an important environmental pollution factor. Viable pathogens in dried and pulverized canine feces can be spread by wind, vehicular traffic and can be a source of infection through hun wrapped food. A relatively small number of reports have focused on the infection risk of populations living in rural or suburban settings (Blaszkowska, Kurnatowski, & Damiecka, 2011). Many of these communities have large populations of free roaming domestic dogs and little access to veterinary care.

OBJECTIVES:
The aim of this study was to evaluate the occurrence of environmental contamination with zoonotic intestinal parasites of dogs from different groups in Ponte de Lima, Portugal.

MATERIALS AND METHODS:
Between December 2011 and November 2012, we collected dog feces from three different sample groups: environmental, shepherd and hunting dogs. Samples were randomly collected from public areas (chosen in preference to the areas attended by people, like green parks and others), sheep and goat farms and hunting dogs packs. Qualitative flotation coprologie analyzes were performed. Positive samples for *Taniidae* eggs were submitted to molecular analyse to evaluate the presence of *E. granulosus*.

RESULTS AND DISCUSSION:
A total of 592 samples was collected from three different groups of faecal samples, 374 (63.17%) were positive for the presence of parasitic forms. In the examined samples, 12 types of intestinal parasites were detected from *Nematoda* (*Ancylostomatidae, Trichuridae, Toxocara* spp., *Toxascaris leonina, Heterakisgallinarum*), *Cestoda* (*Dipyllidium* spp., *Taeniidae*, *Hymenolepis* spp., *Monieziabenedeni* and *Monieziaexpansa*) and *Coccidia* (*Isospora* spp. and *Eimeria* spp.) Classes. *Ancylostomatidae* was the most prevalent with 44.59%, followed by *Trichuridae* (36.49%), *Toxocara* spp. (9.29%), *Isospora* spp. (2.87%), *Eimeria* spp. (1.18%), *Dipyllidium*spp. (0.85%), *Taeniidae* (0.68%), *Hymenolepis* spp. (0.34%), *Toxascaris leonina* (0.34%), *Heterakisgallinarum* (0.34%), *Monieziabenedeni* (0.17%) and *Monieziaexpansa* (0.17%). *Taeniidae* eggs were analysed with the polymerase chain reaction technique and revealed to be from *Taenia* spp. and not *Echinococcusgranulosus*. The majority of dogs was infected by only one type of parasite (34.80%), but we found up to 5 different type of parasites in one sample. Studies from worldwide have a huge range of prevalences, which highlights the importance of promoting research at local scale for planning control

strategies. The overall parasite prevalence found was high. The environmental pollution is the result of poor environmental sanitation and poor hygiene habits, reflecting the population's behavior regarding environmental sanitation (Ulukanligil, Seyrek, Aslan, Ozbilge, & Atay, 2001). A wide diversity of zoonotic parasites were detected (*Ancylostomatidae, Trichuridae, Toxocaridae, Isospora* spp., *Dipyllidium* spp., *Hymenolepis* spp., *Toxascarisleonina, Moniezia* spp. and *Taeniidae*). Katagiri & Oliveira-Sequeira (2008) pointed out that smaller municipalities, like Ponte de Lima is, are exactly where the availability of health care services to humans and pet animals is scarce or even absent. Few studies have been undertaken to determine the prevalence of these organisms and/or their associated diseases in people from the same community. Multidisciplinary approaches will lead to a more complete understanding of the actual epidemiological situation in the country.

CONCLUSION:

The epidemiological investigations conducted in Ponte de Lima revealed a considerable environmental contamination with zoonotic parasites. The high level of environmental zoonotic contamination found, calls for a greater awareness of the problem among the population.

Health education and risk communication should be developed to children and families, farmers, hunters and a closer collaboration between researchers and practicioners (human and veterinary medicine), as well as public health authorities is needed. Prevention of zoonoses require a global commitment, nevertheless, the main task still relies on local populations and requires changes in human behaviours. Populations awareness is a pressing need!

ACKNOWLEDGMENTS:

This work was supported by the Portuguese Science and Technology Foundation (FCT) under the Project PEst-OE/AGR/UI0772/2014.

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Task-based approach to study occupational exposure to particulate matter in cork industry

Authors: Susana Viegas[1,2], Tiago Faria[1]

1 *Environment and Health RG. Lisbon School of Health Technology- Polytechnic Institute of Lisbon*
2 *Center for Malaria & Tropical Diseases (CMDT) - Public Health and Policy, Escola Nacional de Saúde Pública, Universidade Nova de Lisboa, Portugal.*

Presenting Author: Email: susana.viegas@estesl.ipl.pt | Tel.: +351 914 484 676 | Fax: +351 218 980 460

INTRODUCTION:
Environment monitoring has an important role in occupational exposure assessment. However, due to several factors is done with insufficient frequency and normally don´t give the necessary information to choose the most adequate safety measures to avoid or control exposure. A task-based assessment can provide a better evaluation of exposure variability, instead of assessing only personal exposures using continuous 8-hour time weighted average measurements.

Although an important natural resource, cork is also associated to negative health effects for people working in cork industry and workers are at risk of developing an occupational lung disease (suberosis) due to repeated exposure to mouldy cork dust.

OBJECTIVES:
The principal aim of this research was to apply task-based exposure assessment in cork industry in order to identify the task that represents higher exposure to PM. Additionally, and besides mass, particle number concentration was used as exposure metric to better characterize the workplace contamination by PM.

MATERIALS AND METHODS:
The study was developed in a cork industry located in Portugal dedicated to the production of natural and colmated wine stoppers. Environmental monitoring was developed in a single day of February 2014.Measurements were performed using a portable direct-reading hand-held equipment (Lighthouse, model 3016 IAQ). The value of this instrument is its capacity to give information regarding mass concentration in 5 different sizes (PM0.5; PM1; PM2.5; PM5; PM10). Additionally, data related with particle number concentration by each diameter size is also available. In this last case, particles results were given in six different diameters sizes, namely; 0.3 µm, 0.5 µm, 1 µm, 2.5 µm, 5 µm and 10 µm. As mentioned in some literature, this data was also collected because might be more closely correlated with adverse PM health effects (Wichmann et al., 2000; Weijers et al., 2004).

In each workplace were identified the tasks developed. Based on visual observations of work practices on a task-by-task basis, in the time spend in each one, and in professional judgment was define which tasks to sample in each workplace. The main principle in the case of professional judgment was to choose the task that probably involves higher exposure to PM. Five workplaces were studied: mechanical grinding, weighing granulated, pressing, glue and agglomerate mixing zone and cutting and then chosen one or two tasks in each workplace The measurements were conducted near the workers nose and during tasks performance and for each task was done one measurement of 5 minutes.

RESULTS AND DISCUSSION:
PM5 and PM10 presented higher values in all the tasks developed. However, the higher mean value was obtained during the operation of the machine in the Cutting workplace. Regarding particle number concentration results, data showed that were the tasks developed in Pressing and Mixing that present higher values of the smaller particles (0.3 µm) (Figure 1).

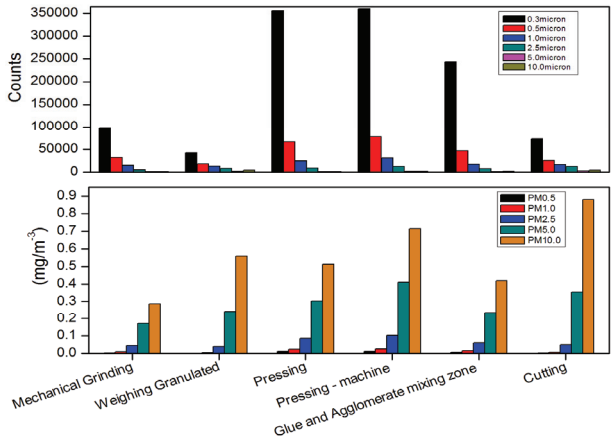


Figure 1 - Particulate matter results with both exposure metrics

In this study, we used a task-based approach to perform exposure assessment. Our PM findings allow us to obtain a detailed contamination characterization by task. Other studies (Penttinen et al., 2001; Nygaard et al., 2004) reported the need to monitor the size distribution and particle number concentration, in addition to mass, particularly when trying to predict the biological effects of PM (Penttinen et al., 2001; Nygaard et al., 2004; Weijers et al., 2004). Smaller particles can reach deeper in the respiratory system meaning that, besides local effects, also systemic effects can be expected related with, for instance, PM chemical composition. Taking in account all these considerations and the results obtained, the tasks of "pressing" (both) and mixing are the ones that involve higher risk for workers health because presented higher number of particles in the smaller dimension (0.3 µm). Additionally, the fact that those tasks are prominent in the workplaces can mean higher risk and, consequently, consider as priorities for the investment in prevention and control of exposure. Therefore, interventions on those workplaces can have substantial impact on total exposure. If only mass data was used we will concluded that cutting was the worst task when trying to relate exposure to a possible health effect. However, is well describe that essentially local effects can be expected from particles bigger than 5 µm (PM10) (Brunekreef and Forsberg, 2005).

Moreover, fungi and their metabolites such as mycotoxins can be present in the dust during all cork industrial process, despite the barks treatments (fungicide and boiling) prior to and during cork processing that can lead to a reduction of fungi load. Therefore, the presence of mycotoxins can represent a real additional risk factor that has being recently revealed in others occupational settings where exposure to organic dust is also a reality (Viegas et al., 2013).In this case, the cork dust can act as a carrier of mycotoxins to the workers respiratory system causing the systemic health effects caused by mycotoxins.

CONCLUSION:

The task-based approach permitted to identify the tasks with higher and more problematic exposure to PM. The particle number concentration data allowed to better characterize exposure and estimate with more detail the possible health effects.

ACKNOWLEDGMENTS:

The study would not have been possible to develop without the institutional support given by Lisbon School of Health Technology.

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Limited knowledge about hydatidosis among farmers in Ponte de Lima - Portugal – and its impact in Public Health

Authors: Teresa Letra Mateus[1,2,3], João Niza Ribeiro[3], António Castro[4], Maria Madalena Vieira-Pinto[2,5]

1 *Departamento de Medicina Veterinária, Escola Universitária Vasco da Gama, Coimbra, Portugal*
2 *CECAV, Centro de Ciência Animal e Veterinária, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*
3 *Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal*
4 *Instituto Nacional de Saúde Dr. Ricardo Jorge, Porto, Portugal*
5 *Dept. Ciências Veterinárias, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*

Presenting Author: Email: tlmateus@gmail.com | Tel.: +351 239 444 444 | Fax: +351 239 437 627

INTRODUCTION:

Three-quarters of emerging human pathogens are zoonotic (Woolhouse, 2002).The anthropogenic changes of the biosphere are causing environmental damage, which favour the emergence of zoonosis locally and its spread globally. According to the World Health Organization, nearly 2 billion people worldwide are infected with soil-transmitted helminthes. *Echinococcus* spp. Are cestoda that live in the small intestine of dogs, causing echinococcosis. Their eggs are discharged with the faeces and can be ingested by mammals or humans. The development of disease caused by larval forms (cysts) in intermediate hosts is known as hydatidosis, an important zoonotic disease that causes high morbility and mortality in humans, is hyperendemic in the Mediterranean region, and in Portugal new human cases are identified each year. Communities involved in raising sheep are described as a priority setting (Carmena, Sanchez-Serrano, & Barbero-Martinez, 2008). *E.granulosus* occurs worldwide wherever there are certain socio-cultural practices, and lack of knowledge about the disease by humans, allowing the perpetuation of this zoonosis (Seimenis, 2003). The development of strategies to inform the community of the risk and prevention measures should be based not only on the results of scientific research of risk factors, but also in analyzing the perception of the problem by the community.

OBJECTIVES:

The aim of this study was to identify the awareness of the sheep and goats farmers about hydatidosis in Ponte de Lima, Portugal.

MATERIALS AND METHODS:

Farmers were visited with the purpose of performing questionnaires. Knowledge, perception, and attitude evaluation was based on a combination of methods, which included questionnaires, focus groups, interviews, and meetings. This has resulted in the final questionnaire, composed of a sequence of questions of multiple choice or open-ended. The questionnaires were reviewed and revised after field testing. Statistical analysis was conducted using appropriate software (Statistical Package for 107 Social Sciences - SPSS version 18.0).

RESULTS AND DISCUSSION:

A total of 279 questionnaires have been collected. Most of the farmers (55.9%) age range between 50 and 70 years old. Two hundred and thirty six (84.6%) farmers had at least one dog, and 69.2% admitted to practice domestic slaughtering, burying the viscera or feeding them to the dog, which allows the perpetuation of the life cycle of the parasite. Regarding to the practices of deworming of the dogs, 54.2% of the farmers reported to do it, nevertheless, the other 45.8% admitted not to deworm their dogs. When we asked about the frequency of the deworming, the majority answered once a year, which may be not enough. Concerning to the product used in the deworming, 64.1% of the farmers do not know what was used. It was then important to know if these dogs have contact with other dogs or livestock, and actually 54.7% have. We finally wanted to know if they have ever heard about echinococcosis - hydatidosis, and 96.8% have never heard about the

disease. Two hundred and ten (75.3%) farmers demonstrated interest in receiving information about hydatidosis. The respondents would like to receive this information mainly, from the veterinary.

CONCLUSION:

It appears that a plan is required to raise awareness amongst sheep and goats farmers. Veterinarians may have an important role in sharing relevant information on zoonosis in general and hydatidosis in particular, actively contributing to the motto "Veterinary sciences for one health".Health education and risk communication should be developed to farmers and a closer collaboration between researchers and practicioners, as well as public health authorities is needed. Prevention of zoonoses require a global commitment, nevertheless, the main task still relies on local populations and requires changes in human behaviours. Populations awareness is a pressing need!

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Occupational lung cancer surveillance of Korea in 2013

Authors: Yoon-Ji Kim[1], Young-IL Lee[2], Jong-Eun Kim[2], Dongmug Kang[1,2]

1 *Department of Preventive and Occupational Medicine, School of Medicine, Pusan National University, Pusan, South Korea*
2 *Department of Occupational & Environmental Medicine, Pusan National University Yangsan Hospital, Yangsan, South Korea*

Presenting Author: Email: harry-potter-79@hanmail.net | Tel.: +82 51 510 8166 | Fax: +82 51 510 8038

INTRODUCTION:
Unlike that of other types of diseases, the incidence of occupational diseases can be reduced if the particular types of jobs that involve exposure to disease-causing substance are fully managed. In developed countries, the need for such management of occupational diseases was already realized at an early stage, and many efforts for efficient management have been made, one of which is the implementation of a surveillance system for occupational diseases.[1)]An example is the surveillance system for occupational cancer, which was established to enable a systematic and continuous data collection, analysis, and management; distribution of information; and so on.

OBJECTIVES:
This study mainly discusses lung cancer, which has an incidence rate that is proportionally highest among occupational cancers. In addition, this study provides information about domestic workers' occupational exposure to risk factors based on the data collected from Korean university hospitals that were selected to be publicly observed in 2013.

MATERIALS AND METHODS:
For the surveillance of occupational lung cancer occurring in Korea in 2013, we divided the nation into 2 districts, selecting 18 hospitals. Through a literature review by the International Agency for Research on Cancer, lung cancer-causing materials and processes were reclassified.[2)]Practical patients with occupational lung cancer were defined based on diagnostic accuracy, carcinogens to which the patients were exposed, latency, and degree of exposure. The degree of exposure was evaluated by 3 detailed criteria, namely intensity of exposure, exposure time of day, and exposure duration. Latency, from the time of exposure to the carcinogen to the onset of disease, was documented during the evaluation; the average latency period was 10 years. The subjects of the survey were selected from among each hospital's inpatients or outpatients, particularly adults older than 20 years who were newly diagnosed with lung cancer through histological diagnosis (except the patients who previously received any treatments such as radiation therapy for the same kind of cancer or others). A survey was conducted through detailed interviews using a questionnaire, which included items on sex, age, smoking history, and occupational history. The results were published on a Website. The relationship between the reported occupations of the patients and the incidence of lung cancer was evaluated using 6 categories, namely definite, probable, possible, suspicious, none, and undetermined.

RESULTS AND DISCUSSION:
A total of 1,404 lung cancer patients were surveyed, among whom 973 (69.3%) were male and 431 (30.7%) were female. Their mean age was 64.6 years, and the average age of the male patients (65.5 years) was a little older than that of the female patients (62.7 years). In a stratified analysis of smoking history according to sex, a sex-related difference was observed; 48.2% of the men (469 patients) and 6.7% of the women (29 patients) were current smokers. As for the relevance of the relationship between occupation and lung cancer, 31 people (2.2%) were classified as "definite" and 102 (7.3%) were classified as "probable."Of all the cases, 9.5% appeared to be definitely or highly related to the patients' occupations. With regard to sex, only the men appeared to be clearly classified as "definite."A total of 24 kinds of work processes were identified as possible sources of hazardous materials or having hazardous effects. In the "definite" cases, the most common hazardous materials used in the work processes included asbestos (14 patients, 1.0%) and

crystalline silica (12 patients, 0.9%). In the “probable” cases, non-arsenic pesticide, diesel engine emission, and asbestos and asbestos-containing talc were found. The results of a histological analysis of the distribution of hazardous substances, which were categorized according to smoking history or experience, indicated that among the patients who had a history of smoking (including current smokers), 283 (29.8%, the highest rate) had squamous cell carcinoma and 282 (29.7%) had adenocarcinoma. In particular, squamous cell carcinoma is known to frequently occur in smokers.[3)]In this study, adenocarcinoma had the highest incidence rate at 41.7% (586 patients). As a result of evaluating only the cases that were highly related to the patients’ occupation, regardless of histological type, non-arsenic pesticide and asbestos were the most common, albeit without significant difference with other substances.

CONCLUSION:

Through this study, the high incidence of work-related lung cancer was confirmed. In this study, the most prevalent cases were related to the patients whose disease had “definite” relevance to their work, and asbestos was the most common lung cancer-causing hazardous substance. While most other domestic research studies in developing countries have focused on passive surveillance systems based on registered data, the present study is valuable in that it conducted an active surveillance on occupational relevance and the incidence of cancer, and evaluated its significance using national data. In the future, regarding exposure to non-arsenic pesticides, which was discussed in this study though not seriously dealt with in previous studies, occupational exposure of agricultural workers has to be assessed. The incidence of lung cancer due to occupational exposure also has to be estimated simultaneously. In such circumstances, the management and prevention of occupational diseases can succeed.

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A study of patients with malignant mesothelioma resulting from environmental asbestos exposure compensated by the Korean Asbestos Damage Relief Act during 2011 to 2012

Authors: Myeong-Ja Yun[1], Dong-Mug Kang[1,2], Jong-Eun Kim[3] , Ju-Young Kim[2], Kye-Sun Kim[2]

1 *Department of Preventive and Occupational Medicine, School of Medicine, Pusan National University, Pusan, South Korea*
2 *Environmental Health Center of Asbestos, Department of Occupational & Environmental Medicine, Pusan National University Yangsan Hospital, Yangsan, South Korea*
3 *Department of Occupational & Environmental Medicine, Pusan National University Yangsan Hospital, Yangsan, South Korea*

Presenting Author: Email: ymjml@naver.com | Tel.: +82 55360 2142 | Fax: +82 55 360 2712

INTRODUCTION:
Korea was the 6th country in the world to compensate patients with diseases originating from environmental asbestos exposure. The purpose of the Korean Asbestos Damage Relief Act is to provide prompt and fair relief to people who suffer from environmental asbestos-related diseases and their survivors. This is carried out through measures such as the payment of medical expenses, allowances, and funeral costs. In this Act, the range of asbestos-related diseases includes malignant mesothelioma (MM), lung cancer, and asbestosis (and will also include diffuse pleural thickening from 2014) according to a corresponding Ordinance of the President of Republic of Korea. This Act activated on January1, 2011.

OBJECTIVES:
Data from patients with MM, who had been compensated through this act between January 2011 and December 2012, were analyzed, and the distribution and features of asbestos exposure leading to disease were described

MATERIALS AND METHODS:
Data on the mode of exposure to asbestos were taken from the application papers of patients with MM and evaluated using an exposure matrix. Occupational exposures were evaluated using an occupational asbestos exposure matrix developed by Choi (1). Environmental exposures were graded in a multicenter study conducted by Magnani et al. (2).

RESULTS AND DISCUSSION:
In total, 556 patients with MM were compensated through the act. Of these, 154 were surviving patients, and 402 were deceased. There were 375 male (67.4%), and 181 female (32.6%) patients. The majority (80.9%) of patients with MM were aged in their fifties, sixties, and seventies. Smokers accounted for 28.9% and non-smokers for 69.1% (no information: 2.0%).With regard to MM location, 71.8% was located in pleura, 24.6% in the peritoneum, and 2.6% in other sites (no information: 1.1%).We found a source of asbestos exposure in 52% cases. Occupational exposure accounted for 24.5% of cases, para-occupational exposure 9.9%, exposure in the home through relatives 15.6%, and exposure from nearby factories or mines 7.7%. We could not find the exposure source from 48.0%.

The average period of latency between the first exposure and disease onset was 30years. This period was the longest environmental exposure cases (38.8 years), followed by occupational, home, and para-occupational exposures, respectively. Environmental exposure cases had the longest mean duration of exposure (17.4 years), followed by home, para-occupational, and occupational exposures, respectively.

CONCLUSION:
The Korean government has compensated patients with diseases arising from environmental asbestos exposure since 2011. We show the demographics, clinical features, and exposure sources for patients with

MM who have been compensated through the Korean Asbestos Damage Relief Act. Details of the act are also described.

Keywords: malignant mesothelioma, environmental exposure, asbestos, Korea

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Personal daily exposure to particulate matter: an elderly study

Authors: Marina Almeida-Silva[1,2], Tiago Faria[1], Susana M. Almeida[1], Hubert T. Wolterbeek[2]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, EN 10, km 139.7, 2695-066 Bobadela LRS, Portugal.*

2 *Faculty of Applied Sciences, Department of Radiation, Radionuclides and Reactors, Section RIH (Radiation and Isotopes in Health), Technical University of Delft, Delft, The Netherlands.*

Presenting Author: Email: marina@ctn.ist.utl.pt | Tel.: +351 219 946 130

INTRODUCTION:

Personal integrated exposure to air pollutants is of considerable importance as it is the key determinant of the pollutants exposure and dose received by an individual and, thus, directly influences the health impacts described by several studies (Almeida-Silva et al., 2014).According to Morawska (2013), up to 30% of the burden of disease from particulate matter exposure can be attributed to indoor-generated particles, signifying that indoor environments are likely to be a dominant factor affecting human health. To study elderly exposure to particulate matter is of special interest since they are considered a susceptible group and, moreover, studies have not been focused on them. Moreover, since 1950 the world is assisting an inversion of age pyramid, with a constant increase on number of elders worldwide. According to the United Nations (2013) the percentage of total population aged 60 years or over in the world was 11% for the year 2010 and 18% for 2050 (United Nations, 2013).

OBJECTIVES:

This work aims to evaluate the elderly personal daily exposure to PM during their daily life, identifying the most problematic micro-environments.

MATERIALS AND METHODS:

This study was developed in the metropolitan area of Lisbon which has an area of 2870 km^2 and has almost 3 million inhabitants. The daily personal exposure to PM_1, $PM_{2.5}$, PM_4 and $PM_{10}$was achieved using a portable dust monitor (DUSTTRAK™ DRX Aerosol Monitor model 8533). The instrument was calibrated just before the study and the zero calibration was done between each participant. The equipment was placed in a back bag in order to perform the sampling point as near as possible of the breathing level. Each participant carried the equipment for 2 to 4 consecutive days, in 24h sampling periods. The participants also fulfilled a daily diary to record their time occupancy.

RESULTS AND DISCUSSION:

Figure 1shows the time spent by the elders in each microenvironment. Due to the lack of differences between week days and weekends, the results are presented for typical 24 h. The results indicate that the elders spend, in average, 95% of their time indoors, splitted between living-room (36%), bedroom (36%), kitchen (12%) and other indoor micro-environments (11%), such as WC, restaurant/coffees, garage and public transportation.

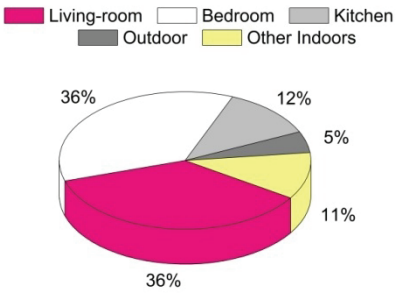


Figure 1 - Time budget data. Values are presented in percentage (%).

Regarding the pollutants concentration, Figure 2a shows the particles concentration for the different micro-environments identified. It is possible to observe that the public transportation is the micro-environment with highest concentration of particles, followed by the office and the kitchen. The site defined by "Others Indoors" is characterized by a PM_{10} concentration of 0.046 mg/m^3 and corresponds to the WC, coffee/ restaurant and an indoor balcony. Inside the dwelling, the micro-environment that presented the highest concentration was the kitchen, which could be explained by the cooking activities.

Figure 2b shows the daily personal exposure, in order to identify the existence of peaks. According to the results it is possible to observe a decrease on particles concentration during the night period. The first peak that occurred between 7:00 and 9:00 corresponds to the uprising and cooking period (breakfast in the kitchen). The other peaks are associated with the public transportations - period of commuting between home and the office.

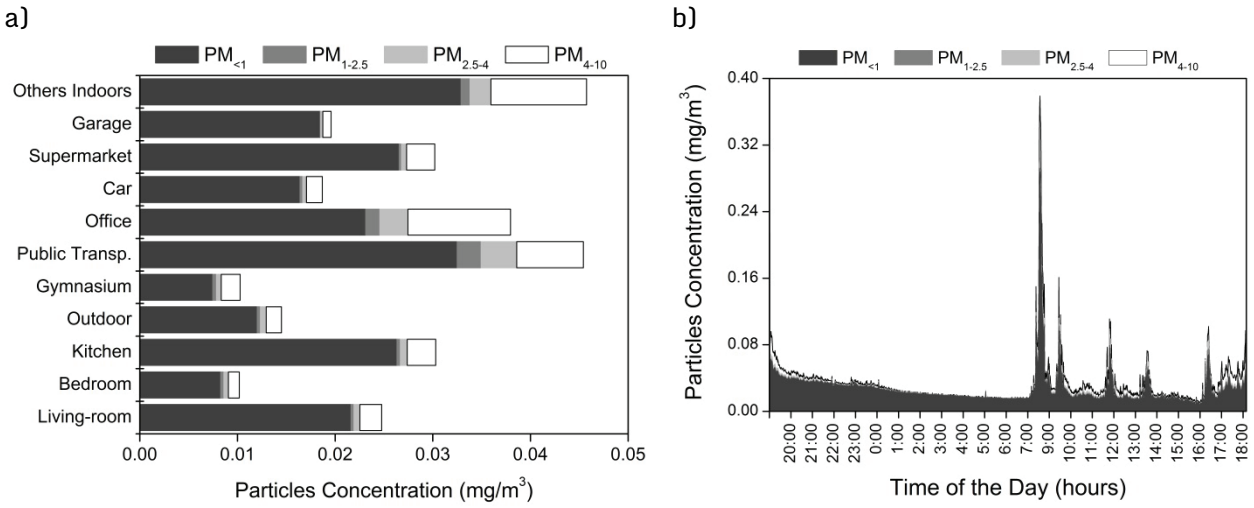


Figure 2 - a) Particles concentration according to each micro-environment. b) Temporal variation (time of the day in hours) of particles. All results are presented in mg.m^{-3}.

CONCLUSION:

This research work focused on the pollutants assessment according to different daily patterns and emphasized the utility of the applied methodology to assess the human exposure. Moreover, the elders' daily exposure assessment to air pollutants proved to fully understand and quantify the magnitude of individual exposure.

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Health risk assessment of local food consumption. The case of two southwest Portuguese coastal villages.

Authors: Paula Vaz-Fernandes[1,2], Ana Paula Martinho[1, 3], Marina Saraiva[4], Sandra Gueifão[4], Inês Coelho[4], Isabel Castanheira[4], Sandra Caeiro[1,5]

1 *Department of Sciences and Technology, Universidade Aberta, Rua da Escola Politécnica, 141, 1269-001 Lisboa, Portugal;*
2 *Center of Public Policy and Management, Institute of Social and Politics Science (CAPP/ISCSP), RuaAlmerindoLessa, 1300-663 Lisbon, Portugal;*
3 *Lead, Laboratory distance education and e-learning, UniversidadeAberta, Portugal;*
4 *Departmentof Food and Nutrition, National Health Institute Dr Ricardo Jorge; Av. Padre Cruz, 1649-016, Lisboa, Portugal;*
5 *CENSE, Center for Environmental and Sustainability Research, Departamento de Ciências e Engenharia do Ambiente.*

Presenting Author: Email: paulavaz@uab.pt | Tel.: +351 213 910 036

INTRODUCTION:

The Sado estuary, located in the West Coast of Portugal, consists of a large basin of great socio-economical importance, also classified as a natural reserve. This coastal area is very important for tourism, local fisheries, aquaculture, maritime transport and upstream large and local farm agriculture. This area is also generally threatened by several sources of anthropogenic pressure from industrial to domestic sources. The presence of potential pollution has originated a moderately contaminated estuary, particularly in areas near industrial areas and the lower estuary. On those locations levels of concern for many contaminants, both organic and inorganic, with adverse toxicological consequences to biota have been found in recent studies(Lobo *et al,* 2008; Caeiro*et al.*, 2009; Ribeiro *et al.*, 2009).Also human health studies conducted in local population confirmed several human exposure routes to Sado estuary contaminants namely trough occupation, water and food consumption. Mira Estuary is another Southwest Portuguese coastal area), with similar fishing and agricultural activities but it is considered one of the estuarine basins least impacted by human pressures in Portugal, without reference to any direct sources of pollution (Vasconcelos et al. 2007). On both of these two estuarine areas small fishing villages coexists with traditional fishing and agricultural practices.

Objectives: The aim of this study was to evaluate the human health risk associated with the local farming products consumption of two Southwest Portuguese Coastal Villages of Sado and Mira Estuaries. The intake of metals and metalloids from the most consumed vegetables, soils and water holes was measured in two coastal villages: Carrasqueira, near the upstream Sado estuary and in Vila Nova de Mil Fontes(VNMF) near the mouth of Mira Estuary. These date was used to analyse the risk assessment according with EFSA guidelines (EFSA, 2009) and the methodology used for estimation of food daily intake.

MATERIALS AND METHODS:

The four more consumed vegetables by the local populations were selected from applied Food Frequency Questionnaire: tomato, lettuce, cabbage and potatoes. To determinate the contamination of metals from local farming products, samples of those vegetables, soils and water holes were collected. Water holes analysis were added since in accordance to early results, the local populations in particular Carrasqueira drink and use this water for cooking and agriculture. Samplings from 3 local farming places in Carrasqueira and also 3 places in Vila Nova de Mil Fontes were selected. Samples were analysed by ICP-MS and ICP-OES preceded by assisted microwave digestion to determine inorganic contents. Composition in chromium, manganese, nickel, copper, zinc, arsenic, selenium, cadmium, lead, were evaluated. In the case of the water hole samples, nitrate and nitrites were also analyse to outwit possible organic contamination. Organic matter was also measured in the soils, by organic carbon loss-on-ignition at 500 ± 50 ºC, as well as fine fraction obtained by hydraulic separation(<63 mm). For the risk assessment of the foodstuff the methodology used was based on: i) the quantification of each metal concentration in the vegetables; ii) the frequency of

consumption of the vegetables according to Food Frequency Questionnaire; and iii) the use of a worst scenario (EFSA, 2009) based on estimation of food daily intake.

RESULTS AND DISCUSSION:

High content of cobalt (Co) was found in the Carrasqueira agriculture soils, although below Canadian Soil Quality Guidelines, also this element was not found in a high content in the analysed foods. All the concentrations found in the vegetables are below limit sof Cd and Pb, according to the Commission Regulation (EC) No 1881/2006 of 19 December 2006, that set maximum levels for certain contaminants in foodstuffs.

The results obtained under appropriated quality control were analysed for risk assessment according with EFSA guidelines and the methodology used for estimation of food daily intake. In the worst Scenario 5 persons (3 children's and 2 adults) from Carrasqueira exceeded the value of EFSA (2009) for As (90% of inorganic As - 0.3 to 8 g/day/kg), above that value there is a potential to cause teratogenic effects. Also 3 person (3 children´s) from VNMF are near the limit value but from Cd (25 µg/kg bw/month according to the JECFA 2011 guidelines). The others metals and metalloids concentrations were below values that can cause effects on human health. These results are of concern in particular for children since only 4 vegetables were analysed and already exceeded the amount of daily intake established by EFSA. The high content of As found in potatoes suggest that further work is necessary to clarify the potential health risks of As to young children of the Carrasqueira. Also, higher Cd concentrations found in VNMF, who are already close to the maximum allowed for this metal, needs further work.

CONCLUSION:

Never the less the results shown that although the contamination is lower on the soils, water and vegetables, there is potential risk in terms of food intake for local population, so showing the relevancy of these methodologies for human health risk assessment, in particular in small and traditional coastal villages. This work also allowed to conclude that human health risk associated with food consumption from local farming is an important line of evidence within an integrative weight of evidence assessment of environmental risk of coastal areas, where the routes of contamination are diverse.

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Evaluation of Food Safety Conditions of Seafood Sales in Coimbra

Authors: Ana Cruz[1], Teresa Mateus[1, 2], Nuno Carolino[1,3], Filomena Ramalho[4], Humberto Rocha[1, 2]

1 *Departamento de Medicina Veterinária, Escola Universitária Vasco da Gama, Coimbra, Portugal*
2 *CECAV, Centro de Ciência Animal e Veterinária, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*
3 *Instituto Nacional de Investigação Agrária e Veterinária, I.P. Polo de Investigação da Fonte Boa, Santarém, Portugal*
4 *Gabinete Médico-Veterinário, Centro Oficial de Recolha de Animais de Companhia de Coimbra, Coimbra, Portugal*

Presenting Author: Email: mv.anacruz@gmail.com | Tel.: +351 961 226 443

INTRODUCTION:

In 2010, the Food and Agriculture Organization (FAO) registered Portugal as the third biggest seafood consumer world-wide (FAO/WHO, 2010). Considering the economic relevance of these consumption levels, there's a need to ensure seafood sales hygiene and safety in Portugal. There are pathogens that can be transmitted between surfaces of structures and equipments, handlers and seafood such as: *Clostridium perfingens, Escherichia coli, Vibrio cholerae, Staphylococcus aureus*, among others (FAO/WHO, 2003). For this reason, the maintenance of structures that make possible the appropriate hygiene and disinfecting procedures, and that avoid contamination of food products is of great importance. Considering these objectives, it is important to, not only have a specific Hazard Analysis and Critical Control Points (HACCP) plan for each establishment, but also that each food operator implements and understands it. To verify HACCP efficiency, verification procedures, either by the own company or by the official authorities are also important (Michele, Antonietta, Alessandra, & Giovanna, 2007).

OBJECTIVES:

This study intended to evaluate the hygiene and food safety practices of seafood selling, which could contribute to contamination with potential hazards to human health, in the city of Coimbra, Portugal.

MATERIALS AND METHODS:

Based on current national laws and European Committee food safety regulations, 20 HACCP pre-requisites were chosen as parameters for this study, and evaluated as "according" (C) or "not according" (N) to these standards. The evaluated parameters in each of the premises, were: "hygiene", "structure adequation", "structure and equipment conservation state", "ventilation", "personnel washing and dressing rooms", "sinks", "hand soap and disinfectant", "disposable hand wipes", "common garbage recipient", " animal sub-products recipient", "lamps", "storage", "traceability", "HACCP", "space organizing", "clothing", "records", "occupational medicine file", "instruction", and "pest control". We evaluated 30 of the 32 establishments known by food safety authorities. These included: 18 seafood stands from the municipal market, 6 hypermarket sections and 6 individual seafood shops. 13 of these establishments sold only fresh seafood, while 10 sold only frozen and dry/salted seafood. The other 7 sold both types of products. The obtained data was analyzed using Microsoft Excel 2010® and Statistical Analysis System® (SAS).

RESULTS AND DISCUSSION:

In the overall seafood sales, the parameters most frequently considered as N were "sinks" (80.00%), "personnel washing and dressing rooms" (73.33%) and "pest control" (71.43%).

Hypermarket seafood selling sections showed N percentages below 40.00% and the most frequently observed N parameters were "ventilation" (33.33%), and "space organizing" (33.33%) of the working place. In this group, the percentages of N were lower comparatively to the other two. This result might be due to stricter rules applied by the companies themselves and by the regulation authorities, being aware that their

products reach a large number of consumers and therefore, the risk of distributing a contaminated product to the general population increases.

Individual seafood shops and municipal market stands scored 100.00% N in ”structure adequation”, “personnel washing and dressing rooms” and “sinks”. In the municipal market, the majority of N’s were related to structural problems.

In the individual seafood shops, the most common N’s were “traceability” (100.00%), poor hygiene state of structures and equipments (83.33%), insufficient number of sinks, or unavailability of hot water (83.33%), and absence of an efficient pest control plan (83.33%). It was also verified that seafood storage and conservation procedures, as to prevent spoilage and contamination, were not adequate (83.33%). In contrast to Pereira (2011) study, in which 0.00% of the establishments in Torres Novas were N for traceability, in Coimbra this value was 100.00% for individual fresh seafood shops.

The relation between N and the type of product sold was also accessed. It was verified that in average, the establishments that sold only fresh seafood, were the ones with higher N percentage (66.44%) comparatively to frozen and dry/salty seafood sellers (46.22%) or the ones that sold simultaneously all types (14.29%). All fresh seafood establishments were classified as N in: “personnel washing and dressing rooms”, “sinks”, “common garbage recipient”, “clothing” and “pest control”. This is a concerning fact, assuming that fresh seafood is manipulated directly to a higher extent and at higher temperatures compared to frozen products. Despite the existence of HACCP implementation in 20 of the analyzed establishments, 13 of these were classified as N for “space organizing”, 12 for “clothing, 12 for “pest control”, 7 for “traceability”, 6 for “records”, 5 for “storage”, 5 for “hygiene”, 3 for “occupational medicine file” and 2 for “instruction”. Therefore, in the majority of these establishments, the HACCP principles were not accomplished, with special emphasis for pre-requisites relative to hygiene good practices in food handling, for example the use of proper clothing and absence of personal decorative objects, adequate storage of cleaning products and personal objects. Considering that 75.00% of the handlers had instruction in the food safety area, the data can also indicate that this instruction might not have been effective. Pereira (2011) verified that only 8.00% of the seafood shops studied in Torres Novas, Chamusca and Golegã (in Portugal) were classified as N to the HACCP parameter. Also, in Torres Novas all the fish shops were classified as C for this parameter.

Solving the most problematic structural issues requires investments in repairing and/or modifying their disposition or conception. Economically, it might be more viable to consider the improvement of the hygienic practices, and procedures that act towards maintaining the “cold chain” of seafood.

CONCLUSION:

The most frequent “not according” parameters in seafood selling establishments of Coimbra were “sinks”, “washing and dressing rooms” and “pest control”. In parameters related to handlers, “clothing” and “space organizing” were the most problematic. Out of 20 establishments that had implemented a plan based on the principles of HACCP, 13 were “not according” for “space organizing”, 12 for “clothing” and also 12 for “pest control”. Fresh seafood establishments showed higher number of “not according” parameters compared to frozen seafood ones. They were in majority related to “washing and dressing rooms”, “sinks”, “common garbage recipient”, “clothing” and “pest control”.

Considering the economic sustainability of small companies, it is considered a priority to invest in new strategies to instruct and inform the seafood handlers, so that HACCP plans can be correctly implemented. It is considered an asset for the scientific community, to extend this study to other cities of Portugal. It would be interesting to repeat the described study in the following years, and verify the percentage of improvement, attaining the objectives of PACE (Establishment Approving and Control Plan).

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Microbiological challenges for safe cold-smoked salmon: studies on smoking processes and inhibitory properties of Lactic Acid Bacteria against *Listeria innocua* and *Listeria monocytogenes*

Authors: Manuela V. da Silva[1,2], Paul A. Gibbs[1,3]

1 *Faculty of Biotechnology, Portuguese Catholic University*
2 *Environmental Health Department* / Centre of Environment and Health - *Allied Health Sciences School of Polytechnic Institute of Porto, Portugal*
3 *Leatherhead Food Research, Surrey, UK*

Presenting Author: Email: m.silva@eu.ipp.pt | Tel.: +351 222 061 000 | Fax: + 351 222 061 001

INTRODUCTION:

Several studies on vacuum-packed cold-smoked salmon stored at 4ºC showed that, at the end of shelf life, 60% of the flora are lactic acid bacteria (LAB) being mainly *Carnobacterium* sp (47%) and *Lactobacillus* sp.(1):also present were Enterobacteriaceae and *Vibrio* sp.. *Listeria monocytogenes* is a foodborne pathogen infrequently present in cold-smoked salmon. The overall incidence of this microorganism in cold smoked salmon was determined to be approximately 10% at the end of shelf life. Contamination could arise from water, raw materials, or the processing chain. The ability of *L.monocytogenes* to grow at temperatures between 4ºC and 10ºC and to endure salt concentrations of up to 12% and pH approaching 4.5 makes it ubiquitous in nature (2). Salting, smoke compounds, and cold or hot smoking processes were not effective for inhibiting *Listeria* growth (2). Nowadays, the general tendency is to decrease the use of chemical additives that could affect the sensory qualities of the product, instead, attention is being given to the use of naturally occurring metabolites produced by selected bacteria that inhibit the growth of undesirable microorganisms.

OBJECTIVES:

Studies on antimicrobial activity were performed using strains of Lactic Acid Bacteria (LAB), isolated from commercial Portuguese cold-smoked salmon and also from laboratory experiments that involved different salting and smoking conditions combined with vacuum and modified atmospheres packaging (VP or MAP),against *Listeria innocua* and *L. monocytogenes.*

MATERIALS AND METHODS:

LAB isolates from Escola Superior de Biotecnologia, Porto (ESB, Portugal) culture collection, isolated from Portuguese commercial vacuum-packed cold-smoked salmon samples and from laboratory experiments, involving different salting conditions (brine and dry salting), smoking (P1: 6h smoking + 2h drying; P2: 2h smoking + 6h drying) and packaging (modified atmosphere, MAP; vacuum, VAC), carried out at ESB. *Listeria innocua* 2030c from PHLS, England, *Listeria innocua*10580 and *Listeria monocytogenes* 37 and 98 from ESB, Portugal were used to assess anti-listerial activities.

For the purpose of studying different kinds of interaction/inhibition between the LAB isolates and *Listeria* strains, the methodology consisted of placing a drop of a LAB strain, side by side, with a drop of the target strain (*Listeria* spp.) on plates with two agar media (Elliker, Tryptone Soya Agar (TSA)+0.6% w/v Yeast Extract). Results were observed after 24h of incubation at room temperature and observations interpreted as: a) both bacteria grow; b) both bacteria grow but, in the intersection, *Listeria* does not; c) LAB grow with very good inhibition of *Listeria* and d) both bacteria are inhibited.

A further methodology was also used:LAB strains were tested by replacing the *L. Innocua* 2030c and *L. innocua* 10580 with two strains of *L. monocytogenes* (37 and 98) by spreading 100µl of broth cultures (overnight culture, 37ªC) of these microorganisms on agar plates of specific culture media (Elliker medium

and TSA+0.6% YE), left to dry and drops (10µl) of different LAB strains were placed on the plates. Inhibition was recorded when the presence of a visible halo surrounding the drops of LAB was observed. For LAB strains that indicated inhibitory properties, bacteriocin activity was study using the methodology proposed by Duffes*et al.*(1999).

For identification ofsome LAB isolates API 50 CH galleries were used following the manufacturers' instructions and specific identification of the LAB isolates was made by SDS-PAGE, by comparing the standards of their total proteins solubilized in SDS with the standards of the reference strains. The methodology used was described by Laemmli (1970) and modified by Kiredjian et al. (1986).

RESULTS AND DISCUSSION:

The behaviour of LAB during storage of cold smoked salmon, was similar between different samples, but differences in initial counts were observed for commercial samples and for cold-smoked salmon samples produced in laboratory experiments. The higher numbers of LAB did not seem to be related with inhibitory properties, but related with the type of smoking processes in combination with packaging storage. The majority of LAB strains isolated from smoking experiments (P2: 2h smoking + 6h drying in combination with packaging in modified atmospheres) showed inhibitory properties against *L. innocua* and *L. monocytogenes* 37. This group of LAB may have different properties and will be characterised by SDS-PAGE (results not shown).

From LAB characterised, some strains showed inhibitory properties against *L. innocua* 2030c and *Listeria innocua* 10580. Compared to inhibition of*L. monocytogenes*, the same LAB showed similar behaviours but fewer numbers of LAB have this capacity. *L. monocytogenes* 98 seems to be more resistant when compared with the other two strains.

The use of this methodology showed different results when the Elliker medium was replaced by TSA+0.6%YE. Yeast extract is frequently used in medium to increase the growth of *Listeria*.

CONCLUSION:

These results can be a useful indication for utilisation of LAB (or their bacteriocins) in the production of cold-smoked fish with improved safety. As the shelf-life of cold-smoked fish is also related to the presence of high numbers of LAB, there would be two objectives for future work (i) reducing the total numbers, but also(ii) selection of those LAB strains with the capacity to inhibit *Listeria monocytogenes*.

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Quantification of total chromium and Cr(VI) in different styles of Lager beers and the influence of packaging material

Authors: Elsa Vieira[1], M. Elisa Soares[2], Isabel M.P.L.V.O. Ferreira[1], M. Lourdes Bastos[2]

1 *Department of Bromatology and Hydrology, Faculty of Pharmacy of Porto University, Portugal*
2 *Department of Toxicology, Faculty of Pharmacy of University of Porto, Portugal*

Presenting Author: Email: elsavieiraf@gmail.com | Tel.: +351 220 428 642 | Fax: +351 226 093 390

INTRODUCTION:

Beer is the third most popular beverage in the world (Granato, Branco, Faria, & Cruz, 2011). In Portugal, in 2012, the consumption of beer was about 53 liters *per capita* (Lichota, 2012). Chromium content results from the composition of ingredients used in the brewing process and also from exogenous sources, namely the contamination from brewery equipment used for beer handling, including fermentation, conditioning, filtration, carbonating and packing material (Pohl, 2008).Chromium in diet may have both beneficial and adverse effects on human health depending on its valence state. For this reason, several European directives have promoted the development of standard methods for Cr(VI) quantification, aiming to limit the release of this element into the environment and to protect consumers (Unceta, Seby, Malherbe, & Donard, 2010). Several studies have been reported to quantify the concentration of chromium in beer. However, the data described in these studies focused only on the total Cr rather than on speciation of Cr(VI), which, to our knowledge, was not done before in beer samples.

OBJECTIVES:

The aim of this work was the determination of total chromium and Cr(VI) in different Lager beers. Furthermore, this work highlighted the variability between different styles of Lager beer and the influence of packaging material. The contribution of beer to daily dietary intake of total Cr and Cr(VI) was also estimated.

MATERIALS AND METHODS:

Seventy beer samples from 38 brands were analyzed, including 56 bottled beers and 14 canned beers. Sampling included Portuguese and imported beers, 1 or 2 samples of each brand packed at different dates that were randomly acquired in local supermarkets in Porto, Portugal. All the samples had at least 3-6 months to the expiration date and were Lager type beers from different styles: Pale Lager, Low Alcohol, Fruit Beer, Dunkel, Schwarzbier, Premium lager, Amber and Heller Bock. For the selective extraction of Cr(VI), a procedure previously adopted to other matrices, using a Chromabond NH_2 column and elution with nitric acid, was adapted and validated for beer analyses. TotalCr and Cr(VI) in Lager beers was quantified by Graphite-Furnace Atomic Absorption Spectroscopy and using validated methodologies. The analytical methods were evaluated according to linearity, repeatability and intermediate precision, limit of detection (LD), limit of quantification (LQ), accuracy and matrix effects. Statistical Analysis were carried out with SPSS (version 17, Chicago IL, USA).

RESULTS AND DISCUSSION:

The methods implemented were accurate and selective to quantify total chromium and Cr(VI) in beer samples.

For total chromium experiments, the linearity range was 0.26-20 μg L^{-1}; the precision was 1.5 and 10.7% for the analytical and the over-all procedures, respectively, and the detection limits was 0.26 μg L^{-1}. For Cr (VI), these results were 0.68-15 μg L^{-1}, 2.5% and 5.9%; and 0.68 μg L^{-1}, respectively. Mean contents of total and Cr(VI) in bottled/canned beer samples grouped according to beer style were quantified and expressed as μg L^{-1}. Please see Table 1. Results showed that the mean content of total Cr ranged between 1.07 μg L^{-1} in canned Pale Lager and 4.32 μg L^{-1} in Low Alcohol beer. The Cr(VI) content in Low Alcohol beer was <2.04 μg L^{-1}. For

other styles (Fruit beer, Dunkel, Schwarzbier, Premium Lager, Amber Lager, Heller Bock and canned beers), Cr(VI) contents were lower than LQ (<1.61 μg L^{-1}). Comparing total Cr contents between Pale Lager canned beers and the same brands of bottled beers, results showed that the first presented significantly lower contents (p=0.045, t test, n=38).These results suggest that the bottling process increased the Cr content of beer.On the basis of a mean daily ingestion of 500 mL of beer, the consumption of this beverage can contribute approximately with 2.28 to 8.64% of women and 1.6-6.17% of men recommended daily intake of chromium.

Table 1 -Contents expressed in μg L^{-1}(Mean Values ± sd), maximum and minimum values of Total Cr and Cr(VI) in bottled and canned beer samples grouped according to beer style

Beer Style	Total Cr (μg L^{-1})	Cr(VI) (μg L^{-1})	Cr(VI)/Total Cr
	Mean±sd	Mean±sd	
Pale Lager (n=24)	3.27 ±3.52	0.94 ± 2.42	0.29
Canned Pale Lager (n=14)	1.07 ± 1.10	<LQ	-
Low Alcohol (n=14)	4.32 ± 4.29	2.04 ± 3.83	0.47
Fruit beer (n=3)	2.13 ± 0.53	<LQ	-
Dunkel/Tmavý (n=5)	1.95 ± 1.31	<LQ	-
Schwarzbier (n=2)	1.26 ± 1.78	<LQ	-
Premium Lager (n=2)	1.96 ± 0.01	<LQ	-
Amber Lager (n=3)	1.58 ± 1.53	<LQ	-
Heller Bock (n=4)	2.38 ± 0.76	<LQ	-

CONCLUSION:

Chromium is a controversial element, whereby its speciation analysis is of the utmost importance. The methods used for quantification of total Cr and Cr(VI) in Lager beers were validated to guarantee its linearity, precision and accuracy. Beer consumption can contribute approximately with 2.28 to 8.64% of women and 1.6-6.17% of men recommended daily intake of chromium, considering an intake of 500 mL of beer.

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Museums and collections nature, use & preservation: long-life for collections(?), life-long occupational health and safety risks. Bridging knowledge towards an integrated management policy.

Authors: Paula Menino Homem[1]

1 *Centro de Investigação Transdisciplinar Cultura, Espaço e Memória, Departamento de Ciências e Técnicas do Património, Faculdade de Letras da Universidade do Porto, Portugal*

Presenting Author: Email: phomem@letras.up.pt | Tel.: +351 226 077 172 | Fax: +351 226 077 182

INTRODUCTION:

Museum buildings and professionals hold and share responsibilities in order to ensure long-term preservation of collections, while in storage, exhibition, transport, research and direct access to publics, for their beneficial use, knowledge and development, pleasure and fun. Museum collections reflect the history of the museum environments and of the methodologies adopted in care and conservation, in order to prevent/ mitigate deterioration, along with their own history and characteristics of nature, behaviour and aging processes. Those reflections may constitute health and safety risks for professionals, mainly, and for those who use the collections. Unfortunately and most of times, they are not clearly perceived and understood. Despite the international research and development of some assessment technologies, the reduced (in)formation, the limited access to and the high costs of the resources, tend to exclude this issue of the policies and practices of research and intervention within museums and other cultural institutions.

OBJECTIVES:

This paper aims to raise awareness of these health and safety risks issues, of interest to different professionals, sharing information and challenging to interdisciplinary approaches towards an integrated risk management policy in museums, in benefit of cultural heritage and the whole community.

MATERIALS AND METHODS:

Some materials that give support to museums and collections are, by nature, a risk to humans as they emit radioactivity and are toxic. Good examples may be found in geological (Lambert, 1994) and biological specimens (Westhorpe, 2008), just for instance. The risk magnitude may vary according to the stability or deterioration process of materials over time.

To prevent/mitigate the deterioration or loss of organic materials by insects, especially since the late 1700s, different methods were used and the application of biocides became a routine. Although knowledge about all of them is incomplete, research proves that arsenic, mercury salts, hydrogen cyanide, DDT, lindane, dichlorvos, methyl bromide, ethylene oxide, naphthalene and paradichlorobenzene were among the ones used in collections. Notwithstanding the preservation of organic materials, the impact of biocides on objects/collections varies according to their specific vulnerability and there are, for instance, also proofs of corrosion as adverse effect on metals. Although nowadays disinfestation methods tend to explore inert atmospheres of anoxia, old residues remain and jeopardize both health and safety and the utility of some specimens for research and general use, as Williams (1999) reviews and discuss.

Other dangerous substances may be left behind due to the use of collections, before entering the museums, and to cleaning methods, once there are inside. As an example, Strahan's (1983) alert for silver cyanide formation, as result of interaction with sodium or potassium cyanide, used as cleaning agent for decades, especially since the 1960s.

RESULTS AND DISCUSSION:

The absence of museums documentation, in terms of old and specific care and conservation methodologies, disguises and camouflages the dangerous legacy. Its imperceptibility and/or misunderstanding enhance the health and safety risks (Makos, 2001).

CONCLUSION:

Beyond adequate legislation, reliable and simple tools for monitoring and evaluation are needed to be developed and made technically accessible and financially affordable, as well as interdisciplinary research projects. They would all give support to the definition of a protocol of action of both protection of professionals and publics and intervention on collections, guided by the cautionary principle.

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Museums and collections

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Evaluation of the 2013 Amadora Heat Response Plan

Authors: F. Montenegro[1], A. Peralta Santos[1,2], D. Ermida[2], D. Pinto[3], J. Silva[4], A. Silva[4]

1 *PublicHealthResident, PublicHealthUnit Amadora, Amadora PrimaryCare Cluster, Portugal*
2 *Center for Research andCreativity in Informatics, Hospital Professor Doutor Fernando da Fonseca, Portugal*
3 *EnvironmentalHealthTechnician, PublicHealthUnit Amadora, Amadora PrimaryCare Cluster, Portugal*
4 *Senior Public Health Specialist, Public Health Unit Amadora, Amadora Primary Care Cluster, Portugal*

Presenting Author: Email: andre.peralta.santos@gmail.com | Tel.: +351 914 248 999

INTRODUCTION:
The adverse effects of heat waves on health are recognized and defined as partially preventable (1,2). Amadora´s Heat Wave Contingency Plan has been developed since 2007 in collaboration with local health stakeholders (Social Services, Civil Protection and Municipality Services). The aim of this plan was to mitigate the adverse effects of the extreme temperatures on the health and well being of Amadora´s population.

OBJECTIVES:
The aim of this evaluation was to describe the procedures in the heat response plan and to quantify the effect of the heat in the health of Amadora´s population. Furthermore we wanted to assess if the impact on mortality expected at the national level had the same pattern at a local level.

MATERIALS AND METHODS:
The2013 contingency plan was implemented in Amadora´s Municipality between 15th of May and 30th of September. The alert levels for heat plan surveillance were defined on a daily basis for the Great Lisbon Region. These were generated based on the ICARO index (3), which estimates the heat´s impact on mortality. The atmospheric temperatures were given by the Instituto Português do Mar e da Atmosfera. The impact on the health services' demand was measured by the number of admissions to the Hospital Emergency Department, and the primary care emergency. We also measured the number of hospital admissions and total deaths in Amadora. To determine the baseline of demand of health services, admissions and mortality we used the variable mean within the days with lowest alert level (green alert). The mean of the variables and the 95% IC was calculated using STATA 13.

RESULTS AND DISCUSSION:
During the 140 days of surveillance of the heat plan we had 3 days of high alert level (red alert), 23 days of mid level (yellow alert) and 114 days of the lowest alert level (green alert). The 3 days of red alert were observed between the 6th and the 8th of July. The mean temperatures are showed in table 1. During the lowest alert level, 530 elders (65 years old plus) were identified by the Social and Municipality Services as a risk group and visited at least once during the contingency plan. Due to lack of interoperability between the databases of different stakeholders it was not possible to do the linkage with the National Health Service databases (primary care and hospitals). Regarding the demand of health services (PC urgency and Emergency admissions) there is no clear increment as we escalated the alert levels. The same was observed in the hospital admissions, although it was possible to identify a dose response relationship between the alert levels and mortality in the hospital and total mortality.

Table1 - Air temperatures in the Great Lisbon area by alert levels

Alert Levels	Max Temp		Mean Temperature		Min Temp	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Green	28.7	27.7-29.7	20.6	20.0-21.2	15.6	15.1-16.1
Yellow	38.2	36.8-39.5	27.2	26.1-28.2	19.2	18.3-20.1
Red	42.0	38.0-46.0	31.7	29.9-33.4	20.0	13.1-27.0

Table 2 - Primary Care urgency, Emergency admissions, hospital admissions and hospital deaths and total deaths in Amadora by Levels of Alert of the Heat Response Plan

Alert Levels	PC Urgency[1]		Emergency Admissions[2]		Hospital Admissions[2]		Hospital Deaths[3]		Total Deaths[4]	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Green (baseline)	11.8	8.9-14.7	130	127-134	11.1	10.4-11.7	2.82	2.51-3.14	3.76	3.38-4.14
Yellow	9.9	4.7-15.1	126	119-134	12.0	10.3-13.6	3.19	2.47-3.91	3.77	2.98-4.56
Red	3.3	0-9.93	127	106-148	11.3	8.5-14.2	3.67	3.01-4.33	5.67	3.92-7.41

1 - Primary care consultations that are not programed; 2 - Emergency and hospital admissions due to cause specific symptoms related heat (respiratory symptoms, cardiovascular and renal symptoms); 3 Hospital deaths due to all causes; 4 - Total deaths

The same mortality pattern was also observed at a national and regional level (4).

CONCLUSION:

More integration between the actions and information systems of the stakeholders is needed to improve the surveillance during the contingency plan. The impact of heat wave in Amadora had little effect on health services demand but had impact on mortality.

ACKNOWLEDGMENTS:

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Quality Control of Water Supply in a Teaching Hospital

Authors: Pedro Norton[123], Natália M. C. Martins[1]

1 *Occupational Health Unit, São João Hospital Centre, Porto, Portugal*
2 *Department of Clinical Epidemiology, Predictive Medicine and Public Health, Faculty of Medicine, University of Porto, Porto, Portugal*
3 *Institute of Public Health, University of Porto (ISPUP), Porto, Portugal*

Presenting Author: Email: pnorton@med.up.pt | Tel.: +351 225 513 652 | Fax: +351 225 513 653

INTRODUCTION:
A Water Quality Control Program was implemented in 2005 in a Teaching Hospital and it provides the analysis of three types of water: hemodialysis, hydrotherapy pool and general water consumption. The first two are mandatory and covered by specific legislation (Despacho nº 14391/2001, of July, 10 and Aviso nº: 1352/2002, of January, 31, respectively). Regarding water for general consumption, there is no legislation regulating its use in hospitals. However, considering that this type of water is used by immunodepressed patients and also to prepare infant's formula milk, the Occupational Health Department has given special attention to assessing its quality.

The reference range initially used for the concentration of Cl_2 at the Hospital was 0.2 to 0.6 mg/L Cl_2, as recommended in the Decreto-Lei nº: 306/2007. However, considering the type of patients treated, the structural dimension of the building (with a 50km water network), and the previous history of the presence of pathogenic bacteria (including *Pseudomonas aeruginosa* and *Legionella spp*), it was decided in 2012 to increase the reference range to 0.8 to 1.5 mg/L of Cl_2.

OBJECTIVES:
To determine the effect of increasing the concentration of Cl_2 used for the disinfection of the water supply, in the detection of *Pseudomonas aeruginosa* and *Legionella* spp.

MATERIALS AND METHODS:
5 ml of water were collected on a daily basis, during 2011 and 2012, at random points of the building. The method used for the detection of *Legionella* spp. in the water is described at ISO standard 11731; the one used for *Pseudomonas* analysis is at ISO 16266. The Cl_2 concentration was measured with a colorimeter. Statistical analysis was performed using SPSS software version 21.0. Chi-square test (χ^2) was used to compare categorical variables and the statistical significance was considered for $p<0.05$.

RESULTS AND DISCUSSION:
6809 and 6082 water samples were collected during 2011 and 2012, respectively. Microbiologically, the proportion of non-compliant samples was 5.9% in 2011 and 3.9% in 2012 (p = 0,005) (Figure 1). Similarly, between 2011 and 2012 the number of isolated *Pseudomonas aeruginosa* declined (9.9% vs 2.0%, p = 0,000) as well as the number of *Legionella spp* (62.5% vs 11.1%, p = 0,003) (Figure 2).

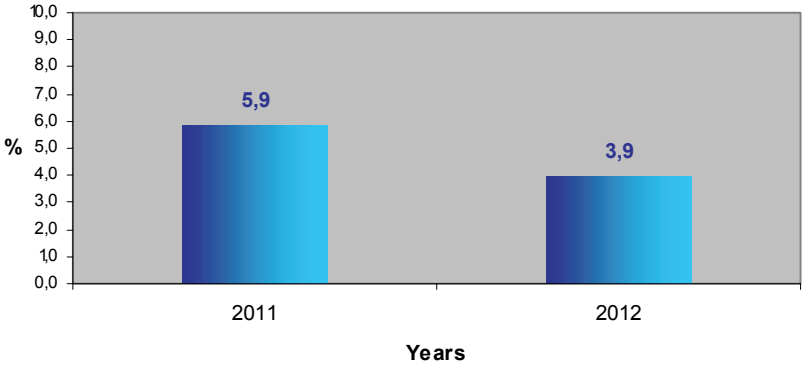


Figure 1 - Proportion of non-compliant samples considering all the microbiologic parameters

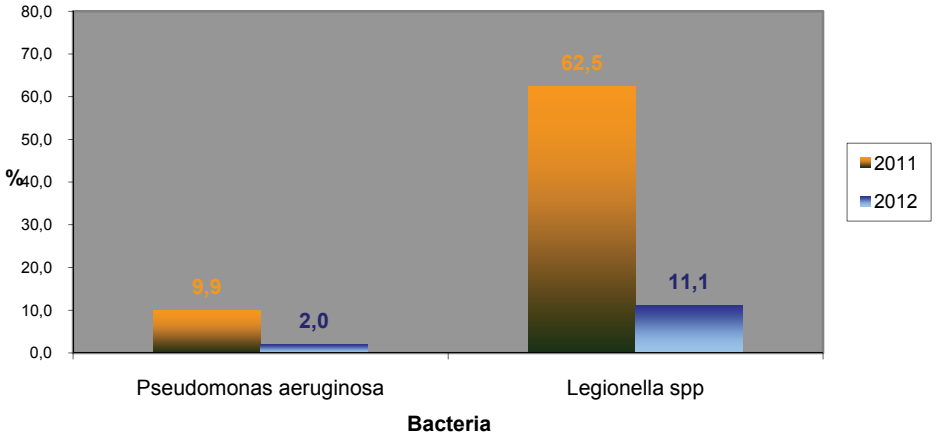


Figure 2 - Proportion of microbiological non-conformances for *Pseudomonas aeruginosa* and *Legionella spp*

CONCLUSION:

Increased concentration of Cl_2 to the range 0.8 to 1.5 mg/L enabled more effective disinfection of water supply. Therefore, the concentration of Cl_2 in the water supply (0.2 to 0.6 mg/L) recommended by Decreto-Lei nº: 306/2007 should be revised.

Biological Air Contamination in Elderly Care Centers: GERIA Project

Authors: Lívia Aguiar[1], Ana Mendes[1], Cristiana Pereira[1], Paula Neves[1,2], Diana Mendes[1], João Paulo Teixeira[1,3]

1 *Environmental Health Department, Portuguese National Health Institute Doutor Ricardo Jorge, Porto, Portugal*
2 *Research Centre of Health, School of Allied Health Sciences of Polytechnic of Porto, Portugal*
3 *Institute of Public Health (ISPUP), Porto University, Porto, Portugal*

Presenting Author: Email: livia7_aguiar@hotmail.com | Tel.: +351 912 720 263

INTRODUCTION:

Healthcare organizations throughout the world have an increasing concern about how to cope with a quickly aging population (Caley & Sidhu, 2011). This trend explains the increasing demand of long-term care services (Damiani *et al.*, 2009) such as Elderly Care Centers (ECCs) (Kembel *et al.*, 2012). In Portugal, the number of ECCs increased 49% between 1998 and 2010. Furthermore, considering that elders often spend a considerable portion of their lives indoors, enhanced by they reduced independency, it is clear that the possibility that adverse indoor climate can influence their health status cannot be ignored (Almeida-Silva *et al.*, 2014). It has been estimated that older persons spend about 19-20 hours per day indoors and the majority spend all of their time inside the ECCs. Older persons may be particularly at risk of detrimental effects from pollutants, even at low concentrations, due to their common and multiple underlying chronic diseases that increases their susceptibility. It is extremely important to assess elderly exposure to biological pollutants due to the adverse health effects caused by biological agents to this susceptible population present in ECCs, enhanced by the time these people spend indoors.

OBJECTIVES:

The study aims were 1) to evaluate the total bacteria and fungi concentrations in a representative sample of ECCs in Porto as compared with the current national standards, 2) to study the variability of this biological parameters among different spaces within ECCs, 3) to identify the main fungi species found in the evaluated areas, and 4) how buildings characteristics may affect the indoor air biological pollutants.

MATERIALS AND METHODS:

Out of a total of 58 ECCs located in Porto urban area, 38% (n =22) accepted to participate in this study. Data were collected for each ECCs in two seasons and the following parameters were measured: building and ventilation characteristics, as well as biological parameters such as total bacteria count, fungi count and identification. It was conducted during summer and winter, from November 2011 to August 2013, at a total of 141 areas within dining rooms, drawing rooms, medical offices and bedrooms (including the bedridden). Air sampling was carried out with a microbiological air sampler (Merck Air Sampler MAS-100) and using *Tryptic Soy Agar* (TSA) for total bacteria and *Malt Extract Agar* (MEA) for fungi. Ambient air samples were also collected for comparison to the indoor measurements. The results obtained were compared with the recently revised Portuguese standards. Classical statistical methods were used to estimate means, medians and frequencies (percentages) in order to obtain insight into the ECCs characteristics and environmental monitoring results within and between buildings. All data were analyzed using IBM SPSS 21.0.

RESULTS AND DISCUSSION:

In the present study, 22 ECCs had an average bacteria concentration within the actual reference terms in both seasons and in all evaluated rooms. Looking back the previous legislation, the values would also be considered in conformity within the limit value. To be noticed that bacteria concentrations were higher in summer for all ECCs evaluated areas.

In summer season, the fungi concentration results, within ECCs and rooms, found mean concentrations all according the references. However, in winter this was not detected. The ECCs indoor fungi mean concentrations, and also in 4 out of 5 assessed areas, presented results above the outdoor concentrations. If the previous legislation were still ruling, fungi concentration would be considered to be within the reference value for ECCs mean and for concentrations found in some evaluated areas.

The most predominant indoor fungi species were *Cladosporium* (73%) in summer, followed by *Penicillium* (52%), *Paecilomyces* (52%) and *Aspergillus flavus* (52%). In winter, *Penicillium* (67%) was the prevalent fungi specie identified, being *Cladosporium* (58%) and *Aspergillus niger* (57%) the other two main species (Figure 1). Nonetheless, indoor vs. outdoor fungi specie identification comparison showed that fungi species indoors were the same as outdoors: *Cladosporium* in majority, followed by *Penicillium*. *Aspergillus fumigatus*, known potential pathogenic/toxigenic species, was also identified.

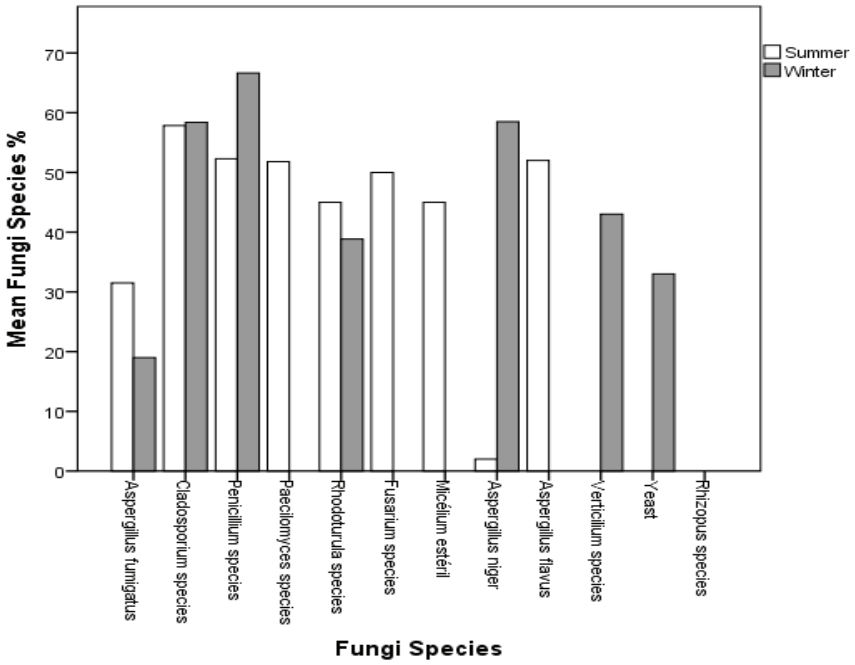


Figure 1 - ECCs main indoor fungi species by season.

Regarding the possible influence of building characteristics on the biological indoor air parameters, our study showed that the presence of bacteria in an ECCs depends on heating/ventilation or the presence of building pathologies, as well as upon the presence of windows sealants and the type of these sealants. On the other hand, fungi concentrations are associated to the building adaption to ECCs, existing sources of pollution, insulation, roof and windows characteristics, and building pathologies such as infiltrations and condensations.

CONCLUSION:

Comparing the results with Portuguese newly legislation, only fungi concentrations in winter did not accomplished the reference levels. Nevertheless, maximum bacteria concentrations were very high in both seasons. It is important to notice that, although biological concentrations were considerable acceptable, in light of Portuguese references, it doesn't assure that there is no risk for people who spend most of the day, or even consecutive days, inside these areas. Especially the elderly people: a group that is known for their impaired immune system and therefore ability to develop diseases, such as respiratory illnesses or airborne infections.

Although fungi main species found were *Cladosporium* and *Penicillium*, considered to be common in indoor air, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger* were also identified, species that produce mycotoxins and therefore can be a cause of several adverse health effects.

For an improvement of IAQ regarding biological agents, control of indoor humidity is essential, eliminating all dampness and mold present in walls, roofs and surfaces. Number of occupants and their habits are also a contributor for the proliferation of microorganisms. Opening the windows, or intake of fresh and clean air, when the room is empty, is another effective and inexpensive measure that can improve IAQ, removing biological agents from its composition.

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Seasonality of vector-borne diseases in the Western Amazon and its relationship with local climate oscillations

Authors: Juliana Lúcia Duarte [1]; Leandro Luiz Giatti [1]; Antônio Carlos Batista [2]; Daniela Biondi Batista [2]; Giuseppe Biondi Batista [3]

1 *Department of Environmental Health, school of Public health, University of São Paulo, São Paulo, São Paulo, Brazil*
2 *Department of Forestry Engineering, Federal University of Paraná, Curitiba, Paraná, Brazil*
3 *Department of Pathology, Federal University of Paraná, Curitiba, Paraná, Brazil*

Presenting Author: Email: jluciaduarte@hotmail.com | Tel.: +55 68 812 46018

INTRODUCTION:
In Brazil, the climate is characterized by alternating cold and warm seasons in temperate zones, located further south, and the alternation of the dry season and the rainy season in tropical areas, located further north and northeast of the country. The Western Amazon is an area located in the north of the country and consists of the states of Amazonas, Acre, Rondônia and Roraima. In this region, the rains generally occur between the months from October to April, while the dry prevails between May and September. During the dry season, local climatic oscillations are intensified by the burning and deforestation.

Deforestation reduces the capacity retention of rainwater, creating a substantial increase in the level of the rivers and surface runoff. Then the floods occur. As a result, the population of this region suffers the impact of climate variability and changes in environmental conditions almost all year. With global climate change, the weather, climate and the environment have been significantly affected over the years, increasing the occurrence and magnitude of extreme weather events. These events can produce many hazards to human health, including increased morbidity and mortality due to climate sensitive diseases (Barcellos et al, 2009; Silva, Barcellos & Bacuri, 2010; World Health Organization [WHO], 2012).

OBJECTIVES:
Studying the behavior of the major vector-borne diseases to climate fluctuations and changes in environmental conditions in a region of the Brazilian Amazon Basin, between the years 2000-2013.

MATERIALS AND METHODS:
The study was conducted in the city of Rio Branco, State of Acre's capital. With more than 336,000 inhabitants, is the city of greatest economic importance to the state. Located on the banks of the Acre's River, is divided into two parts: the first district (left) and second district (right). During periods of rain, the level of the Acre's River increases and exceeds its quota overflow (14 meters) causing flooding involving a good part of town and displace thousands of people. During the dry season, the river level reaches generally less than 1 meter and the supply of water service in the suburbs is impaired. During this period also usually fires occur in urban, rural and forest areas. Thus, the city constantly suffers the impacts of local climate oscillations (Duarte, 2011).

For this study, data on the number of monthly hospitalizations for dengue, malaria and leishmaniasis were collected, the vector-borne diseases with the highest incidence in the region, from January of 2000 until December of 2013 (Ministery of Health of Brazil). The incidences of hospitalizations were calculated considering the annual population estimates for the city. For the same time period, the values of the average monthly rainfall, temperature, relative humidity (National Institute for Space Research) and the Acre's River level (National Water Agency) were also collected. Data were tabulated and organized into Microsoft Excel spreadsheets. Each variable had its monthly average over 13 years selected for the study. Values were tested for association by determining the Pearson correlation coefficients (r), using the statistical program ESTATGRAPHICS CENTURION XVI.

RESULTS AND DISCUSSION:

Positive and statistically significant difference between the incidence of dengue and rain precipitation, relative humidity and the Acre's River level correlations were observed, considering the confidence interval of 95%. There was no statistical association between the incidence of malaria and leishmaniasis with climate and environmental variables studied in this work (table 1).

Tabela 1 - Results of the statistical correlation test between variables.

		Dengue	Malaria	Leishmaniasis
Temperature (ºC)	Pearson (r)	0,1814	-0,0741	0,0372
	p value	0,5726	0,8190	0,9085
	Association	Absent	Absent	Absent
Relative humidity (%)	Pearson (r)	0,7974	- 0,3409	0,1177
	p value	0,0019	0,2781	0,7157
	Association	Positive	Absent	Absent
Amount of rain (mm)	Pearson (r)	0,6587	0,3340	-0,0328
	p value	0,0199	0, 2887	0, 9193
	Association	Positive	Absent	Absent
River level (cm)	Pearson (r)	0,9496	0,4683	-0,0507
	p value	0,0000	0,1247	0,8756
	Association	Positive	Absent	Absent

The results show that dengue, in the studied region, features a seasonal profile with elevated incidence usually between the rainier months, from December to April (Figure 1). This occurs because the seasonal dynamics of dengue vector, which features an aquatic phase in its development, is commonly associated with climatic conditions that favor greater number of breeding (Viana & Ignotti, 2013).

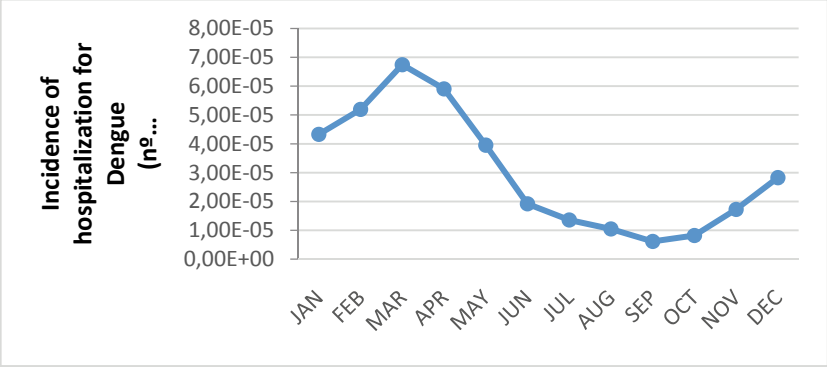


Figure 1 - Illustrative plot of the seasonal profile for dengue in Rio Branco (2000-2013).

CONCLUSION:

Among the major vector-borne diseases in this part of Western Brazilian Amazon region, dengue stands out for being the only one to present typical seasonal profile and incidence highly related to periods of higher rainfall, humidity and concentration of water in the breeding of the mosquito vector.

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Fungal contamination of sandpits from recreational parks

Authors: Carla Viegas[1], João Brandão[2], Márcia Meneses[1], Raquel Sabino[1,2], Cristina Veríssimo[2]

1 *Environmental Health RG - Lisbon School of Health Technology - Polytechnique Institute of Lisbon, Portugal*
2 *MycologyLaboratory - Instituto Nacional de Saúde Dr. Ricardo Jorge ofLisbon, Portugal*

Presenting Author: Email: carla.viegas@estesl.ipl.pt | Tel.: +351 218 980 430 | Fax: +351 218 980 469

INTRODUCTION:
Soils rich in keratinic residues constitute a permanent or occasional reservoir for dermatophytes and other keratinophilic fungi. They are therefore a source of potential infection for man and animals, especially in urban environments where people, residues, wild and domestic life, all come together in high density. Sandpits, used by children and sporters, are frequently visited by animals such as sparrows, pigeons, dogs, cats and rats all year round. These visitors leave organic debris and contaminate the sand directly with the flora they carry. Municipalities in Portugal have the legal obligation to maintain recreational parks in certain hygienic and safety levels. These requirements include sand maintenance, when used.

OBJECTIVES:
This study intended to assess fungal contamination from sand of two recreational parks in the city of Lisbon.

MATERIALS AND METHODS:
One sample from each park was collected and 40 g of each suspended in 100 mL of sterilized water, followed by agitation during 30 min at 100 rpm. Two replicas of 0.2 mL of each sample were inoculated in malt extract agar (2%) with chloramphenicol (0.05 g/L) and incubated during 5 to 7 days at 27.5 ºC and 40 ºC. For dermatophytes analysis, van Breuseghem technique was applied and after incubation during 20 days at 27.5ºC, baby sterilized hair were incubated on mycosel agar with cyclohexamide at same temperature during two weeks. Results were obtained with identification of the isolated fungal species. Microscopy was used to identify isolated fungi, using lactophenol cotton blue tease mount or Scotch tape mount. Morphological Identification was achieved through macro and microscopic characteristics as proposed by specific atlas for fungal identification. Table 1shows the results obtained under the assumption of 1 ml of wash being equivalent to 1g of sand.

RESULTS AND DISCUSSION:
From one of the samples ten filamentous fungi genera/species were isolated with the following distribution of the most present ones: *Fusariumdimerum* (32.4%), *Cladosporium*sp. (23.5%), *Fusariumsolani* and *Phoma* sp. (11.8%), all of which potentially pathogenic. The other sample yielded eight relevant genera/species. Of these eight, all relevant in a public health context, the main three found (and respective distributions) were: *Chrysonilia* sp. (46.6%), *Cladosporium* sp. (28.8%) and *Acremonium* sp. (15.1%). Dermatophytes were not detected in this study.

Table 1 - More prevalent fungi at 27.5 ºC incubation temperature

Sample 1	Freq. (%)	CFUg	Sample 2	Freq. (%)	CFU/g
Fusariumdimerum	32.4	55	*Chrysonilia*sp.	46.6	170
*Cladosporium*sp.	23.5	40	*Cladosporium*sp.	28.8	105
Fusariumsolani	11.8	20	*Acremonium*sp.	15.1	55
*Phoma*sp.	11.8	20	*Fusarium*sp.	4.1	15
Others	20.5	35	Others	5.4	20
Total		170	Total		365

Since there are no legal requirements concerning mycologic parameters, criteria applied by Sabino et al., 2011 for beach sand were used. Considering these requirements, it is important to highlight the identification of potential pathogenic species, such as species from *Aspergillus*, *Scedosporium*, *Scopulariposis*, *Scytallidium, Chrysosporium* and *Fusarium.* The later was found in both samples. *Fusarium* spp. were found to exceed the recommendations by Sabino et al., 2011 (up to 85 CFU/g).

Risk of fungal infection for children must be assumed as sandpits are often used as playground and also due to their increased susceptibility (Rees, 1967). Moreover, domestic canines and felines often have access to public sandpits and may carry keratinophilic fungi asymptomatically. They should therefore be considered asdermatomicosis carriers (Mantovani, 1978), inducing direct and indirect sand contamination.

Although dermatophytes were not found we cannot be certain of their absence since it was a cross-sectional study and longitudinal studies are needed. Also, since only conventional methods were used, intrinsic limitations, such as differences in fungi growth rates and recovery yield result in data underestimation (the later has been estimated in the lab as 1% only - unpublished data) - fungal species with faster growth rates may inhibit others (Bartlett et al., 2004).

CONCLUSION:

Potential risk of exposure to keratinophylic fungi by children has been proven in the sand of these two recreational parks and attention or care is therefore suggested to the frequent replacement and/or chemical treatment of this material. It is essential to maintain good hygiene of sandpits in order to avoid keratinophilic fungal proliferation and infections.

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The effect of socioeconomic status on dietary patterns in representative cohort of Polish girls aged 13-21 years

Authors: Joanna Kowalkowska[1], Lidia Wadolowska[1], Jolanta Czarnocińska[2], Marzena Jezewska-Zychowicz[3], Ewa Babicz-Zielińska[4]

1 *Department of Human Nutrition, University of Warmia and Mazury in Olsztyn, Poland*
2 *Department of Human Nutrition and Hygiene, Poznan University of Life Sciences, Poland*
3 *Department of Organization and Consumption Economics, Warsaw University of Life Sciences, Poland*
4 *Department of Trade and Services, Gdynia Maritime University, Poland*

Presenting Author: Email: joanna.kowalkowska@uwm.edu.pl | Tel.: +48 89 523 3270

INTRODUCTION:
Dietary habits are influenced by many factors of socioeconomic status which determine the food choices and diet quality as well as health [1]. It was found that the low socioeconomic status favored inappropriate dietary habits, i.e. higher consumption of high energy density food, containing a lot of fat and/or sugar, which is cheap and lower consumption of fruit and vegetable as quite expensive products [2].

OBJECTIVES:
To analyze the effect of socioeconomic status (SES) and single factors of SES on dietary patterns in girls.

MATERIALS AND METHODS:
The research involved the representative cohort of 1107 Polish girls aged 13-21. A total of 2104 individuals were randomly selected from the PESEL data base. Interviews were conducted with 52.6% initial group. Socioeconomic status (SES) was based on: place of residence, economic situation, number of children in the family, parenteral education. Based on numerical values assigned to each components, SES index was calculated (SESI). Based on the tercile's distribution girls with low (28.0% total sample), average (34.6%) or high SESI (37.4%) were identified. The validated Food Intake Variety Questionnaire (FIVeQ) and two validated Block's Questionnaires in own modification were used to assess fiber intake (Block Screening Questionnaire for Fruit/Vegetable/Fiber Intake, BSQFVF) and fat intake (Block Screening Questionnaire for Fat Intake, BSQF) [1,3]. The three hypothesis-driven dietary patterns were pre-definied: (1) pro-healthy (acceptable food intake variety, fiber and fat intake), (2) neither pro-healthy nor non-healthy, (3) non-healthy (unacceptable food intake variety, fiber and fat intake). The reference groups in logistic regression analysis were girls with: pro-healthy dietary pattern and low SES or lived in village, low economic situation of household, average economic situation of family, family with 4-8 children, elementary education of father and mother (OR=1.00). The odds ratio were adjusted by age and the significance of odds ratio was assessed by Wald's statistics.

RESULTS AND DISCUSSION:
Non-healthy dietary pattern had more girls with low SES than high SES (Table 1). More girls with non-healthy dietary pattern lived in the village, had a father with elementary education, came from families with 4-8 children.

Table 1 - Dietary pattern prevalence depending on socioeconomic status and its factors in representative cohort of Polish girls and young women aged 13-21 years (%) (n=1107)

Socioeconomic status (SES) and single factors of SES	N	Dietary pattern (%)		
		Pro-healthy (N=48)	Neither pro-healthy nor non-healthy (N=875)	Non-healthy (N=184)
SES***				
low	310	1.7	75.9	22.4
average	383	4.5	78.5	17.0
high	414	6.1	81.9	12.0
Education of mother				
elementary	441	2.8	78.2	19.0
secondary	484	4.9	78.9	16.2
higher	182	6.4	81.5	12.1
Education of father*				
elementary	579	2.8	79.0	18.2
secondary	389	5.7	78.3	16.0
higher	139	6.7	81.2	12.1
Place of living**				
village	521	3.6	78.4	18.0
town	348	2.4	80.6	17.0
city	238	8.6	78.3	13.1
Self-declared economic situation of family				
below average	44	0.0	78.4	21.6
average	885	4.5	78.6	16.9
above average	178	4.2	81.6	14.2
Self-declared economic situation of household*				
poor	91	9.1	74.2	16.6
average	549	3.1	78.3	18.6
good	467	4.8	80.9	14.4
Number of children in the family (<18 years)**				
4-8 children	38	2.8	72.0	25.2
2-3 children	378	6.5	73.9	19.5
0-1 child	691	3.2	82.2	14.6

#Pro-healthy - acceptable food intake variety, fiber and fat intake; & Neither pro-healthy nor non-healthy - weren't met one or two criteria: unacceptable food intake variety and/or fiber intake and/or fat intake; ‡Non-healthy - unacceptable food intake variety, fiber and fat intake; * p<0.05; ** p<0.01; *** p<0.001 (chi^2 test)

The odds ratios (OR) for non-healthy dietary pattern were: 0.23 (95%CI: 0.09-0.54; p<0.001) in girls with high SES, 0.33 (95%CI: 0.14-0.81; p<0.05) in girls of mothers with higher education level, 0.31 (95%CI: 0.12-0.81; p<0.05) in girls of fathers with higher education level, 0.41 (95%CI: 0.21-0.81; p<0.05) in girls of fathers with secondary education level, 0.41 (95%CI: 0.20-0.84; p<0.05) in girls living in the city and 3.27 (95%CI: 1.23-8.69; p<0.05) in girls with average economic situation of household (Figure 1).

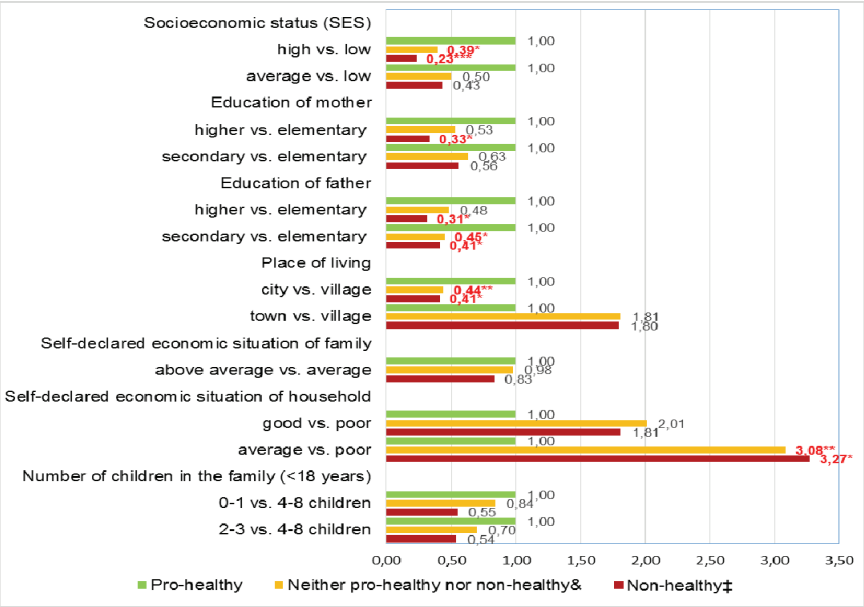


Figure 1 - Odds ratio (OR) of 'non-healthy' or 'neither pro-healthy nor non-healthy' dietary pattern prevalence depending on socioeconomic status and its factors in representative cohort of Polish girls and young women aged 13-21 years (n=1107)

&Neither pro-healthy nor non-healthy - weren't met one or two criteria: unacceptable food intake variety and/or fiber intake and/or fat intake; ‡Non-healthy - unacceptable food intake variety, fiber and fat intake; * p<0.05; ** p<0.01; *** p<0.001 (Wald's statistics)

CONCLUSION:

Higher socioeconomic status of girls was related to lower prevalence of non-healthy dietary patterns such as low food intake variety, inadequate dietary fiber intake and high fat intake. The results prove the positive effect of high socioeconomic status overall as well as single factors of socioeconomic status on diet.

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Pregnant women's attitudes towards congenital neural tube defects prevention

Authors: Dorota Żołnierczuk-Kieliszek, Teresa B. Kulik, Mariola Janiszewska, Joanna Król

Chair of Public Heath, Medical University of Lublin, Poland

Presenting Author: Email: dorota.zolnierczuk@gmail.com | Tel.: +48 79 498 4699 | Fax: +48 81 742 3714

INTRODUCTION:

Folic acid supplementation is an effective method of congenital neural tube defects prevention. Neural tube defects are the most notable birth defects that occur from folate deficiency. Neural tube defects are severe abnormalities of the central nervous system that develop in babies during the first few weeks of pregnancy resulting in malformations of the spine, skull, and brain; the most common neural tube defects are spina bifida and anencephaly.

To prevent these abnormalities every woman in a reproductive age who could become pregnant should supplement folic acid - 0.4 mg per day. Folic acid deficiencies must be corrected before a woman knows she is pregnant because the neural tube forms so early in pregnancy (day 26 to 28). This has led to public health strategies that mandate supplementation in food products, folic acid has been added to white flour, where it finds its way into baked goods like bread. But it has been proved that in children, men, and women beyond their childbearing years, supplementation in the absence of folic acid deficiency has no demonstrated health benefits, and there are worrying signals that it may raise cancer risks, possibly by "feeding" existing cancer. So folic acid supplementation in women of childbearing age becomes the right and reasonable choice.

OBJECTIVES:

The aim of the study was to become acquainted with the attitudes of pregnant women towards congenital neural tube defects prevention.

MATERIALS AND METHODS:

The study was conducted from August to December 2013 and comprised 100 pregnant women from Lublin and Kraśnik (Lublin Province, southeastern Poland). The survey was administered by the researchers among pregnant women in out-patient clinics and women attending classes at a maternity school. An original questionnaire consisting of 23 questions was used. The questionnaire was not validated previously. Explanations were given by the researchers to any respondents' doubts or questions.

The attitudes of pregnant women towards neural tube defects prevention were treated as dependant variables, whereas the women's age, permanent place of residence, education, marital status and the number of past pregnancies were treated as independent variables.

To identify distinctions and dependences between variable chi^2 test was used. For a small number, Yates correction was applied. Values with probability <0.05 were taken as statistically significant. Statistical analysis was conducted using STATISTICA v 10.0 software (StatSoft, Poland).

The women's age varied between 17 and 38 years, mean 28.1; standard deviation 4.7. In the examined group two age groups were distinguished: 17-28 years (48% of respondents) and 28-38 years (52% of respondents). 68% of the polled women were big cities inhabitants, 19% were small town inhabitants, permanent rural dwellers constituted 13% of the examined group.

24% of polled women were classified as women with lower education (secondary and vocational education), whereas 76% of respondents were classified as those with higher level of education (bachelor's degree,

master's degree, postgraduate or doctorate). Married women constituted two thirds of the polled population (66%), women in informal relationship constituted 22% and single women 11% of the examined group.

RESULTS AND DISCUSSION:

In our study all respondents supplemented folic acid during their pregnancy. Nevertheless over half of the women (55%) started folic acid supplementation after conception and three fours of them started the folic acid supplementation in the second month of their pregnancy. Having in mind that folic acid deficiency in the first month of pregnancy can cause neural tube defects of a fetus we must admit that starting the folic acid supplementation in the second month of pregnancy may turn out to be too late to prevent the development of the defects.

Other authors showed lower percentage of pregnant women supplementing folic acid before conception. Hamułka and Wawrzyniak (2005) claimed that only 24% pregnant women from Mazovia Voivodeship supplemented folic acid before they got pregnant, Suliga (2011) estimated this percentage as high as 33% and Gacek (2010) as high as 72.5%.

According to our data all surveyed women strictly followed the rules of folic acid supplementation and took folic acid tablets daily.

The proper folic acid supplementation means starting the supplementation at least for a month before conception and continuing it until the twelfth week of pregnancy.

In our study statistical differences were observed between the respondents age and the time of starting folic acid supplementation. They are shown in table 1.

Table 1 - The relationship between respondents' age and folic acid supplementation before and after getting pregnant.

Age interval	Folic acid supplementation		
	Before getting pregnant	After getting pregnant	Total
17-27	27.1%	72.9%	100.0%
28-38	61.5%	38.5%	100.0%
Total	45.0%	55.0%	100.0%
	$Chi^2 = 11.9722$; $p = 0.0005$		

These findings are consistent with Czech-Szczapa study (2012), who established that younger women more seldom than the older ones supplemented folic acid before conception.

Similar statistical differences were observed according to women's education: the higher the level of education the higher the percentage of women supplementing folic acid before they got pregnant. These differences are presented in table 2.

Table 2 - The influence of respondents' education on folic acid supplementation before and after getting pregnant.

Education	Folic acid supplementation		
	Before getting pregnant	After getting pregnant	Total
Secondary and vocational	26.1%	73.9%	100.0%
Higher	50.6%	49.4%	100.0%
Total	45.0%	55.0%	100.0%
	$Chi^2 = 4.3170$; $p = 0.0377$		

Similar differences were found by other researchers - Godala, Pietrzak, Łaszek, Gawron-Skarbek and Szatko (2012) as well as by Czech-Szczapa (2012).

Statistical analysis of the obtained data showed that women in second and subsequent pregnancy more often started folic acid supplementation before conception in comparison with women in first pregnancy (28.4% versus 92.3% respectively, p<0001).

CONCLUSION:

All respondents followed the rules of congenital neural tube defects prevention and supplemented folic acid during their pregnancy. Nearly half of the respondents had taken folic acid tablets before conception. All pregnant women took folic acid tablets every day.

Women aged 28-38, women with higher education and respondents being in second or subsequent pregnancy followed the rules of congenital neural tube defects prevention more strictly and had taken folic acid tablets before conception significantly more often then the other respondents.

It is necessary to disseminate knowledge on congenital neural tube defects prevention among women in reproductive age.

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Temporary hearing threshold shift at the Valpaços Scouts Fanfare

Authors: Aida Sousa[1], Matilde Rodrigues[1], Joana Calvão[1],Inês Moreira[1]

1 *Research Centre on Environment and Health, Allied Health Sciences School of Polytechnic Institute of Porto, Portugal*

Presenting Author: Email: aidas@eu.ipp.pt | Tel.: +351 222 063 232 | Fax: +351 222 063 232

INTRODUCTION:

Noise is present in our daily life at several places and activities, i.e., at home or at work, or even when we listening to music. Without noticing, we can be exposed, directly or indirectly, to high levels of sound pressure that sometimes are assessed as harmless because they are enjoyable. This is the case of music. In general, music is seen as an enjoyable sound, and can be listened by hobby or due to professional activities. However, when exposed to high levels of sound pressure, health effects can arise, particularly at hearing level (Sataloff, 2006). Noise- induced hearing loss, tinnitus, hyperacusis, diplacusis and implications in performance are some of the effects found in previous studies with professional musicians, particularly in classic musicians (O'Brien *et al.*, 2008). However, other groups of musicians, mainly non-professional musicians can also be exposed to high noise levels. The scouts' fanfare musicians are one of these groups. The noise levels in the course of rehearsals and performances can be high and carried out in places with poor acoustical conditions. Furthermore, most of the musicians making up these groups have low sensibility to the noise problem, and no risk reduction measures are implemented. Bearing this in mind, it is important the analysis of the problem related with fanfare musicians noise exposition.

OBJECTIVES:

This work aims to analyse the temporary hearing threshold shift at the Valpaços Scouts Fanfare due to the noise levels exposition in the course of rehearsals.

MATERIALS AND METHODS:

Measurements of the noise level and audiological evaluation were performed at Valpaços Scouts Fanfare. A total of 42 subjects, 16 females and 26 males, with ages between 13 and 43 years old, took part in this study. Sound pressure levels exposure has been evaluated with acoustic dosimeters during the entire rehearsal. Before and after music performance, audiological evaluation took place including otoscopy, pure tone audiometry, tympanogram and acoustic reflex test.

RESULTS AND DISCUSSION:

The group showed a temporary hearing threshold shift in both genders ($p<0.05$). The frequencies 1000Hz and 2000Hz were less affected in both ears. It was found significant statistical differences between the hearing threshold in both ears when the right ear is exposed to higher sound levels ($p<0.05$). No significant differences exist between both ears ($p>0.05$) when the left ear is the most exposed. The obtained results for the audiological acuity are related with the high noise levels found in the course of rehearsals. The values of LAeq ranged between 94.8dB(A) and 105.0dB(A) and the values LCpico between 135.1dB(C) and 138.3dB(C). In fact, the values of noise levels found in this study are higher than the values found in other research studies with other type of musicians (MacDonald et al., 2008; O'Brien et al., 2008), suggesting that despite the lower time spend in group rehearsals, the noise levels that fanfare musicians can be exposed are also worrying.

CONCLUSION:

The Valpaços Scouts Fanfare members show a temporary hearing threshold shift due to the sound pressure levels exposition in the course of rehearsals. Temporary, yet significant, this auditory changes place this

musicians at risk for hearing loss. In due to prevent it, is necessary to raise awareness to the problem and develop hearing conservation programs directed to this population.

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ETdA model in the health care sector

Authors: Patrícia Campos[1], Isabel Loureiro[2]

1 *Department of Information Systems, School of Engineering, University of Minho, Guimarães, Portugal*
2 *Department of Information Systems, Centro ALGORITMI, University of Minho, Guimarães, Portugal*

Presenting Author: Email: pcs.campos@gmail.com

INTRODUCTION:

According to Wilson (2013), Ergonomics is a discipline and a profession based on systems philosophy and systems approaches. A system approach requires a holistic vision of the ergonomic context, as well as the knowledge of the different system components. Parsons (2000) also highlighted the application of knowledge of human characteristics to the systems' design efficiency.

According to Dul & Neumann (2009), a systemic and integrated perspective of ergonomics, contribute to a positive analysis of the workplaces, especially in health care sector improving the work conditions, the professionals' activities and that way patient's security. In this context, all system' participants are beneficiated from ergonomic outcomes namely, the organization, the management, the workers, the patients and the patient's visitors.

According to Lapão (2005) the current guidelines from the health care sector define that the improvements on this sector must be client-focused with the purpose of increase his participation, responsibility and the attribution of a more active role in his health management. Regarding a particular health care institution; the blood donor centers, a safety objective is related not only to the donors' health and wellbeing as well as to the blood component. In this way, donors' security and donors' participation in ergonomics assessments becomes an important issue to be achieved. The Ergonomic Tri-dimensional Analysis (ETdA) is a new approach developed by Loureiro et al. (2012) for ergonomic assessments and redesign of Common Areas with Free Circulation of People (CAFCP).The CAFCP are areas where people can freely circulate contacting with the professionals when required. This approach considers that clients, patients or users and professionals can be exposed to the same ergonomics risk factors; therefore the participation of the entire organization (manager, professionals and clients) is required to the identification of risk situations.

OBJECTIVES:

This work aims to analyze the adaptation of the ETdA observation tools to be used in the context of health institutions, particularly in blood donors' centers.

MATERIALS AND METHODS:

The ETdA model uses different observation tools as it is showed in table 1. According to the ETdA guidelines this tools can be adaptable to the type of business thus specific terminology can be used or adjusted. This model also allows the introduction of variables to assess certain ergonomic factors previously identified by managers, professionals or related to clients' complain (Loureiro et al., 2012).

Table 1 - ETdA observation tools

Dimension	Observation tools
Clients	ETdAquestionnaire
Professional	ETdA Evaluation forms
Analyst	ETdA checklist

As the observation tools were developed to access commercial areas, an adjustment on the terminology was required.

RESULTS AND DISCUSSION:

After a carefully study of the ergonomic context under study, and by manager suggestion and clients complaints, the analyst decided to include two items for analysis: vibrations and exposure to gas from generator. It is important to notice that generators are necessary to perform this activity. As the ETdA model is very flexible, this was easy to achieve. Experimental procedures were required to assess these items.
Regarding the terminology adaptation of the ETdA questionnaire a pretest was done. Pretest is an important step as it allows seeing the questions' appropriateness and whether the questions are correctly interpreted by the respondents. A sample of 32 donors was used. Table 1 summarizes the most important changes in relation to the ergonomic factor under analysis.

Table 1 - Summarization of the ETdA questionnaire adaptation

Ergonomic factor	Major changes
Ergonomic perceptions	Definition improvement
Restritiveness	Identification of possible causes
workspace	Donors chairs: comfort and accessibility
Physical loading	Definition improvement
Repetitiveness	Change on the scale of evaluation
Risk accident	Specification of eventual causes

CONCLUSION:

The results indicate that the ETdA methodology is a good methodology to identify risk situations in common areas where professionals and donors can be exposure to the same ergonomic risk factors. Indeed, the fact that the observation tools are very flexible and adaptable to the context understudy makes easier the analyst assessments and facilitates the ergonomic analysis.

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Lead in lipsticks: results from a study on products manufactured in Brazil and Portugal

Authors: Ana Carvalhido[1], Kainá Paiva[1,2], Edgar Pinto[3], Patrícia Ramos[1], Agostinho Almeida[1]

1 *REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal*

2 *Department of Pharmaceutical Sciences, Health Sciences Centre-Vitória, Federal University of Espírito Santo, Brazil*

3 *CISA - Research Centre on Environment and Health, School of Allied Health Sciences, Polytechnic Institute of Porto, Porto, Portugal*

Presenting Author: Email: analuisacarvalhido@gmail.com | Tel.: +351 915 560 539

INTRODUCTION:

Lead (Pb) is ubiquitous in the environment and can be present in varying amounts in all raw materials. Due to its well-known toxicity, human exposure to products containing Pb must be limited.

At the end of the 2000s, several reports on the presence of Pb in lipsticks at levels that would pose safety concerns were published in the media and on the internet[1]. A scientific paper published in 2009 [1] also reported very high Pb levels (reaching 3700 ppm) in lipsticks (manufactured in China and available on the Saudi Arabia market). This led to FDA scientists to develop and validate an analytical procedure for Pb determination in lipstick and, later, to perform a survey of the U.S. market. This study, published in 2012 [2], found an average Pb content in the 400 lipsticks tested of 1.11 ppm, with results ranging from the detection limit (0.026 ppm) to 7.19 ppm. In 2013, the results of a European survey on the content of Pb in lipsticks were also published [3]. On average, the Pb content found was 0.75 ppm (max. 3.75 ppm).

OBJECTIVES:

To determine the Pb content in lipsticks manufactured in Brazil and Portugal. Because only one manufacturer was found in Portugal, samples from a supermarket own brand (declared as manufactured in the EU; known to be actually manufactured in Portugal) and products available in low cost stores were also analysed.

MATERIALS AND METHODS:

The analytical procedure as developed and validated by Hepp *et al.* [2], combining a two-steps closed vessels microwave-assisted acid digestion for samples mineralization with inductively coupled plasma-mass spectrometry (ICP-MS) for Pb determination, was used. Samples (˜0.3g) were digested with 7 ml HNO_3 + 2 ml HF (step 1). Boric acid 4% (m/v) was used to neutralize there mainly HF (step 2). After cooling, the solutions were made to the final volume (50 ml) with ultrapure water and analysed by ICP-MS.

RESULTS AND DISCUSSION:

A total of n=96 samples were analysed. Results obtained are summarized in next table.

Table 1 - Lead (Pb) content (µg/g) in lipstick samples

	Portugal					Brazil		TOTAL
	A	**B**	**C**	**D**	**E**	**F**	**G**	
n	*10*	*5*	*3*	*5*	*19*	*6*	*48*	*96*
mean	0.63	0.86	0.17	0.15	0.85	0.95	1.02	0.86
SD	0.43	0.75	0.04	0.06	0.93	1.20	0.84	0.83
median	0.61	0.46	0.15	0.15	0.39	044	0.90	0.65
minimum	0.14	0.15	0.15	0.08	0.02	0.17	0.02	0.02
maximum	1.21	1.69	0.22	0.23	3.21	3.31	3.89	3.89

A = Portuguese manufacturer; **B** = own brand of a supermarket (labelled as manufactured in the EU; known to be actually manufactured in Portugal); **C** = Low cost "youth fashion and beauty stores", manufactured in China; **D** = Low cost "youth fashion and beauty stores", manufactured in Turkey; **E** = Low cost Chinese stores, manufactured in China; **F** = manufactured in Brazil, purchased in Portugal; **G** = manufactured and available in the Brazil market.

[1]http://www.fda.gov/cosmetics/productandingredientsafety/productinformation/ucm137224.htm

CONCLUSION:

Overall, the mean Pb content (0.86±0.83 g/g; max. 3.89 g/g) was quite similar to those levels found in the recent USA and European surveys. Moreover, Pb content was quite below the limits imposed by the Germany (20 µg/g) and Canada (10 µg/g) health authorities for Pb in cosmetics. No important differences were found between the different groups of samples.

Safety assessment: According to "Cosmetics Europe" (formerly Colipa) data, the estimated amount of lipstick typically applied by the consumers is 0.057 g/day, corresponding to a relative amount of 0.90 mg/kg body weight/day [4]. Considering the maximum amount of Pb found in the analyzed products (3.89 g/g) and assuming the total oral ingestion of the applied product, this will representan estimated daily intake (EDI) of Pb of approximately 0.0035 g/kg body weight/day, which corresponds to ca. 0.1% of the tolerable daily intake (TDI) for Pb (3.6 µg/kgbw/day) [5].

This study confirms that when manufactured with raw-materials with adequate quality and according to current good manufacturing practices, it is possible to obtain a product with a very low Pb content, which should pose no risk for human health under normal or reasonably foreseeable use.

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Microbiological monitoring of surfaces and food handlers

Authors: Sílvia Barros[1], Manuela Amorim[1,2], Stephanie Ferreira[1,2], Jorge Condeço[1,2,3], Sandra Mota[1,2], Anabela Moreira[1,2] , António Augusto[1,2,4]

1 *Department of Biomedical and Public Health Laboratory Sciences, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

2 *Research Centre of Health and Environment, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

3 *Portuguese Blood Bank and Transplantation Institute, Portugal*

4 *Biogerm, SA, Maia, Portugal*

Presenting Author: Email: mas@estsp.ipp.pt | Tel.: +351 222 092 128

INTRODUCTION:

Every year, foodborne diseases affects about 48 million people in the United States alone, usually associated with microbiological infection or intoxication The increase in demand for catering services has contributed to cases increase (Kleter & Marvin, 2009; Lund, A.C. Baird-Parker, 2000; Newell et al., 2010). These data show the importance of quality assurance from food production to the consumption. Therefore, it is necessary to take into account special care towards the environment (air), facilities, equipment, surfaces, utensils and handlers throughout the processing by cleaning processes and disinfection (Kleter & Marvin, 2009).

OBJECTIVES:

To evaluate microbiological growth on surfaces, utensil and handler of a coffee establishment.

MATERIALS AND METHODS:

An experimental study was conducted with microbiological sample collection from surfaces, utensils and food handlers from a coffee establishment, in April of 2014, before and after disinfection. Samples were collected under aseptic conditions. Contact plates were used to surface evaluation. These plates were kept against surfaces for about 10 seconds. Handlers and utensils were tested using a swab soaked in Maximum recovery diluent-Histidine Lecithin and Polysorbate (MRD-HLPS) rubbing against parts were food might get retained. The parameters evaluated were total counts of viable microorganisms at 30°C/72h, coliforms at 37ºC/24h, *Escherichia coli* at 44ºC/24h and coagulase positive *Staphylococcus* at 37ºC/48h. Disinfection time was extended for 2 minutes when results were unsatisfactory, and sample collection and analysis were repeated.

RESULTS AND DISCUSSION:

According to the results there was absence of *Escherichia coli* and coagulase positive *Staphylococcus*. Handler and utensils revealed presence of coliforms. Utensils also revealed a high number of total counts of viable microorganisms. Fungi and bacteria were observed on surfaces. To eliminate these microorganisms the exposure time to the disinfectant was increased up to 2 minutes, which proved to be effective at reducing the number of microorganisms. After this procedure the counts results were within the established values.

Table 1

		After 10 seconds disinfection				After 2 minutes disinfection
		Coliformsat 37ºC/24h	***Escherichiacoli*at 44ºC/24h**	**Coagulase positive *Staphylococcus* 37ºC/48h**	**Microorganismsviable at 30ºC/72h**	**Microorganismsviable at 30ºC/72h**
Handlers		Satisfactory	Satisfactory	Satisfactory		
Utensils		Unsatisfactory	Satisfactory		Unsatisfactory	Satisfactory
Surfaces	Counter				Unsatisfactory	Satisfactory
	Table				Satisfactory	
	Fridge				Satisfactory	
	Fridgedoor				Satisfactory	
	Cuttingboard				Satisfactory	

CONCLUSION:

After adjustment in the disinfection time, our results show that the coffee establishment is in compliance with food safety requirements.

ACKNOWLEDGMENTS:

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Research of *Legionella* sp. in the water supply of a hospital in northern Portugal

Authors: Sandra Alves[1], Maria Céu Lamas[1,2,3], António Araújo[1,2,4], Manuela Amorim[1,2]

1 *Department of Biomedical and Public Health Laboratory Sciences, School of Allied Health Sciences of Polytechnic of Porto, Portugal*
2 *Research Centre of Health and Environment, School of Allied Health Sciences of Polytechnic of Porto, Portugal*
3 *Magalhães Lemos Hospital,* E.P.E., Porto, Portugal
4 *Biogerm, SA, Maia, Portugal*

Presenting Author: Email: mms@eu.ipp.pt | Tel.: +351 222 092 128

INTRODUCTION:

The distribution systems of drinking water have characteristics that allow the growth of microorganisms that may be pathogenic to humans, such as *Legionella*. The *Legionella sp.* (aerobic gram-negative bacilli) are widely distributed in aquatic environments, but it is in artificial environments that they hit higher levels due the characteristics propitious (like temperature, pH and biofilms presence) to their development. Human intervention was crucial for the presence of *Legionella* that has become common in building networks of water supply, cooling towers, air conditioning systems, swimming pools, tanks, humidifiers, condensers, ornamental fountains, among others[1,2,3].

The Legionella spp. is a fastidious bacteria that require special isolation techniques. Among the species of *Legionella*, the *Legionella pneumophila* is the most commonly identified specie that causes human disease and the deterioration of health conditions. In Portugal, since 1999 to 2008, 522 cases of legionellosis were reported, predominantly associated with hotel units. It is considered the second leading cause of pneumonia, which have a mortality rate of 40% in the hospital environment[4].

Initially, it was thought that cooling towers was the main source of contamination in hospitals, but many cases were associated with conduct hot and cold water systems. Therefore, the inhalation of contaminated aerosols is the most likely form of contamination, which may be originated from tanks / cooling towers, air conditioning systems, water heating systems, showers, swimming pools, nebulizers and humidifiers systems. So it is important to monitorize these water systems, through physico-chemical and microbiological characterization, in order to reduce and control the number of infections caused by these bacteria[3].

OBJECTIVES:

This work aimed to identify possible sites contaminated by *Legionella sp.* in hospital distribution network and evaluating the distribution system based on some of the physico-chemical parameters (temperature, pH and dissolved oxygen concentration) that are important for the development of *Legionella sp*.

MATERIALS AND METHODS:

Samples were collected in hot water tanks, showers, entry and end of the cold domestic water supply, kitchen and pool area network. At each point were collected two bottles of 1000ml, one of them with a sterile sodium thiosulphate concentration of 80 mg/L for microbiological parameters (*Legionella sp*), and one without additives, for physico-chemical parameters (pH, conductivity and dissolved O2), each being properly identified. After sampling the temperature measurement was made. These determinations were carried out by potentiometry, thermometry and electrometry, respectively. For *Legionella sp* research we used all materials and procedures recommended by ISO 11 731[5].

RESULTS AND DISCUSSION:

In this study, 16 water samples and 2 controls were analyzed. The results of each sample and each parameters analyzed are presented in Table 1.

Have been identified some favorable sites of growth the *Legionella sp*. Those sites exhibit characteristics as temperature < 50 °C and slightly alkaline pH. Although the pH values are within the reference values, these values do not guarantee that *Legionella sp*. does not show growth in the distribution system of the hospital[4]. But different values of these reference values can damage the distribution system[4]. The results of temperature in samples of cold domestic water are in accordance with recommendations by the Portuguese Institute of Quality. While the temperature in the hot water samples were lower than recommended, does not prevent the installation of these bacteria, since it resists these temperatures[4]. Regarding the concentration of dissolved O_2, which is slightly increased, it is contributive to the development of *Legionella sp* in the water network, since large concentrations of dissolved O_2 increases the ability of developing biofilm. For microbiological results, there was no growth of *Legionella sp* in the analyzed samples. Todos os procedimentos utilizados seguiram a norma permitindo garantindo a qualidade dos resultados. All procedures used followed the rule allowing ensuring quality results.

Table 1 - Physico-chemical parameters and research of *Legionella sp*

Local	Water Type	Temperature	pH	O_2 dissolved (mg/L O_2)	Presence of *Legionella* spp.
Unit B tank	HOT	52,4	7,29	5,75	Negative
Unit B shower	HOT	46,8	7,05	5,93	Negative
Unit C tank	HOT	55,7	7,33	5,44	Negative
Unit C shower	HOT	49,6	7,00	5,70	Negative
Unit D tank	HOT	52,4	7,71	5,79	Negative
Unit D shower	HOT	50,1	6,93	5,72	Negative
Unit E tank	HOT	47,0	7,34	5,41	Negative
Unit E shower	HOT	41,3	7,21	5,22	Negative
Pool tank	HOT	44,0	7,38	5,37	Negative
Pool shower	MIXED	37,4	7,40	5,51	Negative
Unit SII Tank	HOT	53,8	7,20	5,67	Negative
Unit SII shower	HOT	30,2	7,41	5,49	Negative
Ordinance (mains input)	COLD	15,1	7,61	6,41	Negative
Kitchen	COLD	17,3	7,48	6,27	Negative
Pool	COLD	16,7	7,76	6,29	Negative
Unit mains output	COLD	16,2	7,58	6,42	Negative
Positive control					Positive
Negative control					Negative

CONCLUSION:

Maintaining the quality of water used in hospitals and the research of *L. pneumophila* is essential to ensure safety in the hospital environment. Currently only the hot water and the cooling systems for buildings have legislation that requires the research of *Legionella sp* with others parameters for the evaluation of water quality. However, there are several guidelines and protocols with parameters for controling other types of environments and water, as well as their limits[3]. These protocols have preventive measures that should be adopted, as the continuous monitoring of water circuits through physico-chemical and microbiological characterization, maintaining the temperatures of the tanks outside the optimum temperature for the bacteria (> 60 ° C <20 ° C), performing regular maintenance, including cleaning and disinfection of the

circuit. This way, can be possible minimize the risk of bacteria development in the network system, since their elimination is difficult[3].

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Microbiological characterization of haemodialysis waters in a dialysis centre at Northern Portugal

Authors: Joana Azevedo[1], Manuela Amorim[1,2], Stephanie Ferreira[1,2], Sandra Mota[1,2], Anabela Moreira[1], António Augusto[1,2,4]

1 *Department of Biomedical and Public Health Laboratory Sciences, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

2 *Research Centre of Health and Environment, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

3 *Biogerm, SA, Maia, Portugal*

Presenting Author: Email: mas@estsp.ipp.pt | Tel.: +351 222 092 128

INTRODUCTION:

The main therapeutics for chronic renal failure are haemodialysis, peritoneal dialysis and renal transplantation, which seek to replace renal function (Coulliette & Arduino, 2013). The high-flux haemodialysis is a sequential process, constituted of water pre-treatment (Entry-EA, reverse osmosis-AROI, after osmosis 1-DROI, after osmosis and ring return -RAD), and water treatment in patient system (Damasiewicz, Polkinghorne, & Kerr, 2012). To ensure treatment effectiveness, it is necessary microbiological levels within acceptable limits, which depend on optimal operating conditions in central distribution of dialysis and water quality used for dialysate (Damasiewicz et al., 2012; Ministério da Sáude, 2001). This requires water microbiological and chemical parameters analysis and an effective monitoring to verify that it is suitable for high-flux hemodialysis (Damasiewicz et al., 2012; Ministério da Sáude, 2001).

OBJECTIVES:

Characterize the evolution and compliance of microbiological parameters in haemodialysis waters of a dialysis centre at Northern Portugal.

MATERIALS AND METHODS:

We realized a retrospective study of records on water analysis of haemodialysis centre, performed once per month between January and December, at points EA, AROI, DROI, DROII and RAD. Analytic parameters included: endotoxins, fungi, colony-forming unit (CFU) 22ºC and 37ºC.

RESULTS AND DISCUSSION:

All results are within acceptable limits for the practice of haemodialysis, with the exception of endotoxins values in July (0,036 EU / mL) corresponding to 0,48% of analyses performed on the RAD, points analysis of major importance. 54,167% of the results show higher concentrations in ARO I than EA. In DROII there is an increase of endotoxin in June. The points DROIII and RAD are the most important to assess water quality (dialysate formation), where water must comply with guidelines. Detected noncompliances indicate the need to take preventive measures such as replacing filters and cleaning the entire system. We found one RAD result above limit reference (0.036 EU/ml), yet borderline. The values of endotoxins in the following months were within limits but not near to zero, normalizing after 6 months. This indicates a need for disinfection and possibly changes membrane. We observed an increase in microbiological values from EA to AROI, this may be due to pre-treatment of water using chlorine which enables destruction of the bacteria and release of endotoxins.

CONCLUSION:

Results evidenced that there is a high effectiveness in the use of high-flux haemodialysis presenting as major contaminants of water at an early stage of water treatment CFU at 22ºC and endotoxins.

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Trends on antibiotics use in Human and Veterinary medicine in Portugal- A Public Health concern

Authors: Anabela Almeida[1,2], Sofia Duarte[1,3], Humberto Rocha[1], Angelina Pena[3], Leonor Meisel[4]

1 *Department ofVeterinary Medicine - Escola Universitária Vasco da Gama, Av. José R. Sousa Fernandes, 3020-210 Coimbra, Portugal*

2 *Center for NeuroscienceandCellBiology, Universityof Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal*

3 *Group of Health Surveillance, Center of Pharmaceutical Studies, University of Coimbra, Polo III, Azinhaga de Sta Comba, 3000-548 Coimbra, Portugal*

4 *INFARMED, I.P. - National Authority of Medicines and Health Products, 1749-004 Lisboa, Portugal*

Presenting Author: Email: almeida.anabela@gmail.com | Tel.: +351 239 444 444

INTRODUCTION:

Antibiotics are bioactive compounds, of both natural and semi-synthetic nature, belonging to the antimicrobial group. More than six decades of the large-scale use of antibiotics have resulted in bacterial resistance, which is currently a major global public health concern. A correlation between the use of antibiotics and bacterial resistance has been demonstrated for various pharmacotherapeutic groups, as highlight by Meyer et al. (2013). Therefore, to improve the detection, monitoring and characterization of drug-resistant infections in humans and animals it is important to get a profile of their actual use in human and veterinary medicine.

OBJECTIVES:

The main goal of this study was to identify the most used active substances in human and veterinary site, estimating and comparing the specific amounts in each practice.

MATERIALS AND METHODS:

The most recently available official Portuguese data used in both human and veterinary medicine was assessed for the year 2011. For veterinary medicine, data was collected from the national report of monitoring of antimicrobial consumption, issued by the National Authority for Animal Health - DGAV (DGAV, 2011). For human medicine, information was provided by INFARMED (INFARMED, 2011). Antibiotics for topic and hospital use were not included in this study. To simplify the comparison between the amounts used in both medicines the unit of measurement chosen was the tonnage. Only amounts over 1.0 ton were considered.

RESULTS AND DISCUSSION:

The most used active substances are showed in Figure 1 and 2, for veterinary and human medicine, respectively. The comparison between pharmacological groups used in both human and veterinary medicine are displayed in Figure 3.

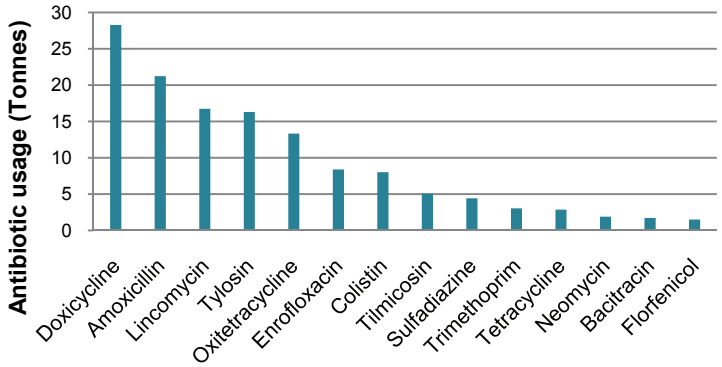


Figure 1 - Estimated usage of antibiotics in human medicine in 2011 (consumptions only above 1 ton were considered)

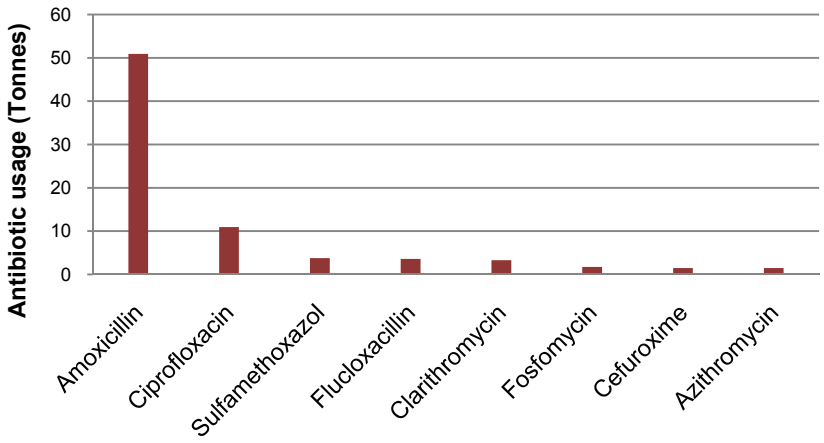


Figure 2 - Estimated usage of antibiotics in veterinary medicine in 2011 (consumptions only above 1 ton were considered)

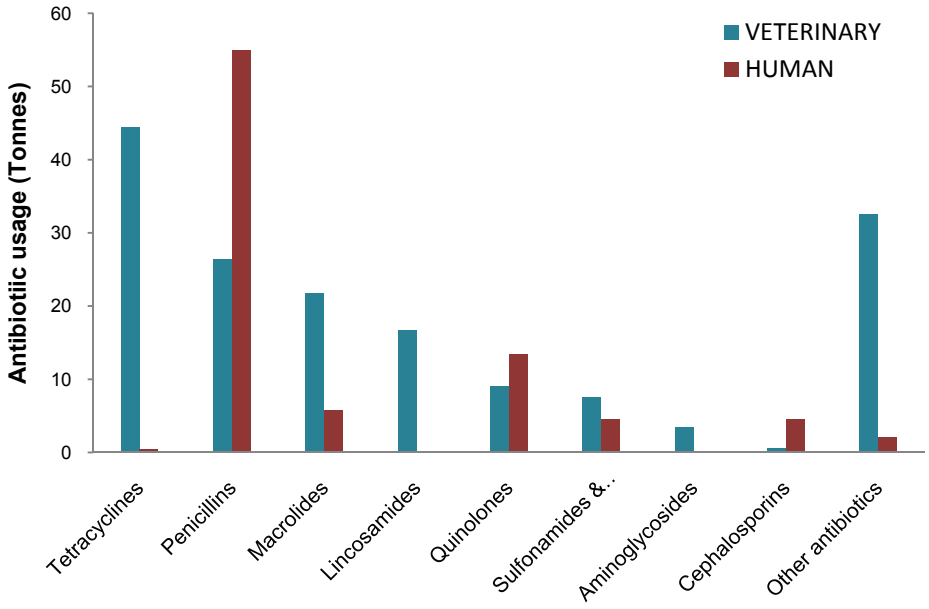


Figure 3 - Comparison of most used antibiotic groupsin Human and Veterinary Medicine in 2011.

Overall two-thirds of the consumed antibiotics in Portugal were used in veterinary practice whereas only one-third in human medicine. The year of 2011 revealed a consumption of 86.0 and 163.0 tonnes for human and veterinary medicine, respectively. Only 8 active substances have been used in amounts over 1.0 tonne in human medicine whereas 14 active substances in veterinary. Due to the increase of emergence of multi-drug resistance of bacteria, the human antibiotics are being increasingly subjected to restricted prescription in ambulatory. Nevertheless, the penicillin-group, quinolones and cephalosporins demonstrated a higher consumption in human medicine (Figure 3). Tylosinand tilmicosin are specific macrolides only used in veterinary medicine which represent the 4th and 8th most used antimicrobial agent (Figure 1). Interesting lyquinolones occupy the 2nd(ciprofloxacin) and 6th(enrofloxacin) position in the human and veterinary medicine ranking, respectively. This is noteworthy as ciprofloxacin is an active metabolite of enrofloxacin.

CONCLUSION:

This raking may be an important tool in public health.

ACKNOWLEDGMENTS:

We thank the support of Escola Universitária Vasco da Gama.

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ENVIRONMENTAL AND SUSTAINABILITY ABSTRACTS

Mobility of Cd and Pb in a groundwater-soil-plant system: a risk assessment

Authors: Edgar Pinto[1,2], Agostinho Almeida[3], Isabel M.P.L.V.O. Ferreira[1]

1 *REQUIMTE, Department of Chemical Sciences, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal*

2 *CISA - Research Center in Environment and Health, ESTSP-IPP*

3 *REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal.*

Presenting Author: Email: ecp@estsp.ipp.pt | Tel.: +351 916 941 708

INTRODUCTION:

Cadmium (Cd) and lead (Pb) are ubiquitous elements and are among the most dangerous metals in the environment. Long-term human exposure is an important public health problem and is associated with kidney failure, neurotoxicity and several types of cancer. The general population is exposed to these metals from several sources, namely food, air and water. Food is the dominant source of exposure to Cd and Pb [1, 2]. Plants can uptake Cd and Pb present in the soil. The mobile fraction of Cd and Pb in soil is very low compared to other elements [3]. Various soil extraction procedures were developed for the determination of plant-available, mobile and potentially mobile pools of trace elements in soils. These procedures offer a reliable approach to investigate possible relationships between water, soil and plants [4].

OBJECTIVES:

The aim of this work was to: (1) assess the Cd and Pb concentration of the groundwater used for irrigation; (2) evaluate the efficiency of three extraction methods (DTPA, Mehlich 3 and NH_4NO_3) to estimate the phytoavailability of Cd and Pb for lettuces; and (3) estimate the daily intake of Cd and Pb from lettuce consumption.

MATERIALS AND METHODS:

The study approach involved an experimental field with 100 lettuces (*Lactuca sativa*) planted in 3 different agricultural fields (A1, A2, A3). To avoid metals contamination from any other sources, groundwater was the only source of irrigation. Soil, water and plants were collected from January to February 2012 every 2 weeks. Determination of Cd and Pb content in groundwater, soil and harvested edible tissues was performed by ICP-MS after microwave acid digestion.

RESULTS AND DISCUSSION:

Groundwater Cd concentration showed no significant spatial variations between the 3 experimental fields. Overall, Cd concentrations were below 0.1 µg/L. As regard to Pb, significant differences were observed between the 3 experimental fields; A1 groundwater showed the highest Pb concentration (1.48 µg/L), which was 25 times higher than the Pb concentration in A2 and A3 groundwaters.

As regard to soil, the total Cd content was 1.12 ± 0.09, 0.68 ± 0.08 and 2.21 ± 0.12 mg/kg in A1, A2 and A3 soils, respectively. A3 soil had the highest Cd content while A2 the lowest, which were significantly different. The total Pb content was 17.0 ± 0.73, 24.2 ± 0.93, 18.9 ± 0.73 mg/kg in A1, A2 and A3 soils, respectively. Significant differences were observed with A2 soil showing the highest Pb content, whereas A3 the lowest.

Cd content showed a steady decreased during the plant growth period with a final mean content of 5.6, 8.6 and 9.7 µg/kg in A1, A2 and A3 lettuces, respectively. However, it should be notice that Cd content in the initial stages of growth was around 40 µg/kg. In a similar way, Pb content decrease during the plant growth

period in both A1 and A3 lettuces (final Pb content of 6.5 and 4.8 µg/kg for A1 and A3 lettuces, respectively). By contrast, the level of Pb in A2 lettuces increased, reaching a Pb content in the final product of 35.6 µg/kg.

Cd and Pb content in soil and lettuce were significantly and positively correlated with each other in all three soil extraction procedures (DTPA, Mehlich 3 and NH_4NO_3). The increased of Pb content in A2 lettuces seems to be related to the increase of Pb availability, estimated by the different extraction methods.

A Provisional Tolerable Weekly Intake (PTWI) of 2.5 and 25 µg/kg body weight has been established for Cd and Pb, respectively [1, 2]. In the present study, lettuce consumption only contributes less than 1% of the established PTWIs. Therefore, the consumption of this plant food can be considered safe as long as Cd and Pb levels are around the values obtained in the present study.

CONCLUSION:

The content of Cd and Pb in the groundwater of all the experimental fields was very low and do not seem to contribute to the accumulation of these metals in soils or lettuces. Each extractant (DTPA, Mehlich 3 and NH_4NO_3) provide useful information in the study of Cd and Pb mobility in the groundwater-soil-plant system. However, the NH_4NO_3 extractant showed the higher correlation between the soil extractable Cd/Pb and the Cd/Pb content of lettuces. Thus, this extraction method was considered to be the most suitable to estimate Cd and Pb phytoavailability.

ACKNOWLEDGMENTS:

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Occurrence, removal and risk assessment of eleven pharmaceuticals in Portuguese wastewaters

Authors: André M.P.T. Pereira[1], Liliana J.G. Silva[1], Celeste M. Lino[1], Fátima Alpendurada[2], Leonor M. Meisel[3,4], Angelina Pena[1]

1 *Group of Health Surveillance, CEF, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal*
2 *Instituto da Água da Região Norte (IAREN), Rua Dr. Eduardo Torres, 229, 4450-113 Matosinhos, Portugal*
3 *INFARMED, I.P. - National Authority of Medicines and Health Products, 1749-004 Lisboa, Portugal*
4 *Pharmacology Department, Pharmacy Faculty, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal*

Presenting Author: Email: amptpereira@gmail.com | Tel.: +351 239 488 400 | Fax: +351 239 488 503

INTRODUCTION:
The main source of pharmaceuticals residues in the aquatic environment is from human excretion, consequently, the widespread presence of pharmaceuticals in environmental samples is most likely to occur from wastewaters treatment plants (WWTPs) point source discharges, which incompletely remove these compounds, leading to the contamination of surface waters, seawaters, groundwater and some drinking waters (Kümmerer, 2010).

In Portugal the highest prescription and consumption regard, among others, alprazolam, lorazepam and zolpidem (anxiolytics and hypnotics), azithromycin and ciprofloxacin (antibiotics), simvastatin, bezafibrate and gemfibrozil (lipid regulators), ibuprofen, diclofenac and paracetamol (non-steroidal anti-inflammatories and analgesics) (INFARMED, 2011). As their use cannot be avoided, a sound risk assessment of their presence in the environment is a key problem.

OBJECTIVES:
Evaluate the occurrence, removal and risk assessment of eleven pharmaceuticals, selected from the highly prescribed and chronically consumed, in Portuguese wastewaters, during summer season.

MATERIALS AND METHODS:
Wastewater influents (WWIs) and wastewater effluents (WWEs) of 15 different WWTPs, located in 5 Portuguese regions, North, Center, Lisbon and Tagus Valley, Alentejo and Algarve, were collected. These WWTPs are designed for 6850 to 756000 population equivalents, representing 26.1% of national population (10526700 in 2012) and sampling campaign was carried out in 11 July/14 August 2013 (24-h composite samples).

The method used for identification and quantification of these pharmaceuticals was based on the methodology reported by Sousa et al. (2011). Extraction and cleanup was performed through an Oasis MAX (500 mg, 6 mL) cartridge and instrumentation analysis was performed in a LC-MS-MS (ion trap).

RESULTS AND DISCUSSION:
Method detection limits values ranged from 0.4 to 60.0 ng L^{-1} in WWE and from 0.5 to 61.2 in WWI. Method quantification limits ranged from 1.4 to 200 ng L^{-1} in WWE and from 1.7 to 204.1 ng L^{-1} in WWI.

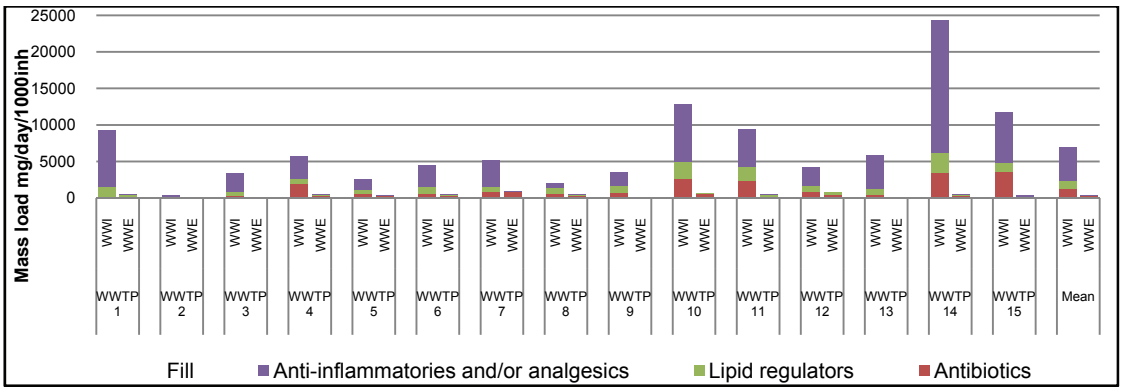


Figure 1 - WWTP occurrence of the selected pharmaceuticals in WWI and WWE

Overall, the results showed that, as expected, the frequencies of contamination, concentration levels and mass loads, were higher in WWI samples, although some exceptions were observed. All samples were contaminated with at least one, and up to 7 pharmaceuticals, and from the 11 targeted pharmaceuticals, only anxiolytics were not detected in any sample (Figure 1).

Lipid regulators were the most frequently found in WWI and WWE (1074.6 and 114.8 mg/day/1000 inhab., respectively), followed by anti-inflammatories (4635.8 and 59.9 mg/day/1000 inhab., respectively), and by antibiotics (1300.2 and 262.4 mg/day/1000 inhab., respectively) (Figure 1). The mean removal efficiency achieved was of 94%, nonetheless, between the different therapeutic groups, as well as within each group, a greater variation in removal efficiencies was observed, going from not eliminated to 100% (Figure 2).

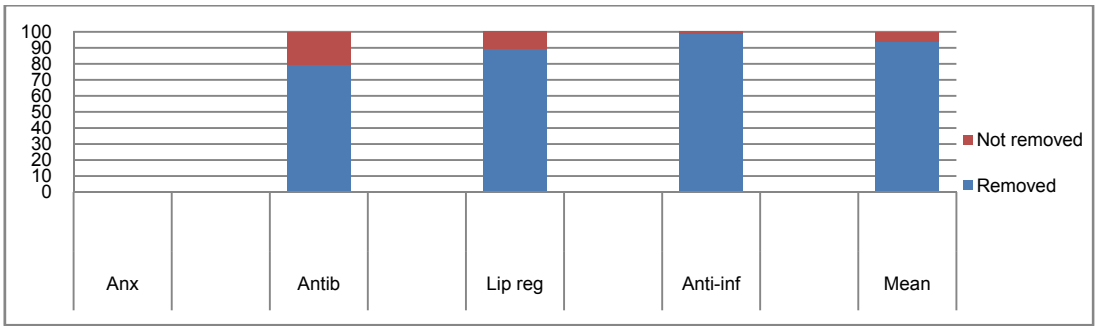


Figure 2 - WWTPs removal percentage of therapeutic group (Anx – anxiolytics and hypnotics; Antib - antibiotics; Lip reg - Lipid regulators; Anti.inf - anti-inflammatories and analgesics).

Table 1 - Maximum environmental concentrations (MEC) (ng L^{-1}) in WWE, predicted no effect concentration (PNEC) (ng L^{-1}) and risk quotients (RQs) for algae, daphnids and fish for the studied pharmaceuticals.

Therapeutic group	Pharmaceutical	MEC	PNEC algae	RQ algae	PNEC daphnids	RQ daphnids	PNEC fish	RQ fish
Antibiotics	Azithromycin	200.0	1 874 [a. b]	0.107	3 023 [e. b]	0.066	21 945 [e. b]	0.009
	Ciprofloxacin	9 800.0	1 000 [c, d]	9.800	180 000 [c, d]	0.054	13 131 424 [e. b]	0.001
Lipid regulators	Bezafibrate	2 400.0	1 352 [a. b]	1.775	2 300 [c, d]	1.043	26 435 [e. b]	0.091
	Gemfibrozil	1 500.0	312 500 [c, d]	0.005	7 800 [c, d]	0.192	150 [c, d]	10
Anti-Inflammatories and/or analgesics	Diclofenac	670.0	1 000 000 [c, d]	0.001	20 000 [c, d]	0.034	50 [c, d]	13.400
	Ibuprofen	1 370.0	4 010 [b]	0.342	102 000 [c, d]	0.013	41 561 [e. b]	0.033

a - EC50 was estimated with ECOSAR; b - UF=1000; c - long-term data; d - UF=10; e - LC50 was estimated with ECOSAR

According to these results, the pharmaceuticals ciprofloxacin, bezafibrate, gemfibrozil and diclofenac showed risk quotients (RQs) higher than one, in the range of 1.043 to 13.400, for at least one trophic level, posing a risk to algae, daphnids and fish. Therefore, it is expected that these pharmaceuticals might be a threat for the aquatic ecosystem.

CONCLUSION:

The occurrence of 11, of the most consumed pharmaceuticals, in 15 Portuguese WWTP influent and effluent samples was investigated and high frequency and levels were found for several compounds. In influents, higher contamination levels were observed for anti-inflammatories, whereas in effluents antibiotics were the therapeutic group found in greatest concentrations. Although the high removal efficiencies for most of the pharmaceuticals, RQs higher than one were found for ciprofloxacin, bezafibrate, gemfibrozil, and diclofenac, therefore, it is expected that these pharmaceuticals might be a threat for the three trophic levels in the aquatic ecosystem.

ACKNOWLEDGMENTS:

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Phytoextraction of heavy metal polluted soils using Sedum plumbizincicola inoculated with metal mobilizing Phyllobacterium myrsinacearum RC6b

Authors: Ying Ma, Helena Freitas

Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Portugal

Presenting Author: Email: cathymaying@gmail.com | Tel.: +351 910 802 125 | Fax: +351 239 855 211

INTRODUCTION:

Currently, the hyperaccumulators have been extensively used to remove various toxic metals from contaminated soil and hence serve as an important resource for research into the unique physiological and molecular mechanisms responsible for metal hyperaccumulation and detoxification (Ma et al., 2011). *Sedum plumbizincicola* is one of the hyperaccumulators, which has a remarkable capacity to withstand the metal stress in polluted soils and recent experiments have also demonstrated its potential for heavy metal phytoextraction (Jiang et al., 2010). Although there is much interest in increasing the phytoremediation efficiency of *S. plumbizincicola*, effects of interactions of metal mobilizing microbes and S. plumbizincicola on the heavy metal phytoremediation, to our knowledge, have not been investigated.

OBJECTIVES:

The objectives of our study were to isolate and characterize metal mobilizing plant growth promoting rhizobacteria (PGPR) from the rhizosphere of *S. plumbizincicola* and to investigate the effects of metal mobilizing PGPR on plant growth and Cd, Zn and Pb uptake by*S. Plumbizincicola* in multi-metal contaminated soils.

MATERIALS AND METHODS:

Among a collection of metal resistant bacterial strains isolated from rhizosphere of S. plumbizincicola grown on Pb/Zn mine spoils in Chunan city of Zhejiang, Southeast of China, strain RC6b was specifically chosen as a most favorable metal mobilizer based on its capability of mobilizing high concentrations of Cd, Zn and Pb in soils. Characterization of metal mobilizing strain RC6b was performed by 16S rRNA sequencing, resistance to heavy metal (Cd, Pb and Zn) and various antibiotics and determination of plant growth promoting potential(production of 1-aminocyclopropane-1-carboxylic acid deaminase, indole-3-acetic acid, siderophore and solubilization of insoluble phosphate). For pot experiments, soil samples were collected from Fuyangcity of Zhejiang Province, China, which were contaminated with Cd, Pb and Zn. The seedlings of *S. plumbizincicola* were obtained from Pb/Zn mine spoils as mentioned above. The fresh shoot samples were cleaned and rooted in a half-strength Hoagland's nutrient solution for 7 d. Roots of precultured seedlings were surface-sterilized before the inoculation. The roots of seedlings were soaked for 2 h in the bacterial culture or sterile water (controls) and transplanted into plastic pot containing 750 g of metal polluted soil. The plant seedlings were allowed to grow in a greenhouse at 25 ± 5°C and a 16:8 d/night regime. Each treatment was performed in five replicates. After 75 d, the plants were carefully removed from the pots and the root surface was cleaned several times with distilled water. Plant root and shoot length, fresh and dry weight were measured. The accumulation of metals (Cd, Zn and Pb) in root and shoot system was quantified as described by Ma et al. (2009).

RESULTS AND DISCUSSION:

Among a collection of metal-resistant bacteria,*P. myrsinacearum* RC6b was specifically chosen as a most favorable metal mobilizer based on its capability of mobilizing high concentrations of Cd, Zn and Pb in soils. *P. myrsinacearum* RC6b exhibited a high degree of resistance to Cd(350 mg L^{-1}), Zn (1000 mg L^{-1}) and Pb (1200 mg L^{-1}) (Figure 1). Furthermore,*P. myrsinacearum* RC6b showed multiple plant growth beneficial

features including the production of 1-aminocyclopropane-1-carboxylic acid deaminase, indole-3-acetic acid, siderophore and solubilization of insoluble phosphate (Figure 2). Inoculation of *P. myrsinacearum*RC6b significantly increased *S. Plumbizincicola* growth and organ metal concentrations except Pb, which concentration was lower in root and stem of inoculated plants (Table 1).The results further suggested that activities of *P. myrsinacearum* RC6b in the rhizosphere soils can significantly improve the phytoremediation potential of plants in metal polluted soils through increasing two factors that control this parameter, i.e., plant biomass production and its metal accumulation.

Table 1 - Influence of P. myrsinacearum RC6b on the plant growth and the uptake (mg kg-1) of Cd, Zn and Pb by S. plumbizincicola

Treatment	Root length (cm)	Shoot length (cm)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Cd concentration		Zn concentration		Pb concentration	
					Root	Shoot	Root	Shoot	Root	Shoot
Control	4.6±0.3	17.2±1.2	46±2	4±0	35±6	93±4	889±57	1072±38	99±11	101±11
RC6b	12.8±4.2*	21.8±1.7*	58±9*	5±1	47±5*	146±2*	1310±174*	1435±31*	15±0	5±1

Average ± standard deviation from five samples. An asterisk (*) denotes a value significantly greater than the corresponding control value according to Fisher' s protected LSD test (p< 0.05).

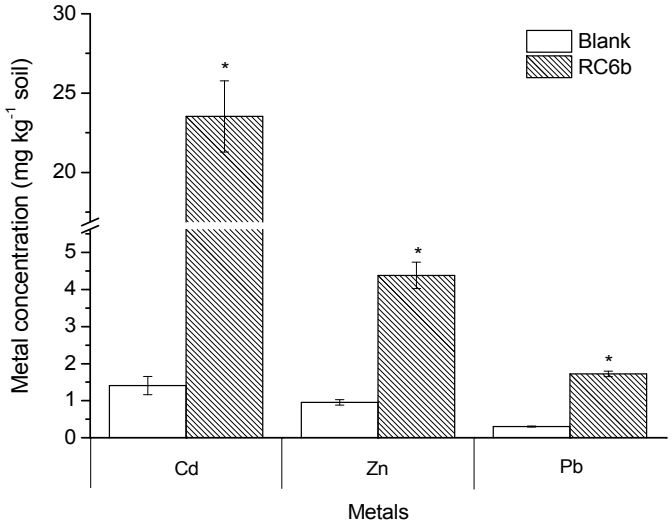


Figure 1 - Effect of inoculation with P. myrsinacearum RC6b on the solubilization of Cd, Zn and Pb in soil

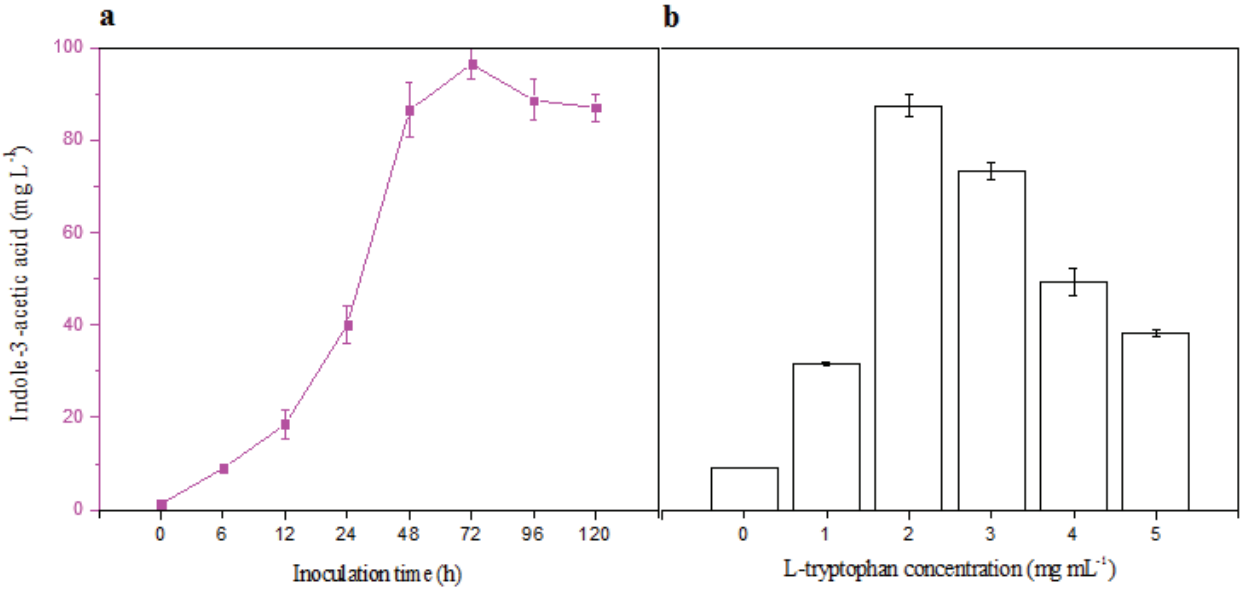


Figure 2 - Effect of inoculation time (a) andL-tryptophan concentration (b) on IAA production of RC6b

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Spatial distribution and contamination assessment of heavy metals in surface soils from an industrial area

Authors: J. Lage[1,2], S.M. Almeida[1], H.Th. Wolterbeek[2]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, 139.7 km, 2695-066 Bobadela LRS, Portugal*

2 *Faculty of Applied Sciences, Department of Radiation, Radionuclides and Reactors, Section RIH, Technical University of Delft, Delft, The Netherlands*

Presenting Author: Email: joanalage@ctn.ist.utl.pt | Tel.: +351 967 648 123

INTRODUCTION:

Following over 200 years of industrialization, soil contamination is a widespread problem in Europe. The most frequent contaminants are heavy metals and mineral oil likewise radionuclides, chlororganic compounds and other toxicants, with origin in emissions of industrial plants, thermal power stations, vehicular traffic and road infrastructures. According to European Environment Agency the number of sites where potential polluting activities have been carried out in the EU is approximately three million and, of these, an estimated 250,000 sites may need urgent remediation. Pollution by heavy metals and organic contaminants are probably the most serious problem as the contamination is practically irreversible and can remain present in the pedosphere for many years even after removing of the pollution sources. This contamination can affect human health either through direct contact or by ingestion through the food chain.

This work was developed within a project aiming the assessment of human exposure to environmental heavy metals in the surrounding of an industrial area. For that an integrated approach was used including the monitoring of soil, sampling of aerosol particles and biomonitoring approaches.

OBJECTIVES:

The objectives of this work were: 1) to identify elements content in soils samples from an industrial area composed by a steelwork, a cement industry, a power plant and a harbor located in Gijón, North of Spain; 2) to study soil contamination using different approaches; 3) to find out large scale variability of the elements; and 4) to identify emission sources.

MATERIALS AND METHODS:

The sampling of the soil from the studied area was carried out according to a regular grid previously defined. This grid was composed by 61 georeferenced sampling points plus 7 sampling points located inside the area with intensive industrial activity (Figure 1.). In each point the topsoil was collected and prepared in laboratory. Afterwards, element content in these samples was performed using the Instrumental Neutron Activation Analysis (INAA) with the k_0 methodology, in the Portuguese Research Reactor.

The effects of this industrial complex in the local soils were further assessed using geoaccumulation index (I_{geo}) (Müller, 1969), contamination factor (Cf) (Fernández and Carballeira, 2001) and enrichment factor (Ef) (Taylor and McLennan) speciation. Distribution patterns of the concentration of the main pollutant metalsin the whole area, and also contamination and enrichment factors, were performed using a Geographic Information Systems, ArcGIS 10.1. Principle component analysis was used to identify pollution sources and to apportion natural vs. anthropic contribution.

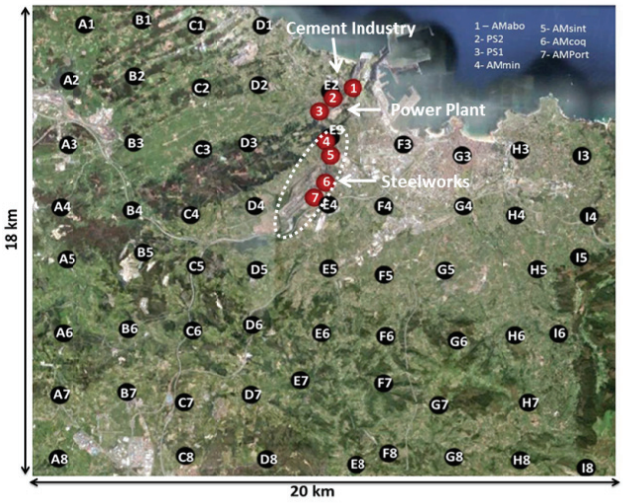


Figure 1 - Grid of sampling points in the area of study, in Gijón, North of Spain.

RESULTS AND DISCUSSION:

Statistical treatment of elements concentrations in soils samples showed a strong influence of this industrial area. Highest geoaccumulation index, contamination factors, enrichment factors were observed in the vicinities of the industries. As shown in figure 2, elements as As, Br, Sb, Sr and Zn have EF>1 which means an enrichment of the soils. This can be due anthropogenic activities, with main influence of the industrial area. The geographical distribution of the elements in soils presented similar patterns as the obtained in a biomonitoring study applied in this same grid showing that there is an important inter-relation between air and soil pollution.

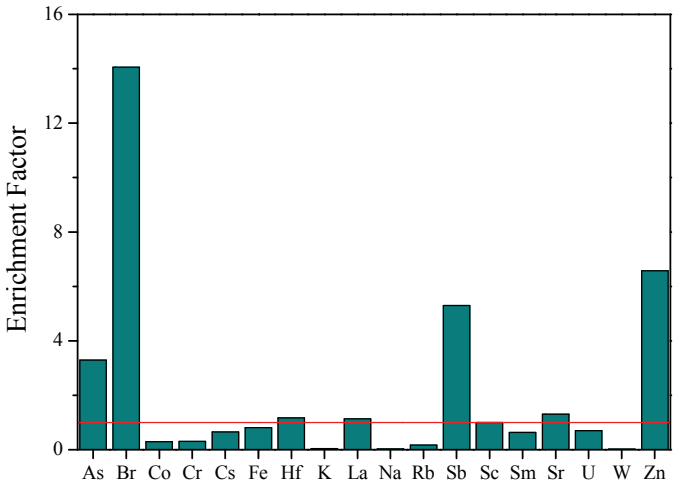


Figure 2- Enrichment factor using Sc as a reference element Mason soil composition.

CONCLUSION:

Results showed a strong impact of the industrial area on soil pollution. Data confirms an enrichment of elements as As, Br, Sb, Sr and Zn in soils. These elements are strongly associated with industrial activities. Currently, we all acknowledge the significance of pollutants in the air or in the water to the poor health. Unequivocal associations between air quality and morbidity and mortality are often reported, and the impacts of the lack of access to safe drinking water, or of an industry discharging pollution into rivers and lakes, are also well documented. However, until recently, the impacts of soil pollution on our health have had much lower attention. Nevertheless, many studies from heavily-contaminated sites around the world, particularly in developing countries, indicate possible health impacts of high levels of soil contamination. Despite the importance of soil pollution for our society the EU legislation specially targeting the protection of soil is very scarce. Furthermore, assess the soils' state in Europe is a hard issue once there is no legal

requirement to collect information in a harmonized manner, which point out the difficulty to compare monitoring soil results of various countries.

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Effectiveness of seed coating with microbial inoculants as an alternative to agrochemicals in sustainable agriculture

Authors: Inês Rocha[1], Aleš Látr[2], Miroslav Vosátka[3], Helena Freitas[1], Rui S. Oliveira[1,4,5]

1 *Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Portugal*
2 *Symbiom Ltd., Lanskroun, Czech Republic*
3 *Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice, Czech Republic*
4 *Research Centre on Health and Environment, School of Allied Health Sciences, Polytechnic Institute of Porto, Portugal*
5 *CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Portugal*

Presenting Author: Email: ines.sousa.rocha@uc.pt | Tel.: +351 239 855 210

INTRODUCTION:

Intensive agriculture is highly dependent on agrochemicals and in order to increase crop production, environment integrity is disturbed with biodiversity loss, emergence of pathogens, and soil and water resources negatively affected. The abuse of agrochemicals in agriculture constitutes a major worldwide problem as it does compromise environment and public health. Reducing the use of chemical fertilisers with increased application of organic onesis considered a required and natural route to ease the pressure on the environment derived from agricultural practices (Malusá et al., 2012).Plant growth-promoting soil microorganisms (PGPM) such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) may replace or reduce many destructive, high-intensity practices in agriculture. AMF are probably the most abundant fungi that are commonly present in agricultural soils. AMF support plant growth directly by enhancing nutrient (P, Zn and others) and water uptake and indirectly by improving soil structure and resistance to certain root pathogens (Nadeem et al., 2014).On the other hand, PGPR play a significant role in enhancing plant growth and development both under non-stressful and stressful conditions by a number of direct and indirect mechanisms. The mechanisms that promote plant growth include: N fixation, production of siderophores and phytohormones and solubilisation of minerals such as P(Ramasamy et al., 2011).The microbial interactions and associations might be crucial for sustainable agriculture as they mainly depend on biological processes rather than agrochemicals to maintain plant growth and development of a proper soil health under stressful conditions.Direct inoculation of free PGPR cells into the soil aiming atcolonise plant roots has proven not to be efficient due to PGPR vulnerability to environmental variations (edaphic conditions, salt stress, pH, temperature and others) (Wu et al., 2012). Large scale inoculation with AMF in agriculture is limited due to the relatively high cost of inoculum and the need to attain a sufficient number of propagules per plant (Vosátka et al., 2012). Both AMF and PGPR application by broadcasting in open agricultural fields is not economically feasible because non-target spreading of inoculum over large areas results in high cost per plant. The solution may lie on seed coating, a technique in which finely-ground solids or liquids are adhered around the seed with a sticky material that has been promptly developed and gained great acceptance by the seed industry. Coating seeds can improve performance and physical properties of seeds, but more importantly, can allow the use of minor amounts of inoculum resulting in a reduction of costs and efficiency increase.

OBJECTIVES:

In this study there were two main goals: the first, is through seed coating technology reduce the amount of inoculum needed for an effective inoculation, making the application of these beneficial soil microorganisms in open field extensive agriculture possible; and the second is to minimise the dependence and use of agrochemicals in agriculture by using PGPM, such as PGPR and AMF.

MATERIALS AND METHODS:

Being one of the most diffused crops worldwide due to its high economic and nutritional value maize was selected for the seed coating tests. *Pseudomonas fluorescens* F113 (PF113) was grown Luria Bertani medium and it was selected as the PGPR, not only due to its proprieties as a plant growth promoter, but also because it is consider to be a mycorrhiza helper bacterium. The inoculum of *Glomusintraradices* BEG140, the selected AMF, was prepared in open pot cultures. Maize seeds were coated with either single BEG140 isolate (BEG) or mixture of BEG140 and PF113 (Mix). Plants were maintained in a greenhouse under controlled environmental conditions and the experiment was performed with sterile agricultural soil. For the greenhouse trials four treatments were tested: seed coated with BEG140; non-coated seed inoculated withBEG140 (positive control); seed coated with BEG140 and PF113; and non-coated seed inoculated with BEG140 and PF113 (positive control). After 4 weeks of plant growth, maize roots were stained and root colonisation by AMF evaluated by microscopy.

RESULTS AND DISCUSSION:

Maize seeds were successfully coated with the microbial inoculants. After 1 week of growth the maize seeds presented a high rate of germination and after 4 weeks it was possible to evaluate the percentage of colonisation of the roots. The AMF colonisation was evaluated by the presence of vesicles, arbuscules and hyphae. All maize seeds inoculated with BEG140 had their roots colonised by AMF. Treatments with single BEG140 isolate and Mix (BEG140+PF113) presented no significant differences. The percentage of root colonisation was higher than 40% for all the treatments and statistically the coated seeds had the same result than seeds with normal inoculation (positive control). These results indicate that seed coating is a good tool for using low amounts of AMF inoculum. In Figure 1 maize plants after 4 weeks of growth are presented, while Figure 2 shows stained roots with and without AMF colonisation.

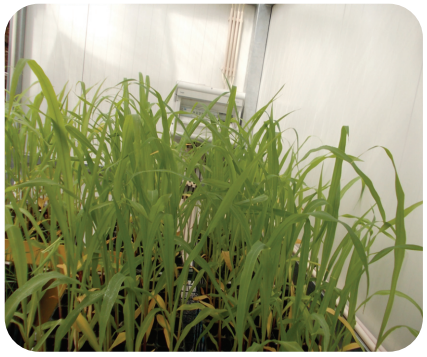


Fig.1 - Maize in the greenhouse trials.

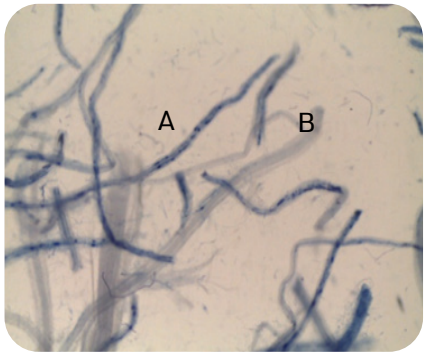


Fig.2 - Colonisation of maize roots by BEG140. **A**- colonised roots; **B**- non-colonised roots.

CONCLUSION:

Seed coating may be the key for a more efficient application of AMF and PGPR, which may allow the large scale application of these beneficial microbial inoculants while aiming to reduce the input of agrochemicals. Seed coating represents an innovative approach to enhance sustainable agriculture, which might result in economic and environmental benefits.

ACKNOWLEDGMENTS:

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Bacterial community changes during bioremediation of petroleum-contaminated soil

Authors: Magdalena Pacwa-Płociniczak[1], Tomasz Płociniczak[1], Grażyna Płaza[2], Zofia Piotrowska-Seget[1]

1 *Department of Microbiology, University of Silesia, Katowice, Poland*
2 *Department of Environmental Microbiology, Institute for Ecology of Industrial Areas, Katowice, Poland*

Presenting Author: Email: tomasz.plociniczak@us.edu.pl | Tel.: +48 322 009 470 | Fax: +48 322 009 361

INTRODUCTION:

Contamination of soil by petroleum hydrocarbons is a serious environmental problem. One of the most promising biological methods for cleaning up polluted environments is bioaugmentation (Mrozik& Piotrowska-Seget, 2010). Inoculation of contaminated soils with bacteria with the ability to degrade hydrocarbons and produce biosurfactants increase the bioavailability of hydrophobic compounds and accelerate hydrocarbon biodegradation (Pacwa-Płociniczak et al., 2011). However, it is being observed that bioaugmentation does not always bring the desired effects. One of the reasons for the failure may be the interactions between the indigenous populations of microorganisms and the introduced strains (Mao et al., 2012). Therefore, one of the challenges of modern soil microbiology is detailed description of shifts in soil bacterial community structure occurring during bioaugmentation with hydrocarbon degrading and biosurfactant producing bacteria.

OBJECTIVES:

The aim of study was to assess the shifts in the structure of soil autochthonous bacterial communities resulting from bioaugmentation of petroleum-contaminated soil with the hydrocarbon-degrading and biosurfactant-producing bacterial strains *Bacillus subtilis* T'-1 and *Pseudomonas* sp. P-1 strains and their consortium.

MATERIALS AND METHODS:

The petroleum polluted soil was obtained from an industrial area located around refinery in Czechowice-Dziedzice (Upper Silesia, Southern Poland). The bioaugmentation study was carried out under laboratory conditions. Bacterial strains used for bioremediation were earlier isolated from hydrocarbon-contaminated soil. The experiment had a completely randomized block design with three replications that had four treatments: (1) soil inoculated with strain T'-1, (2) soil inoculated with strain P-1, (3) soil inoculated with mixture of T'-1 and P-1 strains and (4) control soil treated with sterile saline solution instead of bacterial suspension. 400 g of contaminated soil was placed into pots and then 40 ml of the bacterial solutions of T'-1, P-1 and T'-1+P-1 strains were poured into soil to reach the number of 10^7 bacterial cells g^{-1}dry weight of soil. The pots were incubated for 91 days at room temperature. The total petroleum hydrocarbon concentration in soil was quantified before and after bioremediation study as hydrocarbons with carbon number between 10 and 40 (TPH_{c10-40}) following ISO 16703:2011 standard. During the bioremediation experiment soil samples of 15 g were taken on 1, 7, 42 and 91 days, and immediately stored at -20°C until further analyses. The impact of introduced bacteria on the structure of soil bacterial communities in bioremediated soil was determined using phospholipid fatty acid (PLFA) and community-level physiological profiling (CLPP) analyses.

RESULTS AND DISCUSSION:

The soil subjected to bioaugmentation had a high initial TPH concentration (7775.67 mg kg^{-1} dry weight of soil). Quantification of these pollutants after the bioremediation process showed the greatest decrease (value of 1173.59 mg kg^{-1}d.w. of soil) in the hydrocarbon content in the soil inoculated with the mixture of T'-1+P-1strains. In the soils treated with the single T'-1 and P-1 strains the loss of petroleum hydrocarbons was lower and reached 435.14 and 374.53 mg kg^{-1}d.w. of soil, respectively. Analysis of PLFA profiles, based

on the marker fatty acids, showed that in the soil bioaugmented with T'-1 strain 7 days after inoculation the content of fatty acids characteristic for Gram-positive bacteria was higher, as compared to the non-inoculated soil. At the same time increase in the amount of cyclopropane, unsaturated and hydroxylated fatty acids characteristic for Gram-negative bacteria was estimated. Similarly, increase in the content of these fatty acids was observed 7 days after treatment of soil with P-1 strain. At the end of the experiment, in soils bioaugmented with T'-1 and P-1 strains the structure of microbial communities was similar to that observed in the non-inoculated soil. Obtained results indicate that bacteria used in the bioremediation experiment did not have a long-lasting effect on the autochthonous bacterial community, which may result from the fact that they were members of the existing population. Similarly, no long-lasting changes in the PLFA profiles were observed in the soil bioaugmented with the mixture of the strains. In turn, it has been observed that CLPP of bacterial communities obtained for bioaugmented soils at the end of the experiment differed from the profile obtained for the control soil. In these profiles higher percentage of carbohydrates, organic acids and amino acids utilization was estimated, as compared to control soil. Moreover, higher values of average well colour development (AWCD) and substrate richness (R_S) and lower result of substrate evenness (E_H) were detected in bacterial communities isolated from treated soils after bioremediation process. Obtained result indicated that introduced strains enhanced metabolic activity of bacterial communities in soils. Furthermore, these communities specialised in degradation of specific growth substrates.

CONCLUSION:

Results obtained in this study showed that *Bacillus subtilis* T'-1 and *Pseudomonas* sp. P-1 strains, used for bioremediation of petroleum-contaminated soil, did not affect the structural biodiversity of indigenous soil bacteria, nevertheless they had impact on the functional diversity of these microorganisms.

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Cyanobacteria in freshwater: Influence in liver morbidity in Alentejo's

Authors: Fernando Bellém[1], Manuela Morais[2], Carlos Santos[3], Elisabete Carolino[4]

1 *Lisbon High School Health Technology - Lisbon Polytechnic, Portugal*
2 *Évora University, Portugal*
3 *National School of Public Health - Lisbon New University, Portugal*
4 *Lisbon High School Health Technology - Lisbon Polytechnic, Portugal*

Presenting Author: Email: fernando.bellem@estesl.ipl.pt | Tel.: +351 218 980 400 | Fax: +351 218 980 460

INTRODUCTION:
Water pollution, underground aquifers over-exploitation, freshwater inappropriate use and rain absence have caused water scarcity in many regions, and several dams or artificial reservoirs were built to provide water needs. In those reservoirs, eukaryotic organisms (microscopic algae) and photosynthetic prokaryotic organisms (cyanobacteria) constitute phytoplankton community.

These organisms get nutrients from water column and blooms may occur when specific environment conditions are associated with nutrients enrichment (eutrophication). Cyanobacteria can dominate blooms and promote health problems because some gender are toxins producers, and may cause liver diseases after exposure.

In Portugal between 2000-2008 several cyanobacteria blooms dominated by cyanotoxins producers have been reported in Alentejo's reservoirs used for potable water production, and no specifics treatments were adopted to eliminate cyanobacteria or produced cyanotoxins.

OBJECTIVES:
Assess cyanotoxins exposure through cyanobacteria characterization in Alentejo's reservoirs blooms between 2000-2008 and evaluate if those blooms had an impact in exposed population liver function.

MATERIALS AND METHODS:
In order to develop one historical prospective study (2000-2010), seven Alentejo's reservoirs used to produce drinking water in Beja and Évora districts were evaluated between 2000-2008 (physical, chemical and phytoplankton population features) and two population groups were formed (exposed and non-exposed people), assessed and compared in their laboratory data for hepatic disease.

Relative risk to get liver cancer if exposure occurs and attributable risk connected to exposed factor were calculated and compared as well. Exposure or non-exposure to cyanotoxins was useful to define population among Alentejo Region residents (specifically in Beja and Évora districts) and a convenient sampling model was adopted.

RESULTS AND DISCUSSION:
Selected reservoirs have presented physical and chemical parameters that favored cyanobacteria blooms and eutrophic classification. Several cyanobacteria blooms have been reported and liver toxins producers genders were often dominant. Water average consumption pattern was kept through studied period with higher levels consumption in exposed population.

Laboratory data showed that exposed population presented generally higher liver enzymes values when compared to non-exposed population (statistically significant differences), meaning a more serious liver compromise condition. Other liver injury causes like alcohol consumption or virus infection were also

evaluated but according data, their contribute was not relevant. Liver cancer incidence rate in exposed population was more expressive and in some years this group presented over 80% of cancer diagnosis.

Relative risk of getting liver cancer and have been exposed reached 4,4% when compared with non- exposed population and attributable risk in all population (exposed and non-exposed) 63% which means that 63% off all liver cancers diagnosed in population that year, could be assigned to cyanotoxins exposition.

CONCLUSION:

In Portugal, Alentejo's reservoirs presented between 2000-2008 physical and chemical conditions to promote cyanobacteria blooms and eutrophication conditions. In exposed population the influence of cyanotoxins in liver function is attested by liver enzymology laboratory data and relative risk to get liver cancer in this population touch 4,4%. Attributable risk related to cyanotoxins exposition reach 63% in general population.

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Following-up Antidepressants in Wastewaters across Portugal: A One-Year Study

Authors: Liliana J.G. Silva[1], Leonor M. Meisel[2,3], Celeste M. Lino[1], Angelina Pena[1]

1 *Group of Health Surveillance, Center of Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Polo III, Azinhaga de Stª Comba, 3000-548 Coimbra, Portugal*

2 *INFARMED, I.P. - National Authority of Medicines and Health Products, 1749-004 Lisboa, Portugal*

3 *Department of Pharmacology, Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal*

Presenting Author: Email: ljgsilva@hotmail.com | Tel.: +351 239 488 400 | Fax: +351 239 488 503

INTRODUCTION:

Selective serotonin re-uptake inhibitors (SSRIs) antidepressants are amongst the most prescribed pharmaceuticals throughout the world. They occur in different environmental compartments and their incomplete removal by wastewater treatment plants (WWTPs) has been reported. A better knowledge of SSRIs occurrence and fate will allow a proper risk assessment. This major problem must be tackled to meet the Water Framework Directive of the European Union. The use of antidepressants is highest in Portugal, doubling that of the EU average (15%)(Silva, Lino, Meisel, & Pena, 2012).

OBJECTIVES:

Study the occurrence, fate, seasonal influence and environmental risk assessment of four SSRIs, citalopram, fluoxetine, paroxetine and sertraline, in 15 WWTPsacross Portugal.

MATERIALS AND METHODS:

Influent and effluent samples, collected during the winter, spring, summer and autumn of 2013 campaign, in 15 WWTPs, of 5 regions, have been analysed. After acidification and filtration, 100 mL of 24-h composite samples were extracted through Oasis HLB cartridges (200 mg). For LC-Msn analysis, a QLIT mass spectrometer in the ESI+ mode was used, with SRM acquisition. Validation was performed to assure the fitness for purpose of the analytical method. Risk evaluation was assessed calculating the risk quotient (RQ) for three trophic (algae, daphnids and fish), between measured environmental concentration (MEC) and predicted no-effect concentration (PNEC).

RESULTS AND DISCUSSION:

Results showed that citalopram was the SSRI most frequently found, in both influents and effluents, with mean mass loads ranging between 3.46 and 0.81 mg/day/1000 inhabitants, respectively. Fluoxetine and sertraline were only detected in influent samples, in lower mean mass loads (0.73 and 0.02 mg/day/1000 inhab., respectively), whereas paroxetine was found in influent and effluent samples (0.64 and 0.32 mg/day/1000 inhab., respectively). WWTPs were not capable of completely remove these pharmaceuticals; nonetheless, the mean removal efficiency was 82.24%. Removal efficiency was lower in winter (74.21%) and summer (72.02%), when compared to spring (100%) and autumn (81.19%)(Table 1).Our results translate the variations in SSRIs prescription and use between the 5 Portuguese regions in study. Influent contaminated samples were found in WWTPs from Lisbon, Alentejo, Center and North (9.42, 7.13, 4.14 and 3.48 mg/day/1000 inhab., respectively). In Algarve region no contaminated samples were found. A seasonal pattern in the presence of SSRIs in influent wastewaters was observed (Figure 1A). The SSRIs concentration in influent wastewaters was higher in autumn, followed by winter, spring, and summer (Figure 1B).

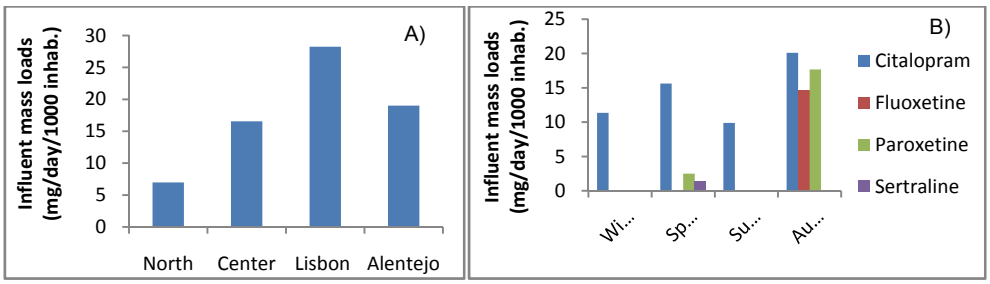


Figure 1 - Regional (A) and seasonal (B) variations on the occurrence of the selected SSRIs in influent wastewater.

Finally, on what concerns the RQ of different trophic levels of aquatic organisms, exposed to the effluents studied, citalopram, and paroxetine, the only SSRIs found in effluent wastewaters, presented RQ lower than 1. According to the results, algae appeared to be the most sensitive species followed by fish and daphnids (Table 2).

Table 1 - Detected concentrations (ng L^{-1}), frequency (%), mass loads (mg/day/1000 inhab), and removal efficiency (%) of SSRIs in WWTP influents and effluents.

Sampling		**Concentration**		**Mass Loads**		
WWTP	**Period**	**WWI**	**WWE**	**WWI**	**WWE**	**Removal**
Citalopram						
WWTP2	Winter	137.40	87.10	3.89	2.47	36.50
	Spring	101.20	n.d.	1.36	n.d.	100.00
	Summer	172.00	n.d.	3.87	n.d.	100.00
	Autumn	213.60	n.d.	3.74	n.d.	100.00
WWTP3	Winter	99.20	n.d.	21.0	n.d.	100.00
	Autumn	158.30	n.d.	18.1	n.d.	100.00
WWTP5	Winter	125.70	82.80	5.50	3.60	34.55
WWTP7	Spring	167.20	n.d.	25.30	n.d.	100.00
WWTP8	Winter	110.50	n.d.	7.80	n.d.	100.00
WWTP10	Autumn	162.20	89.70	37.70	20.90	44.56
WWTP11	Winter	100.50	n.d.	18.60	n.d.	100.00
WWTP13	Spring	179.70	n.d.	20.24	n.d.	100.00
	Summer	167.70	94.90	15.90	8.90	44.03
	Autumn	170.30	95.60	20.90	11.70	44.02
Fluoxetine						
WWTP2	Autumn	120.70	n.d.	2.11	n.d.	100.00
WWTP6	Autumn	157.40	n.d.	17.10	n.d.	100.00
WWTP10	Autumn	105.80	n.d.	24.60	n.d.	100.00
Paroxetine						
WWTP2	Spring	186.40	n.d.	2.50	n.d.	100.00
WWTP2	Autumn	185.60	n.d.	3.25	n.d.	100.00
WWTP10	Autumn	137.90	81.10	32.10	18.90	41.12
Sertraline						
WWTP2	Spring	100.40	n.d.	1.36	n.d.	100.00
All SSRIs						
Frequency		25	8.33	-	-	-
Range	-	99.20 - 213.60	81.10 - 95.60	1.36 - 37.70	2.47 - 20.90	34.55 - 100.00
Mean±SD	-	147.97±33.95	88.53±6.03	14.28±10.90	11.08±7.65	82.24±27.92

Table 2 - Worst-case scenario MEC of SSRIs in effluent wastewaters, PNEC and calculated RQ for algae, daphnids, and fish.

SSRI	MEC (ng L^{-1})	PNEC (ng L^{-1}) algae	RQ algae	PNEC (ng L^{-1}) daphnids	RQ daphnids	PNEC (ng L^{-1}) fish	RQ fish
Citalopram	213.60	360.00 [a, b]	0.59	80000 [c, d]	0.0027	4467[b,e]	0.048
Paroxetine	81.10	260.00 [a, b]	0.31	22000 [c, d]	0,0037	3293[b,e]	0.025

[a] EC50 was estimated with ECOSAR
[b] AF=1000
[c] long-term data from Henry, Kwon, Armbrust, & Black(2004)
[d] AF=10
[e] LC50 was estimated with ECOSAR

CONCLUSION:

The occurrence of 4 SSRIs in 15 Portuguese WWTP influent and effluent samples was investigated and citalopram was the one found in higher frequency and levels. Our results translate the variations in SSRIs prescription and use between the 5 Portuguese regions in study. Seasonal variations in terms of occurrence and removal efficiencies were observed. Most of the selected compounds in influents were removed during the treatment. RQ were lower than the unity in effluent wastewaters. Algae appeared to be the most sensitive species followed by fish and daphnids.

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Environmental Impact due to Coal-mining in Colombia, a Global Public Health Problem

Authors: Tammy Irina, Pulido Iriarte[1]

1 *Master Public Health candidate, Public Health Observatory Research Group, CES University, Medellín, Colombia*

Presenting Author: Email: tampulidoi@gmail.com | Tel.: +57 301 683 7576

INTRODUCTION:

The environmental impact due to mining coal in Colombia and its influence on environmental health with public health consequences are presented. As a developing country, Colombia's both current and future economic developments depend largely on mining. It owns the world's largest opencast coal mine with the largest coal reserves in Latin America, estimated in 16,992MTon, 42% of them already verified. It is believed that the reserve would last more than 100 years at the current mining rate, thus suggesting that the environmental impact due to mining coal may become more critical in the future.

The coal, used as energy source, shows the cheapest fossil fuel price and a high profit margin. It represents an appreciated economic tread for large multinational thermal plants in developed countries. However, coal is most responsible for carbon dioxide (CO_2) emissions in the World, near 11 billion tons each year. Its pollution effect produces an enormous environmental impact that includes climate change, water shortages, drought and smog that causes pulmonary lung disease and mercury from the acid rain that harms the neurological development in children, among other public health and QOL problems. Being the environmental issues similar in all countries with coal mines, as the consumption increases yearly it also do the environmental and health problems. It is here proposed that any possible solution to this environmental problem must be of global nature (according to Declaration of Ottawa[1)]) including an effective follow-up by recognized international organizations with international agreements for the use of clean energy.

OBJECTIVES:

Who should pay for the environment damage caused by the whole coal chain, from mining, transport up to energy production? Who should pay for the health cost care and harm to human beings and to the environment caused in economic and financial terms?In this paper it will argue that there are close interrelations and responsibilities among all countries involved in the entire process, from mining through combustion up to waste disposal. As case study: Colombia. The aim of this paper is to introduce to a problem for its comprehension to a global level.

MATERIALS AND METHODS:

This document is a preliminary product of a three years' exploratory investigation of primary source on coal mining: its environmental impact and external costs on public health and QOL[2,3)].Here will formulate the problem and the need to be solved. Finally, it will be briefly expose a way of dealing with, and express hope for a fairly and necessary global agreement. Facts and evidence of current life condition in the coal mining regions in Colombia after 40 years of coal mining production are presented, intended to provide elements for governmentdecision-making in economics and environmental policy from the perspective of public health and environmental impact in terms of health care costs and the irreparable damage to the ecosystem. This is a new perspective on covering the environment impact, social, public health, and economy, taking advantage of Colombia as a representative case.

Hereto is a brief presentation of the impact on health attributable to coal mining and its economic importance derived from the export of this mineral. Furthermore, it is suggested to apply the socio-ecological model of Health OTTAWA Charter in pursuit of a stable ecosystem with sustainable resources: a healthy ecosystem. Colombia is one of the most bio diverse countries in the world, ranked among the 17 megadiverse

countries of the world, and considered the most megadiverse per square kilometer (Potes, L,"Colombia is the Second Most Bio Diverse Country in the World", in Spanish) Unfortunately, there are no enough control tools to implement safety measures to safeguard this natural wealth against mining. The extractive industry is proud to meet the highest environmental standards, although for the government is clear that there is no control infrastructure to enforce the existing measures. This creates a violation on ecological and safety procedures that affects miners and surrounding communities, even leading to human rights violations.

Colombia is the world's tenth largest producer of hard coals and the 4thworld largest coal exporter after Australia, Indonesia and Russia, even before South Africa, USA and China (World Coal Institute, "Coal Facts 2007"). Due to Colombian coal low-cost and little sulfur content, its increasing production (by 80% since 1999) is exported mainly to European markets, thus stating the international nature of the pollution issue. According to the International Energy Agency, in 2011 the total international coal trade was 1.139 billion tons out of a total global demand of nearly 7.384 billion tons.

It is important to expose the “true cost of coal”, including the remarkable human and environmental damage to human health and the social conditions of communities living near mines, plants and waste sites, some of them even rendering the surrounding land uninhabitable. Coal mining severely messes up ecosystems and contaminates water supplies with carbon and toxic chemicals that give off other pollutes gases (nitrogen oxide, methane) that causes health problems like black lung disease. All these damage costs called “External Cost (EC)” are unavoidably paid by the poorest communities and society in general. If this EC were charged to power plants owner, their profits were so lower than would make the business unattractive.

RESULTS AND DISCUSSION:

The human health cannot be treated separately from the well-being and socio-environmental change and impact to the ecosystem. For this reason, it is interesting to apply the principle of responsibility of Hans Jonas, an ethic of modern technology for sustainable development and the relationship human being/nature. It is clear that dust from coal affects health since it produces pneumoconiosis (black lung) and lung cancer, reducing considerably the life expectancy of the miners and the nearby communities, being children the most affected by these diseases with no cure.

In relation to the environmental impact, the harmful effects to the environment due to the removal of coal are mainly due to the use of large areas leaving a large hole (the greatest coal mining hole is 347 m deep), surrounded of mountains of sterile material (waste) with a marked change of the landscape and soil topography that eliminates the existence of vegetation. It also destroys the genetic profile of the soil; its degradation and desertification negatively affects the existing habitat and wildlife, with deterioration of the air and water sources due to high level of particulate material, contamination by dust, methane, other toxic gases (carbon monoxide CO), and miners drains acids (DMA) or sulfurous. It is clarified that the worst damage caused by the emission of CO_2 occurs during its conversion into energy in the thermal power plants.
The environmental impact on the water sources implies not only variations in the quality of water, but also in the amount of available water for human consumption due to the great amount used for coal washing, as noticed in the water body of Caño Chacon in El Cerrejón, ILSA[4)] (2009). The Cerrejon company now seeks to divert the Ranchería River at a distance of 25 km, to expand the existing mining under the river. Climate and atmospheric changes; the conflict by water that involves the pollution of the Rancheria River and modification of streams; ecosystems and soils as a result of coal mining in the Cesar, has been deeply treaty by Fierro[5)] et-al (2010), where it was shown the negative impacts on the water resource both underground and surface, degraded soils and the high degree of desertification without having improved social indicators of the surrounding population, in spite of taxes and fees paid to these municipalities.

With regard to the social impact, perhaps the best known through the media is the end of cultural traditions and patterns rooted in the life of the communities. This is originated by the implicit violence due to forced displacement by mining, also called (euphemism) megaprojects for relocation of communities.

CONCLUSION:

The ecosystem is fundamental to human health because it is the life-support system of the planet, human species and any form of life (animal or vegetable).We must keep pace with a vision and holistic perspective to deal with a global problem that harms the human beings. It is suggested to apply the socio-ecological Ottawa Charter health model, in pursuit of a stable ecosystem and sustainable resources: health of the ecosystem, with a close link between people and environment towards a socio-ecological health approach. It is also suggested an International Agreement to ensure the preservation of natural resources in the world as a global responsibility and equity in environmental health.

The greatest hope is that we may live in the future with clean energy without coal consumption. "Everything is continuously speeding up". ***We simply must stop digging the ground and look up at the sky***; it is sufficient ***"to tame the sun"*** and ***"for us to write the rest of our history together"***. Herewith is an invitation to use natural resources in a responsible and conscious way to achieve one of the Millennium Development Goals, make the world a sustainable development ***"home"***, thriving in abundant resources and wealth for the present and future generations.

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Endophytic Bacteria Associated with *Hieracium piloselloides*: their Potential for Hydrocarbon-Utilizing and Plant Growth-Promotion

Authors: Małgorzata Kukla, Zofia Piotrowska-Seget

Department of Microbiology, University of Silesia, Katowice, Poland

Presenting Author: Email: malgorzata.pawlik@us.edu.pl | Tel.: +48 322 009 442

INTRODUCTION:

Petroleum hydrocarbons are widely distributed environmental contaminants that have negative biological effects, therefore there is an urgent need to remove these compounds from soil. The different biological methods are apromising alternative to conventional physico-chemical soil clean-up technologies. Among them phytoremediation isa cost-effective and environmentally friendly technology that has been developed recently. It has been found that the efficiency of phytoremediation may be enhanced by endophytic bacteria that have plant growth-promoting and hydrocarbon-degradation activity (Khan et al.,2013). Endophytic microorganisms colonize the healthy internal tissues of plants without causing symptomatic infections or negative effects on their host. Recent studies have described that these plant-associated bacteria play a key role in a plant's adaptation to a hydrocarbon polluted environment. Inoculation of plants with endophytes could reduce phytotoxic effect and increase efficiency of pollutant removal (Khan et al., 2013). Therefore, it is extremely important to know the biochemical capabilities, their natural competence for xenobiotic degradation, and character of the endophytic bacteria and plant relationship (Hardoim et al., 2008; Germaine et al.,2008; Khan et al.,2013).

OBJECTIVES:

The aim of this study was the isolation and characterization of endophytic bacteria from the tissues of *Hieracium piloselloides.* Plant growth-promoting mechanisms, hydrocarbon degradation potential, and detection of genes encoding the 1-aminocyclopropane-1-carboxylate deaminase (ACCD) enzyme, enzymes involved in aliphatic and aromatic hydrocarbons were evaluated.

MATERIALS AND METHODS:

Hieracium piloselloides was harvested from a petroleum-polluted area around a refinery in Czechowice-Dziedzice, Silesia, Poland. The endophytic bacteria were isolated from roots, stems, and leaves after surface disinfection. Hydrocarbon-degrading bacteria were selected on a mineral medium with crude oil as the sole carbon source. Identification of endophytic strains was carried out on the basis of their cellular fatty acid methyl esters (FAMEs) profiles using MIDI-MIS method.

Evaluations of plant growth-promoting activities were performed on selective media. The IAA (indole 3 acetic acid) production was estimated on LB medium supplemented with 500 µg/ml L-tryptophan. Siderophore secretion was assessed by the Chrome azurol S (CAS) method. For the determination of HCN(hydrogen cyanide) production, the strains were grown on a TSA medium, enriched with glycine (4.4 g/L). The ability to produce HCN was determined according to the colour change of filter papers soaked in sodium picrate. The phosphate-solubilizing activity of isolates was determined by measuring the zone size formed by solubilisation of insoluble phosphate on the Pikovskaya's agar plates. Extracellular cellulase activity was tested on indicator medium with a carboxymethyl cellulose (CMC). The motility of the cultured bacteria was examined on a nutrient broth containing 0.2% agar. After a 24-48 h incubation, the ability for bacterial motility was observed macroscopically by a diffuse zone of growth that spread from the center of the plate. The ability of isolated strains to produce biosurfactants was determined by the estimation of emulsification index (EI24) and "oil spreading" test.

By PCR reaction endophytic bacteria were screened for the presence of gene *acdS* encoding the ACCD enzyme. Only strains with a positive reaction to specific *acdS* starters were chosen for the measurement of ACCD activity. ACCD activity was determined by measuring the production of α-ketobutyrate generated by the cleavage of ACC by ACCD. The activity was expressed in μmol of α-ketobutyrate mg^{-1} h^{-1}.

The degradation potential of endophytic isolates was assessed by PCR method and primers the following genes: *alkH* (alkane hydroxylase), *alkB* (alkane monooxygenase), *C230* (catechol-2,3-dioxygenase), *P450* (cytochrome P450-type alkane hydroxylase, CYP153) and *pah* (alpha subunit of the PAH-ring hydroxylating dioxygenases).

RESULTS AND DISCUSSION:

From root, stem, and leaf of *Hieracium piloselloides* 18 strains of endophytic bacteria were isolated. They were able to degrade crude oil and showed multiple plant growth-promoting abilities. Most of them belonged to the genus *Pseudomonas.* Generally, it is known that *Pseudomonas* strains are involved in the degradation of hydrocarbons.

Isolated strains possess a number of properties that are thought to be responsible enhanced plant growth and development such as production of IAA, HCN, siderophores, as well as solubilize of phosphate. It is important to note that, all tested endophytic bacteria showed ability to produce significant amount of IAA in the range from 1 to 30 μg/ml. This suggests that plants are more effectively inhabited by IAA-producing endophytes which could enhance plant growth and development by altering the hormonal balance.

In this study, the ability of isolates to motility and cellulaseproduction were showed. It is well established that these traits are important in colonization process for endophytic bacteria.

Five endophytic strains exhibited emulsification activity resulting from the production of biosurfactants where EI24 was higher than 50%. Generally, it has been proved that the biosurfactant-producing bacteria play a key role in hydrocarbon degradation in environment. Biosurfactantsmay enhance bioavailability of these compounds.

ACCD gene (*acdS*) was found in almost half number of isolated strains. The activity of ACCD in isolates ranged from 2 to 50 μmol of α-ketobutyrate mg^{-1} h^{-1}, which suggests that these strains may be able to modulate ethylene levels and enhance plant growth. Moreover, it has been observed that a low level of ACCD activity, approximately ≥20 nmol α-ketobutyrate mg^{-1} protein h^{-1}, is sufficient to permit a bacterium to grow on ACC and to act as a PGPB (Płociniczak, Kukla, Wątroba, & Piotrowska-Seget, 2013).It has been proposed that ACCD activity plays a crucial role in microbe-plant associations. This bacterial enzyme regulates ethylene level in plants and consequently contributes to the production of longer roots and a greater root density.

In the present study gene-encoding hydrocarbon-degrading enzymes were found in 90% of the endophytic isolates, which indicates that these bacteria may play a great role in reducing the toxicity of the hydrocarbons that are taken up by plants (Phillips, Germida, Farrell, & Greer 2008).

CONCLUSION:

The application of well-characterised endophytic strains that possess the ability to degrade petroleum hydrocarbons will be a useful tool for further studies on endophyte-mediated phytoremediation of soils polluted with petroleum hydrocarbons. One or more strains that showed also multiple plant growth-promoting abilities might be potentially used for the production of a bacterial inoculum that could enhance the efficacy of phytoremediation.

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Persistent hydrophilic ethers (1,4-dioxane and glymes) in surface – and drinking waters in Germany

Authors: Wilhelm Püttmann, Konrad Magg, Daria Demers-Stepien

Goethe-University Frankfurt am Main, Institute of Atmospheric and Environmental Sciences, Department of Environmental Analytical Chemistry, Altenhöferallee 1, 60438 Frankfurt am Main, Germany

Presenting Author: Email: puettmann@iau.uni-frankfurt.de | Tel.: +49 69 798 40225 | Fax: +49 69 798 40240

INTRODUCTION:

In the present study three hydrophilic ethers (1,4-dioxane, triethylene glycol dimethyl ether (triglyme), and tetraethylene glycol dimethyl ether (tetraglyme) were investigated with respect to their distribution and behaviour in surface, ground, and drinking water at several locations in Germany and Poland. The selected ethers are highly miscible in water and have Henry's law constants ranging from 1.04×10^{-14} to 1.64×10^{-3}atm m^3mol^{-1} ensuring low volatility from aqueous solutions. Based on their octanol-water partition coefficients (log P_{ow}= - 1.03 to 1.92) they exert negligible potential for bioaccumulation and are only weakly retarded by sorption to soil particles or suspended solids [1]. Therefore, the main target compartment of these ethers is presumed to be the hydrosphere. Their physiochemical properties combined with their low biodegradability imply difficult removal from water and wastewater, which greatly increases the potential for surface, ground, and drinking water contamination. United States Environmental Protection Agency (USEPA) and the International Agency for Research on Cancer assigned 1,4-dioxane to a group B2 as a possible human carcinogen.

OBJECTIVES:

The occurrence of 1,4-dioxane, trigylme and tetraglyme at relatively high abundance (up to µg/L range) in surface waters is well known from several studies. The present study has been aimed at the identification of sources of these ethers and the behaviour in the aquatic environment. Particularly, studies with respect to the attenuation of the compounds in wastewater treatment plants (WWTPs), during bank filtration and drinking water production has been studied at several locations in Germany.

MATERIALS AND METHODS:

Solid phase extraction (SPE) in combination withgas chromatography/mass spectrometry (GC/MS) was used to analyze the hydrophilic ethers. Further analytical details have been described previously[1].

RESULTS AND DISCUSSION:

1,4-Dioxane has been detected in rivers such as Rhine and Oder with mean concentrations of approximately 0.7 µg/L and peak concentrations of about 2 µg/L (2). Triglyme and tetraglyme were present in high abundance (µg/L rang) only in the Oder river. In the Rhine River, the concentrations of 1,4-dioxane have been measured in 24-h composite samples for a period of two weeks. The results showed an inverse relationship of 1,4-dioxane concentration to the water discharge (m^3/s), suggesting its continuous entrance with wastewater effluents. In Main River, the effluent of a WWTP has been identified as one important source for 1,4-dioxane discharging on average3.5 kg per day into the river. In this WWTP technical methanol contaminated with 1,4-dioxane is added to the waste water as carbon source in the final denitrification step (3). The dominating source for triglyme and tetraglyme in Oder river was found in a sewage treatment plant in Poland that receives wastewater from the flue gas sulfurization of a Kupferschiefer processing applying Glymes for the removal of sulfur oxides from the flue gas in the so-called Solinox-process (4). Along the big rivers in Germany at many places artificial bank filtration is carried out for drinking water production. Studies at two river bank filtration sites have shown the removal rate for 1,4-dioxane during bank filtration is low and even treatment technology of the raw water (ozonation and activated charcoal filtration) during drinking

water production is not able to remove a major proportion of 1,4-dioxane from the water. Consequently, 1,4-dioxane concentrations of up to 0.5 µg/L have been measured in drinking water.

CONCLUSION:

The results of the conducted study confirm the need for 1,4-dioxane monitoring and regulation. The high concentrations detected in the surface waters and its resistance to natural attenuation poses a threat to drinking water produced through bank filtration of river water. Further studies have to clarify whether a similar assessment is also valid for triglyme and tetraglyme.

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Metropolitan Region of São Paulo – native forests and water quality: providers and recipients of environmental services

Authors: Ana Karina Merlin do Imperio Favaro[1,2], Silvana A. Cutolo[1,2], Ana Cláudia Sanches Baptista[1,2], Natasha Ceretti Maria[1,2], Juliana Barbosa Zuquer Giaretta[1], Leandro L. Giatti[1,2]

1 *Department of Environmental Health, School of Public Health, University of São Paulo, Brazil*
2 *INCLINE - INterdisciplinary CLimate INvEstigation Center, University of São Paulo, Brazill*

Presenting Author: Email: anakarinafavaro@usp.br | Tel.: +55 11 30617898 | Fax: +55 11 30617898

INTRODUCTION:

Most natural ecosystems have been converted as a result of other land use types and occupation. Such use may influence the characteristics, properties, components and processes of ecosystems. The realization that the goods and services provided by the ecosystems are a key to wellness is recent. The increased urbanization accelerates the environmental degradation and, therefore, the loss of environmental services. The climate regulation, carbon sequestration and biodiversity maintenance (global scale) and regulation of hydrological flows and fresh water (regional scale) are some of the main services affected by the loss of green areas in the urbanization process. Thus, it is necessary to identify green areas responsible for the maintenance of environmental services (providers) and their users (receivers), providing information for decision in relation to urban sustainability. In a metropolitan context, this information can be very useful for a necessary management aiming improving resilience and adaptability to environmental changes as well as promoting environmental health.

OBJECTIVES:

To identify among the 39 municipalities of the Metropolitan Region of São Paulo (MRSP), which ones are possibly the largest providers and the largest recipients of the environmental services selected for the study.

MATERIALS AND METHODS:

A documentary survey was conducted online in databases available in the Forest Foundation and the Forest Institute, as well as the State of São Paulo Forest Information System. In Cetesb (Company of Environmental Sanitation Technology of the State of São Paulo) reports were found offering data on water quality and water consumption in SNIS (National Information System on Sanitation) and finally, data on areas and population of municipalities in IBGE (Brazilian Institute of Geography and Statistics) reports.

RESULTS AND DISCUSSION:

Based on the amount of green areas, the environmental services that are commonly analyzed are: i) climate regulation; ii) regulation of hydrological flows; iii) carbon sequestration; iv) fresh water and; v) biodiversity - which is very rich in the studied areas because they belong to a rainforest ecosystem. To find out which municipalities are relevant in provisioning or receiving these services we analyzed the native forest remnants areas, the fresh water quality (WQI - water quality index), the fresh water demand and amount of population to identify the providers and recipients of these services (Table 1). The municipalities were ranked 1 to 10 in ascending order and in accordance with the values available for each category. Colors were used for better visualization of values, being red the largest recipients, yellow intermediate values as both recipients and providers and green the largest providers of environmental services. For the category of the native forest remnants, the municipalities that had $\geq 150 km^2$ of area were considered the major providers (green color) and the remainder were considered as intermediaries providers (yellow color). For population category, the five municipalities with larger populations were considered the major recipients (red color) and the others, as intermediary (yellow color). For the Water Quality Index, green color was used to the great and good values and red color to bad and very bad values. For the water consumption, the red was used to the five largest

consumers (recipients) and the yellow to the five intermediate consumers. The Municipalities of the MRSP that had no relevance to the selected categories were excluded from the analysis.

Table 1 - Metropolitan Region of São Paulo, providers and receivers municipalities of environmental services

Municipality	Total area (1) (km²)		Native forest remnants area (2) (km²)		Population (3)	Water Quality Index (4)						Water consumption x10⁶l/ ano		Water consumption per capita (l/hab/dia)	
						River/Reservoir	Best		River/Reservoir	Worst					
São Paulo	1.523,28	2	321,28	1	11.253.503	Braço do Itaquacetuba (BITQ00100)	3	81	Córrego do Ipiranga (IPIR04900)	1	14	1	748.080,5	5	184,6
Cajamar	131,40		16,92		64.114				Juqueri River (JQRI03800)	7	23				
Mairiporã	320,93	8	121,25		80.956	Paiva Castro Reservoir (JQJU00900)	5	75							
Biritiba-Mirim	317,15	9	112,17		28.575	Biritiba-Mirim River (BMIR02800)	6	74							
Guararema	270,60		38,83		25.844			unavailable						7	180,2
Guarulhos	319,19	10	98,67	2	1.221.979				Baquirivu-Guaçu River (BQGU03850)	4	17	2	67.062,1		
Itaquaquecetuba	82,97		7,89	10	321.770				Rio Jaguari (JGUA 03950)	3	16				
Mogi das Cruzes	713,29	4	166,50	7	387.779	Rio Tiête (TIET02090)	10	60				5	40.292,0	1	309,3
Salesópolis	424,97	6	144,58		15.635			unavailable							
Suzano	206,61		31,88		262.480	Reservatório Taiaçupeba (PEBA00900)	2	84	Rio Tiête (TIET03120)	8	26				
Mauá	61,30		7,30	6	417.064			unavailable				8	18.796,0		
Diadema	30,84		1,54	8	386.089			unavailable				7	22.096,0		
Ribeirão Pires	98,75		32,80		113.068	Reservatório do Rio Grande (RGDE02200)	9	67	Ribeirão Pires (PIRE02900)	10	35				
Santo André	174,94		64,77	4	676.407			unavailable				4	43.236,5	9	173,9
São Bernardo do Campo	408,77	3	193,12	3	765.463	Reservatório Billings BILL02900	1	84				3	46.256,9	10	171,0
São Caetano do Sul	15,37		0,00		149.263				Ribeirão dos Couros (CORU04950)	2	14			2	241,6
Cotia	323,10	5	146,22		201.150	Reservatório das Graças (COGR00900)	4	79	Ribeirão das Pedras (PEDA03900)	9	27				
Embu-Guaçu	154,94		71,12		62.769	Reservatório Capivari-Monos (CAMO00900)	7	71							
Juquitiba	522,06	1	399,39		28.737	Rio Juquiá (JUQI00800)	8	69						4	189,2
São Lourenço da Serra	186,40	7	127,39					unavailable						8	179,1
Barueri	66,14		5,35		240.749				Córrrego São João do Barueri (SJBA04950)	6	23	10	16.263,3	6	183,0
Carapicuíba	34,60		1,14	9	369.584							9	18.121,5		
Osasco	64,03		1,56	5	666.740			unavailable				6	40.257,2		
Pirapora do Bom Jesus	108,78		28,92		15.733	Reservatório de Pirapora (TIPI04900)			Reservatório de Pirapora (TIPI04900)	5	22				
Santana de Parnaíba	179,80		43,38		108.813			unavailable						3	190,6

SOURCES: (1) AND (3) IBEG, 2010; (2) FUNDAÇÃO FLORESTAL AND INSTITUTO FLORESTAL, 2013; (4) CETESB, 2012; (5) SNIS, 2011.

CONCLUSION:

Thus, the potential largest providers of the environmental services selected for this study are: in the first place the municipality of Juquitiba (with the largest extension of green area and good WQI); second place São Paulo (with the second largest area and high WQI) and; third place São Bernardo do Campo (with good extension of green area and high WQI). The potential largest recipients of these environmental services as regards the number of inhabitants are: São Paulo, Guarulhos and São Bernardo do Campo. Concerning the water consumption, present the municipalities of São Paulo, Guarulhos and São Bernardo do Campo (with the highest total consumption of liters per year) and Mogi das Cruzes, São Caetano do Sul and Santa de Parnaíba (with the highest consumption of liters per capita).

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Challenges in Health Impact Assessment in Brazil: a retrospective case study

Authors: Simone Georges El Khouri Miraglia[1], Anna Paula Soares Ribeiro Martins[1], DiegoVellosoVeronez, Micheline de Sousa Zanotti Stagliorio Coelho[1,2], Karina Camasmie Abe[1]

1 *Universidade Federal de Sao Paulo, UNIFESP, São Paulo, Brazil.*

2 *Centre for Air Quality and Health Research and Evaluation, Woolcock Institute of Medical Research, New South Wales, Australia*

Presenting Author: Email: miraglia@terra.com.br | Tel.: +55 11 331 93592

INTRODUCTION:

The Health Impact Assessment (HIA) has been developed in many countries and has its origin in Environmental Impact Assessment (EIA) studies. However, EIA did not comprehend fully health impacts derived from environmental aspects and HIA came to overcome it. The World Health Organization has defined HIA, since 1999, as a combination of procedures, methods and tools by which a policy, program or project may be judged as to its potential effects on the health of a population, and the distribution of those effects within the population (Gothemburg Consensus, 1999).HIA must add value to decision making and lead to better decisions than would have otherwise been made. In Brazil, there are no legislative rules or guidelines to address how the professional should conduct a HIA.

OBJECTIVES:

Analyze the network of health impacts regarding a national hydroelectric.

MATERIALS AND METHODS:

This work presents a suggestive approach of health impact network concerning a national hydroelectric, analyzing the most common and potential health effects due to environmental impacts on local population. The method was based in literature review and discussion analyses based on the Brazilian Ministry of Health data considering the local scenario.

RESULTS AND DISCUSSION:

In this case study, we have found positives and negatives aspects affecting population health. Regarding the positive aspects we could cite creation of permanent jobs, increase of income and urban development. Some of the negative impacts were: increase of migration, overflow, noise, air pollution, sexual diseases, increase of deaths due to car and bike accident and increase of tropical diseases by vector. All of those impacts have health effects on local population, decreasing the quality of life and increasing the health costs in health services near the hydroelectric construction. The grid of impacts on health is very important to mitigate negative impacts and help to consider the health services in future similar projects. This study opens a new challenge in national health impact assessment.

CONCLUSION:

The experience in Brazil shows that the HIA process has been a useful mechanism for raising broader public health issues on the energy policy agenda, and it has already had positive results for policy formulation.

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Impacts of climate change and its vulnerability in Bangladesh: It should be a major policy issue

Authors: Shakeel Ahmed Ibne Mahmood, G U Ahsan

Department of Public Health, North South University, Dhaka, Bangladesh

Presenting Author: Email: shakeel@icddrb.org | Tel.: +880 191 351 9511

INTRODUCTION:

Climate change is one of the most important issues to tackle this generation and possibly any generation in history. Climate change is taking a toll on not only the ecology of nations around the world, but also their political, economic and social stability, with the poorest nations and the poorest of the rich nations being the worst sufferers. A one meter rise in sea level could, for instance, flood seventeen per cent of Bangladesh's land area; threaten large parts of coastal cities such as Lagos, Cape Town and elsewhere and overwhelm, along with storm surges, small Island developing States from the Maldives to Tuvalu.

OBJECTIVES:

To evaluate the impact of drought in Bangladesh and recommend measures to be adopted for building its resilience and reducing the risk of climate change, which represents national development.

MATERIALS AND METHODS:

This study was carried out by employing a general review of literature on drought, focusing on its effects in Bangladesh, and the results of specific research recently conducted by the author.

RESULTS AND DISCUSSION:

Among the IPCC's other findings in 2007 was that storms and cyclones have become more intense over the past 30 years and that droughts, especially in the tropics and sub-tropics, have become more frequent with implications for food security.Bangladesh is one of the top 10 nations mostly vulnerable to climate change, said CRI, 2011 report. By the end of century, Bangladesh is set to disappear under the waves as mentioned by NASA space agency. IPCC predicted that by 2050, Bangladesh is on course to lose 17% of its land and 30% of its food production and as a result poverty will increase. The country has already begun to feel the effects of the climate change as flood periods have become longer and the cyclones, droughts and earth quakes that hit the country cause greater devastation and adversely affecting the country's agriculture and land, and challenging water resources, occupational dislocations, food, health, energy and urban planning. About 53% of the coastal areas are affected by salinity. Millions of people in northern Bangladesh are threatened by riverbank erosion and severe droughts.

Please see Table 1

Table 1 - Changes in climate parameters with respect to base year 1990

Parameters	**2030**		**2070**	
Winter	Winter	Monsoon	Winter	Monsoon
Temperature (oc)	2	0.65	3	1.5
Evaporation (%)	10	20	16	5
Precipitation (%)	-3	11	-37	28
Discharge (%)	-5	20	-67	51
FAP completion	60	60	100	100
Sea level rise (cm)	30	30	70	70



E. Islam I Figure 1 - River erosion, Lalmohon Vola

CONCLUSION:

Climate change needs top-down flow of information and communications. The people, in general, and the communities in the rural areas (including farmers, mountain enterprisers) in particular, need to be given information and knowledge about the impacts of drought and matters concerning the mitigation of the problem, adaptation of knowledge, and how successful practices can be replicated. So that they develop resilience to combat it and adapt themselves. There should be a disaster Management and National Calamities Department, fully equipped with modern equipments, information, long term training program, and dissemination of information for the people of Bangladesh.

Bangladesh government can take steps to prepare for a warmer earth. This involves limiting construction along low-lying coastal areas, building dykes to protect the coastal areas from flooding, developing new crop varieties which can grow in drier conditions, protecting wildlife and improving water storage to continue farming in drought condition. The only way to enhance the accountability of public administration is if Bangladesh Government should immediately translate its National Environmental Policy into action to benefit the people of this country. Further more it needs institutional and financial frameworks, along with a good evidence base and monitoring and evaluation technical skills for evaluating drought mitigation and adaptation policies, paving a practical way forward so that countries evolve sustainably and grow its economy in a way that keeps humanity's footprint within planetary boundaries.

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An empirical study about the variables that influence the social acceptability on the biogas

Authors: Telma Dias[1], Matilde A. Rodrigues[1], Ilídio Pereira[1,2], Celina P. Leão[3]

1 *Research Centre on Environment and Health, Allied Health Sciences School of Polytechnic Institute of Porto, Portugal*
2 *Department of Biomathematics, Biostatistics and Bioinformatics, Allied Health Sciences School of Polytechnic Institute of Porto, Portugal.*
3 *R&D Centro Algoritmi, Engineering School, University of Minho, Guimarães, Portugal*

Presenting Author: Email: tdias1990@gmail.com | Tel.: +351 918 408 808 | Fax: +351 222 061 001

INTRODUCTION:

Due to the excessive use of conventional energy sources, renewable energy technologies have becoming a potential alternative to provide a sustainable development of society. Biogas has been considered as one of the most environmental beneficial source of energy that contributes for reducing greenhouse gas emissions and global warming (Jiang *et al.,* 2011). Furthermore, the production of biogas can be highly important in rural areas, where there are many treatment stations of organic wastes, being a good way to solve problems related to release gases into the atmosphere, as well as related to the bad smells. However, the society opposition, disinterest and lack of knowledge about the advantages of biogas production and its use, can be a barrier to the availability of the implementation of these renewable technologies. In this context, more studies are necessary to clarify the public acceptability of these technologies (Zyadin *et al.*, 2012), in order to get further information to help into design an intervention strategy.

Several studies have been conducted in order to evaluate the public acceptability of energy sources, because the acceptability is not considered always positive due to some critical factors that can influence it (see e.g. Yuan *et al.*, 2011). However, no study related with the biogas acceptability was found.

OBJECTIVES:

This study aims to assess the public acceptability of biogas, analysing the key variables that can have influence on it.

MATERIALS AND METHODS:

The study was developed in a rural area, belonging to north of Portugal. This area was selected because it have a high proportion of temporary crops and pastures associated with permanent crops, and a high agricultural production that contributing to a large production of waste with organic fraction, which can be used for biogas production (Bond & Templeton, 2011).

A total of 230 local residents were interviewed in their home or at workplace. 50.4 % of these participants were female and 49.6% were male. The majority of participants (56.1%) had age range between 20 and 39 years, 22.2% were older than 50 years, 17% had age range between 40 and 49 years and 4.8% were younger than 20 years.

In order to fulfil with the proposed objectives a questionnaire was developed and applied. The questionnaire included two main parts. The Group I included socio-demographic questions: gender, age, level of schooling, professional activity and distance of their housing to the closest station wastewater treatment. The Group II was structured in 22 sentences that aimed to analyse which variables influence the acceptability of such energy. The 22 questions were related with five other variables: Benefit Perception, Risk Perception, Trust in regulatory institutions, Knowledge and Experience with the technology. Almost the variables were measured on a 5-point degree scale adapted from Bronfman *et al.* (2012),excepting some questions related to Knowledge and Experience with the Technology, which were measure with a dichotomous scale. The validity

and reliability of the questionnaire were analysed using this sample. Some models were tested using the LISREL software.

RESULTS:
The results show that biogas acceptability is not so high as expected. The higher acceptability of the biogas production was related to the environment, particularly due to the high benefits found. However, for society and even for employees of the companies, the acceptability of this energy source is lower due to the high level of risks perceived related to the production of this technology.

Participants demonstrate total trust in employees of companies. Regarding to the regulatory institutions, they rely more in the inspection authorities of biogas plants, than in the Energy Services Regulatory Entity of Portugal. Regarding to the knowledge about biogas, the results showed that respondents, in generally, do not have a good knowledge, since their majority was no right in all-technical questions analysed. The respondents do not have also much experience with biogas.

Different models were tested to analyse the relationship between biogas acceptability and the other variables in analysis. The model founded with best-fit show that all the variables in analysis have a direct and indirect effect on risk acceptability, i.e., as the higher are the benefits perceived, the trust on involved parties, the knowledge and experience of the respondents and lower the risks associated to the technology, more acceptable is the biogas technology.

DISCUSSION:
According the results obtained, the acceptability of biogas technology is not so favorable and high as predicted. Other studies related with other renewable technologies obtained more favorable results (Yuan *et al.*, 2011; Bronfman *et al.*, 2012). For example, Yuan *et al.* (2011) verified that people accept renewable technologies, in particularly solar water heater, and they also demonstrate a high level of awareness about them. Bronfman *et al.* (2012) pointed out that renewable energy technologies are usually acceptable, since they are known as sources that do not have many risks associated.

In this study, the lower acceptability of biogas technology was pointed to be related with the high risks perceived for the workers and the society in general. In fact, different studies found risk acceptability and risk perception to be inversely related (Bronfman *et al.*, 2012). However, it can still be verified that there is a certain degree of biogas acceptability in relation to the environment, which should be associated to the fact that this new technology be a source of renewable energy with high benefits for the environment, as verified in previously studies with different technologies (Bronfman *et al.*, 2012). These results, along with the low knowledge about this technology and experience indicate that in general, the laypeople have some resistance to this type of energy, situation which could be changed with awareness interventions, showing that the risks can be controlled and the benefits for society are higher than the currently perceived. It is also important to increase the level of trust in the involved parties, as trust was found to be related with risk acceptability in the same way as in previous studies (Bronfman *et al.*, 2012).

CONCLUSION:
This study analyzed the social acceptability of biogas and the variables that influence it. According to results obtained, biogas acceptability was not very high. It was also verified that benefit perception, risk perception, trust, knowledge and experience were the variables that influenced the decision of the people to accept or not the biogas technology.

In general, this study emphasize that it is necessary to enhance awareness and understanding of population regarding to biogas, providing a greater guidance to their perceptions about the risks and benefits related to this technology, in order to increase its acceptability.

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Determination of Synthetic Musks in Beach Sands by QuEChERS extraction followed by GC-MS analysis

Authors: Vera Homem[1], Inês Magalhães[1], Arminda Alves[1], Lúcia Santos[1]

1 *LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal*

Presenting Author: Email: vhomem@fe.up.pt | Tel.: +351 220 414 947 | Fax: +351 225 081 449

INTRODUCTION:

Synthetic musks are organic compounds produced in large quantities and extensively used in daily human life in a wide variety of cosmetics, personal care products and household products (Mottaleb et al., 2012). They are continuously released into the environment through a variety of direct and indirect routes as swimming and bathing in rivers or sea, from showering, washing/cleaning and via wastewater treatment plants (WWTPs). Due to their lipophilic, persistent and bioaccumulative nature, synthetic musks are easily adsorbed by particular organic matter and accumulate in sediments (Martinez-Giron et al., 2010) that become another potential contamination source. Few studies have been conducted to assess the occurrence of synthetic musks in sediment samples, namely beach sands (marine sediment) and, for that reason, there is a lack of global monitoring data on sedimentary concentrations of these compounds.

OBJECTIVES:

The aims of the present research were: to investigate the application of QuEChERS extraction coupled to GC-MS analysis to the determination of synthetic musks in beach sands, to characterize the musk residues in this matrix and to understand their distribution in the Oporto coastal area.

MATERIALS AND METHODS:

Sand samples were collected from 23 beaches on the Oporto coastal area. Samples were spiked with AHTN-d_3 and MX-d_{15} (surrogate standards) and extracted using the following procedure: 3 mL of acetonitrile was added to the sample and then, the mixture was vortexed for 3 min and sonicated for 10 min. The first QuEChERS ($MgSO_4$ and $NaCH_3COO$) were added and the mixture was vortexed for another 3 min. Samples were centrifuged at 3700 rpm for 10 min, and the solvent layer was transferred into the tube containing the second QuEChERS ($MgSO_4$, PSA bonded silica and C_{18}). Once again, the samples were vortexed and centrifuged. The supernatant was collected and evaporated to dryness under a gentle stream of N_2. Then, the extract was reconstituted in 50 µL of acetonitrile before GC-MS analysis. Chromatographic analyses were performed by a Varian Ion Trap GC-MS system, using an electron impact ionization mode. The separation was carried out using a CP-Sil 8 CB capillary column (50 m × 0.25 mm i.d., 0.12 µm) from Agilent Technologies. For these analyses, the GC oven was programmed from 60 ºC (hold for 1 min) to 150 ºC at 6 ºC/min (hold for 10 min), to 225 ºC at 6 ºC/min and, to 300 ºC at 20 ºC/min; (total analysis time = 45 min). For quantitative analysis of target compounds, selected ion storage (SIS) mode was applied.

RESULTS AND DISCUSSION:

The performance of a previously validated QuEChERS-GC-MS methodology (Homem et al., 2013) for the determination of musks was assessed for this new matrix - beach sands. Low detection limits and high recoveries (>80%) were determined. Galaxolide and tonalide were the most frequently detected musk fragrances. Nitromusks were rarely present and, when detected, their levels were low.

CONCLUSION:

In this work, the overall contamination status of synthetic musk fragrances in Oporto coastal area (beach sands) was investigated. Different musk levels were detected, but polycyclic musks were the most common group found. In general, concentrations of galaxolide were the highest, followed by tonalide.

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Carbon footprint of the academic community of Lisbon School of Health Technology, Portugal

Authors: Sérgio Almeida[1], Vítor Manteigas[2]

1 *Lisbon School of Health Technology - Polytechnic Institute of Lisbon, Portugal*
2 *Scientific Area of Environmental Health of Lisbon School of Health Technology - Polytechnic Institute of Lisbon, Portugal*

Presenting Author: Email: vitor.manteigas@estesl.ipl.pt | Tel.: +351 218 980 430 | Fax: +351 218 980 460

INTRODUCTION:
With growing concerns about different environmental issues and the need to address climate change (Larsen et al., 2011), institutions of higher education should create knowledge and integrate sustainability into teaching programs and research programs, as well as promoting environmental issues for society (Geng et al., 2012).

OBJECTIVES:
The aim of this study is to determine the carbon footprint of the academic community of Lisbon School of Health Technology (ESTeSL) in 2013, identifying possible links between the Carbon Footprint and the different socio-demographic variables.

MATERIALS AND METHODS:
Data collection was carried out through the application of a questionnaire online, forwarded to the email addresses of the classes, the teachers and staff. The completion of the questionnaire was effected through the Lime Survey ® program. The questionnaire consisted of 6 groups of questions: general, home, car, public transport, bike &flights and lifestyle. The first group (general) was formed by the independent variables for sample characterization (gender, age, district of residence, education level, occupation, degree, graduation year). The remaining groups were drawn up on the basis of Carbon Footprint calculators available online. All calculators used contained emission/conversion factors based on standards and accredited international organizations (Caixa Geral de Depósitos, 2009; Carbonica, 2012; Carbon Footprint, 2013). The answers to the questionnaire resulted in a carbon footprint for each individual, expressed in tones of carbon dioxide equivalent. Was performed statistical analysis.

RESULTS AND DISCUSSION:
The sample is composed of 67 individuals of the academic community of ESTeSL, consisting of females (n=55) and male (n=12), aged between 18 and 54 years. The sample is representative of 10 Portuguese districts, being Lisbon (61.19%) the district more represented, followed by Setúbal.

On average, a person of the academic community of ESTeSL obtained a carbon footprint of 6,54tCO2eq. There was a statistically significant difference for the variable Year of Course Degree (Table 1).

Table 1 - Carbon Footprint by year of course degree (tCO2eq.)

Year of Course degree	Carbon Footprint
1 (n=12)	6,33
2 (n=16)	7,71
3 (n=17)	6,72
4 (n=13)	5,39

In fact, the 4th year is one that has the lower carbon footprint with the value of 5.39 tCO2eq. A positive correlation was found between kilometers and carbon footprint.

Respondents were not aware of the theme electricity, leading to present less accurate results.

CONCLUSION:

This study concluded that the average value of the carbon footprint of the academic community of Lisbon School of Health Technology is substantially below the corresponding in Europe (National Geographic, 2013) but it is suggested that School perform studies based on input-output analysis and lifecycle assessment, for a more detailed and accurate Carbon Footprint that exists in ESTeSL.

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Are anti-inflammatories and/or analgesics efficiently removed by Portuguese wastewater treatment plants?

Authors: Liliana J.G. Silva[1], André M.P.T. Pereira[1], Celeste M. Lino[1], Leonor M. Meisel[2,3], Angelina Pena[1]

1 *Group of Health Surveillance, Center of Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Polo III, Azinhaga de Stª Comba, 3000-548 Coimbra, Portugal*

2 *INFARMED, I.P. - National Authority of Medicines and Health Products, 1749-004 Lisboa, Portugal*

3 *Department of Pharmacology, Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal*

Presenting Author: Email: ljgsilva@hotmail.com | Tel.: +351 239 488 400 | Fax: +351 239 488 503

INTRODUCTION:

Non-steroidal anti-inflammatories and analgesics are amongst the most prescribed pharmaceuticals throughout the world. In Portugal the highest prescription and consumption regard, among others, ibuprofen, diclofenac and paracetamol (INFARMED, 2011). After intake, these highly active compounds undergo metabolic transformations, with subsequent excretion of significant fractions of the unmetabolized or of active metabolites to raw sewage and wastewater treatment plants (WWTPs). Scientific studies have already demonstrated their incomplete removal by WWTPs, being these facilities considered as the major environmental source since their effluents are discharged to the surrounding water bodies, with inherent and documented acute and chronic toxicity (Santos et al., 2010).

OBJECTIVES:

Evaluate the removal efficiency of the three anti-inflammatories and/or analgesics, in 15 WWTPs with different treatment processes across Portugal.

MATERIALS AND METHODS:

24-h composite samples of wastewater influents (WWIs) and effluents (WWEs) of 15 different WWTPs were collected during 14 May/14 August 2013 (Figure 1). The population equivalent treated ranges between 6850 and 756000, representing 26.1% of national population (10526700 in 2012). WWTPs average loads ranges from 349 to 140000 m^3/day and have their discharge points in the main Portuguese rivers and Atlantic Ocean. They are designed to treat domestic, hospital and industrial wastewaters, operating with secondary (activated sludge - AS; biofiltration - BF; lagoons - LG; trickling filters - TF) or tertiary treatments (ultraviolet - UV).

For identification and quantification, extraction and cleanup was performed through an Oasis MAX cartridge and instrumentation analysis was performed by LC-MS-MS (ion trap) (Sousa et al. 2011). Removal efficiency of the selected pharmaceuticals was evaluated by means of: Removal efficiency (%) = $((m_{inf}-m_{eff})/m_{inf})x100$. Where m_{inf} is the load of pharmaceutical in WWI and m_{eff} is the load of pharmaceutical in WWE.



Figure 1 - Map of the studied WWTPs.

RESULTS AND DISCUSSION:

WWTPs were not able to completely remove these pharmaceuticals, however, in terms of the overall mean values the removal efficiency was of 99% (46%, 94% and 100% for diclofenac, ibuprofen and paracetamol, respectively). Taking into account Figure 2, it is noticeable a great variation in removal efficiencies, especially between diclofenac and the others, ranging from not eliminated to 100%.

Systems that use an AS process are still widely employed for wastewater treatment, mostly because they produce an acceptable quality WWE at reasonable operating and maintenance costs. However, this type of treatment has limited capability of removing pharmaceuticals from wastewater (Vazquez-Roig, Andreu, Blasco, & Picó, 2012). Accordingly, our results show that this secondary treatment was not efficient in removing diclofenac from WWTP 1 and 9, leading to a non elimination in the mean results. On the contrary, ibuprofen and paracetamol were efficiently removed by this process. The addition of a tertiary treatment with UV significantly increased diclofenac removal (48%).

Regarding the remaining treatment processes, although the results regarded only one sample for each, it was observed that the removal efficiency for the TF process was lower for diclofenac and ibuprofen (21.3% and 84.0%, respectively) when compared to paracetamol (98,9%). For BF/UV and LG/UV the data observed showed improved removals for all the compounds.

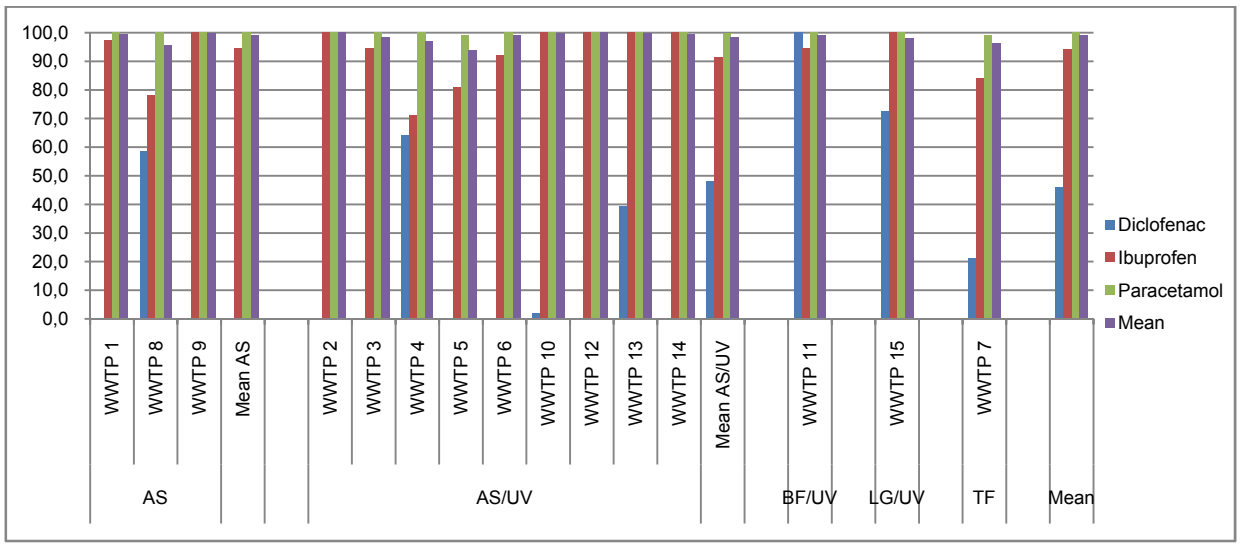


Figure 2 - Removal efficiencies and correspondent treatments of the 15 WWTPs (AS - activated sludge; UV - ultra violet; BF - biofiltration; LG - lagoons; TF - trickling filters).

Although, pharmaceuticals concentrations in sludge or suspended solids was not considered nor measured, one should note that good removal rates obtained in aqueous phase do not imply degradation to the same extent. Moreover, the conversion of a given pharmaceutical to transformation products other than the analyzed might lead to lower pharmaceutical levels in WWE samples, and to an apparent removal (Jelic et al., 2011).

CONCLUSION:

Overall, removal efficiencies were high and similar for all of the employed treatments processes; however this is mainly due to the high values for paracetamol. Nevertheless, diclofenac presented a different behavior with lower removals or even not being eliminated, especially with only secondary treatments.

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Uncertainties and Environmental Health: a challenge for participatory approaches

Authors: Leandro Luiz Giatti[1, 2]

1 *Department of Environmental Health, School of Public Health, University of São Paulo, Brazil*
2 *INCLINE - INterdisciplinary CLimate INvEstigation Center, University of São Paulo, Brazil*

Presenting Author: Email: lgiatti@usp.br | Tel.: +55 11 3061 7978 | Fax: +55 11 3061 7712

INTRODUCTION:
Social-environmental issues of high impact on human health are attuning intensely to contemporary transitions and dilemmas, also in respect of the classic science, in ways of backing political decisions. This context asks for the participation of the subjects of risks in the production and control of the quality of knowledge, as well as in the processes of governance in the face of the increasing scale of uncertainties and decision stakes. The promotion of dialectic processes linking scientific and popular knowledge has been considered fundamental in academic movements and initiatives which aim at intervening in real and complex situations (Santos, 2007), as the contexts that aggregates social, environmental and health questions. Post-normal science argues for the need of extended peer communities, the whole society, to be involved in the production of knowledge and the management of uncertainties that offer high degree of risk (Funtowicz & Ravetz, 1993).

OBJECTIVES:
To provide a panorama about how participatory approaches have been developed in studies focused on environmental health questions, considering the functionality of the participatory processes and the integration of a perspective of uncertainties.

MATERIALS AND METHODS:
A systematic bibliographic review was the employed strategy; it started with a search for papers in the Thompson Reuters´ Web of Science. This source was chosen because of its general recognition as a pan-disciplinary bibliographic database. The keywords applied for the search in topics were: 'health' and 'environment' or 'environmental' plus 'participation' or 'participatory' or 'post-normal' or 'governance' or 'ecohealth'. The first selection brought 1.212 papers published since 1999 and the last year of the time span was 2012. These papers were subsequently filtered in a process with a careful reading of the abstracts concerning aspects of a real application of a participatory process within an environmental health background. 170 papers were finally included and then a classification was applied through the three following categories on respect of the objectives and functionality of the participatory approaches: diagnosis; problem solving; and uncertainties/emergent phenomena.

RESULTS AND DISCUSSION:
Figure 1 brings the distribution of papers through the categories applied for the functionality of the participatory approaches. The first category of the papers´ classification through functionality considered is diagnosis that accounted 78 articles (45.9%). The features of this category are delimited within a collaborative process in which researchers and social actors work together searching for an interdisciplinary description of certain environmental health context. The problem solving category implies in a framework with the same kind of collaborative approach aiming at intervening and finding solutions to an environmental health problem. This second category accounted 76 papers (44.7%). Considering that a diagnosis stage is essential for a problem solving approach then it is reasonable to understand that an evolving process occurs from the stepwise manner: 1st understanding; 2nd finding solutions; and 3rd interacting with complexity of uncertainties in a prospective scenario. In consideration of a full development of a participatory approach dealing with environmental health questions, uncertainties and emergent phenomena were admitted as the

highest level of functionality for analysing the participatory processes. On regard of this last category only 16 papers were found (9.4%), among them the perspective of dealing of prospective scenarios was a key concerning for inclusion. The papers considered as the functionality directed to uncertainties/emergent phenomena were understood as being capable of transcending the diagnosis and the problem solving approaches. Dealing with uncertainties and promoting a future vision was also understood as a better possibility of preparing contingency plans and offering opportunities to identify necessary preventive or mitigatory measures (Godet & Durance, 2009).

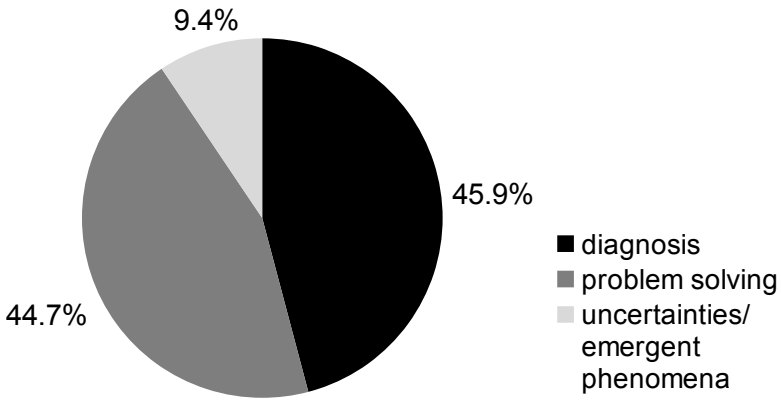


Figure 1 - Classification of papers through functionality of the participatory approaches

CONCLUSION:

By means of the evidences offered by the bibliographic review, it was possible to identify that a new paradigm of relations between science, society and politics is just beginning. The higher degree of participatory processes dealing with uncertainties related to environmental health questions can be considered with a challenge since this kind of approach appeared as the minority of the experiences analyzed.

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Ecological footprint as an indicator of sustainability at Lisbon School of Health Technology, Portugal

Authors: Sara Francisco[1], Gonçalo Costa[1], Vítor Manteigas[2]

1 *Lisbon School of Health Technology - Polytechnic Institute of Lisbon, Portugal*
2 *Scientific Area of Environmental Health of Lisbon School of Health Technology - Polytechnic Institute of Lisbon, Portugal*

Presenting Author: Email: saracfrancisco@gmail.com | Tel.: +351 925 797 782

INTRODUCTION:

Higher education institutions, has an active role in the development of a sustainable future (Côrrea, 2003) and for this it is essential that are environmentally sustainable institutions applying methods such as the ecological footprint analysis (Klein-Banaia & Theisb, 2011).

OBJECTIVES:

This study intent is to strengthen the potential of the ecological footprint as an indicator of the sustainability of students of Lisbon School of Health Technology, and identify the relationship between the ecological footprint and the different socio-demographic variables.

MATERIALS AND METHODS:

Data collection, in sample of 678 students, was carried out through the application of a questionnaire online. The first part of the questionnaire was formed by the independent variables for sample characterization: age, gender, province of residence, level of education, profession, scientific area, level of education, course degree, year of course. The second part of the questionnaire consisted of questions adapted from the Group' s questionnaire of environmental studies at the school of Biotechnology, the Catholic University Portuguesa. Later it was applied a conversion table for global hectares. It was applied descriptive statistical analysis, verification of normality for all variables, and the existence of association between the socio-demographic variables and the value of the ecological footprint.

RESULTS AND DISCUSSION:

By calculating the ecological footprint of the students of Lisbon School of Health Technology it reached a value of 6,01gha. On average, female individuals have a lower ecological footprint than males. There is significant difference between the different districts, and individuals residing in the District of Castelo Branco (Portugal) have, on average, the lowest ecological footprint. It was observed that the average of the lowest ecological footprint was obtained by degree in Prosthetists and Orthotists followed by Environmental Health and the higher average was obtained by students of Radiology degree followed by Physiotherapy (Table 1).

Table 1 - Ecological Footprint by Course Degree

Course Degree	Ecological Footprint (gha)
Biomedical Sciences in Histocellular	5,90
Pathology	5,94
Cardiopneumology	5,66
Clinical Analysis and Public Health	5,84
Dietetics and Nutrition	5,58
Environmental Health	5,79
Nuclear Medicine	5,93
Orthoptics	5,93
Pharmacy	6,10
Physiotherapy	5,45
Prosthetists and Orthotists	6,11
Radiology	5,88
Radiotherapy	

The ecological footprint of the students of Lisbon School of Health Technology is superior than the value of Portugal, and above the national biocapacity (National Footprint Network, 2010).

CONCLUSION:

With this study, we conclude that the students of the Lisbon School of Health Technology is not environmentally sustainable.

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Phytoremediation of metal polluted field soils using *Brassica juncea* and *Ricinus communis* inoculated with plant growth promoting serpentine bacteria

Authors: Ying Ma, Helena Freitas

Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Portugal

Presenting Author: Email: cathymaying@gmail.com | Tel.: +351 910 802 125 | Fax: +351 239 855 211

INTRODUCTION:

Microbially enhanced method of phytoremediation has recently been proposed as a viable strategy for removing/inactivating heavy metals in polluted soils (Ma et al., 2011). Some progress has been made towards an understanding of the serpentine microbial diversity and their beneficial role on plant growth and phytoremediation in artificially Ni polluted soils (Ma et al., 2009; Rajkumar et al., 2009), however, little is known about the role of serpentine bacterial isolates on the growth and heavy metal accumulation potential of plants in metal polluted field soils.

OBJECTIVES:

In this study using Ni rich serpentine soil (SS) as a model for metal polluted field soils, we assessed the effects ofinoculation of Ni resistant serpentine isolates *Pseudomonas* sp. A3R3 or *Psychrobacter* sp. SRS8 on the biomass production and Ni, Zn and Fe accumulation potential of *Brassica juncea* and *Ricinuscommunis* plants.

MATERIALS AND METHODS:

The surface sterilized seeds of *B. juncea* and R*. communis* were exposed to full sunlight during the day and allowed to germinate in sterilized non-contaminated garden soil (GS). For pot experiments, the soil collected from the serpentine area, Bragança, north-east of Portugal was mixed with GS at four different proportions: 0% SS (0% SS + 100% GS), 25% SS (25% SS + 75% GS), 50% SS (50% SS + 50% GS) and 75% SS (75% SS + 25% GS). The mixture was based on the dry weights of the soils. The endophytic bacteria *Pseudomonas* sp. A3R3 (GenBank accession no. GU550663), and the rhizobacteria *Psychrobacter* sp. SRS8 (accession no. FM205059) originally isolated respectively from root interior and rhizosphere of *A. serpyllifolium* grown in SS in Bragança, north-east of Portugal were used in this study. The inoculated and non-inoculated seedlings were transplanted into plastic pot containing 300 g of 0%, 25%, 50% or 75% SS. The plants were allowed to grow in a greenhouse. After 45 days, plants were harvested and separated into roots and shoots. The growth parameters such as root and shoot weight (fresh and dry) were determined. The metal contents (Ni, Zn and Fe) in the plant tissues were measured by atomic absorption spectrophotometery. Further, both total and diethylenetriaminepentaacetic acid (DTPA)-extractable fractions of heavy metals (Ni, Zn and Fe) in the soil were also measured. The metal concentration determinations in plant tissues and soils were used to estimate the translocation factor (TF) and the bioconcentration factor (BCF).

RESULTS AND DISCUSSION:

In pot experiment, regardless of inoculation with bacteria, the root and shoot biomass of both plant species decreased with increase of the proportion of SS. Inoculation of plants with bacteria significantly increased the plant biomass and the heavy metal accumulation in *B.juncea* and *R. communis* compared with non-inoculated control in the presence of different proportion of SS, which was attributed to the production of various plant growth promoting and/or metal mobilizing metabolites by bacteria. However, the rhizobacteria *Psychrobacter* sp. SRS8 showed a maximum increase in the biomass of the test plants grown even in the treatment of 75% SS. In turn, the endophytic bacteria *Pseudomonas* sp. A3R3 showed maximum effects on the accumulation of heavy metals in both plants (Table 1). Both *Pseudomonas* sp. A3R3 and *Psychrobacter* sp. SRS8 led to higher values of the Ni TF indicating that the inoculated PGPB played an important role on Ni

accumulation in plant shoots. Besides, inoculation of plants with *Pseudomonas* sp. A3R3 or *Psychrobacter* sp. SRS8 significantly increased the BCF of the metals (Ni, Zn and Fe) in both *B. juncea* and *R. communis* compared with the respective non-inoculated control. However, regardless of inoculation of bacteria and proportion of SS, both plant species exhibited low values of translocation and bioconcentration factors (<1) for Ni, Zn and Fe. This study demonstrates that the serpentine isolates *Pseudomonas* sp. A3R3 and *Psychrobacter* sp. SRS8 played an important role in promoting the plant growth and which could be used as inoculants to increase the rate ofrevegetation and phytostabilization by *B. juncea* and *R. communis* in metal contaminated soils.

Table 1 - Metal concentrations in shoots and roots of *B. juncea* and *R. communisgrown* in serpentine soils

Treatment		Shoot (mg kg $^{-1}$dw)			Root (mg kg $^{-1}$dw)		
Proportion of SS (%)	**PGPB strain**	**Ni**	**Zn**	**Fe**	**Ni**	**Zn**	**Fe**
B. juncea							
0	Control	2.2 ± 0.2 i	9.5 ± 0.6 h	67.0 ± 5.3 f	13.2 ± 1.3 e	17.8 ± 2.7 e	170 ± 23.3 f
	A3R3	4.4 ± 0.5 hi	10.1 ± 0.8 h	75.3 ± 4.5 f	14.9 ± 1.5 e	24.8 ± 4.5 e	196 ± 22.2 f
	SRS8	5.3 ± 0.3 h	11.1 ± 1.0 h	75.7 ± 7.3 f	17.2 ± 2.0 e	26.0 ± 3.9 e	215 ± 34.8 f
25	Control	29.7 ± 1.3 g	37.6 ± 1.9 g	86.0 ± 9.3 e	127 ± 6.5 d	138 ± 17.9 d	352 ± 32.4 e
	A3R3	34.0 ± 1.9 f	41.9 ± 4.6 f	109 ± 15.5 d	140 ± 2.1 c	176 ± 16.8 c	461 ± 51.8 d
	SRS8	39.7 ± 2.1 e	44.3 ± 5.1 f	117 ± 12.5 d	147 ± 8.3 c	189 ± 11.3 c	525 ± 37.3 d
50	Control	36.6 ± 2.0 ef	58.3 ± 3.4 e	115 ± 5.3 d	146 ± 6.5 c	177 ± 23.4 c	510 ± 42.9 d
	A3R3	39.7 ± 2.8 e	61.7 ± 5.2 de	135 ± 15.2 c	163 ± 14.3 b	219 ± 21.8 bc	640 ± 46.1 bc
	SRS8	42.8 ± 4.2 d	64.0 ± 5.5 d	198 ± 11.0 b	167 ± 6.5 b	232 ± 26.8 bc	667 ± 43.5 bc
75	Control	46.7 ± 1.7 c	72.3 ± 6.9 c	188 ± 17.8 b	173 ± 8.3 b	261 ± 12.7 b	615 ± 44.0 c
	A3R3	50.0 ± 1.9 b	83.3 ± 8.0 b	234 ± 15.2 a	187 ± 15.0 ab	287 ± 35.2 ab	706 ± 6.4 ab
	SRS8	54.4 ± 4.1 a	90.6 ± 5.1 a	244 ± 25.0 a	193 ± 13.1 a	304 ± 35.2 a	733 ± 86.8 a
R. communis							
0	Control	1.7 ± 0.4 e	11.5 ± 0.8 f	26.4 ± 4.2 e	9.5 ± 0.7 f	28.5 ± 1.6 f	95.3 ± 3.1 g
	A3R3	3.0 ± 0.9 e	13.1 ± 1.0 f	34.1 ± 3.8 e	12.7 ± 0.8 f	40.0 ± 3.2 f	116 ± 8.6 g
	SRS8	3.3 ± 0.7 e	14.5 ± 0.9 f	33.5 ± 4.2 e	14.2 ± 0.8 f	44.3 ± 5.4 f	105 ± 9.2 g
25	Control	13.2 ± 1.2 d	23.9 ± 3.3 e	46.9 ± 3.9 d	90.4 ± 6.9 e	184.5 ± 8.2 e	157 ± 11.5 f
	A3R3	19.8 ± 3.4 c	34.3 ± 4.1 d	56.3 ± 5.9 c	109 ± 8.1 d	220 ± 24.8 d	187 ± 7.2 e
	SRS8	21.5 ± 3.4 c	35.9 ± 3.0 d	57.9 ± 5.0 c	112 ± 14.2 d	234 ± 5.4 d	205 ± 10.9 de
50	Control	22.1 ± 4.0 c	41.3 ± 4.9 cd	58.9 ± 7.9 c	113 ± 12.3 cd	219 ± 12.5 d	224 ± 10.1 d
	A3R3	29.2 ± 1.7 b	51.1 ± 4.0 b	70.0 ± 8.6 b	127 ± 4.9 bc	283 ± 16.6 c	265 ± 22.4 c
	SRS8	30.1 ± 2.9 b	53.2 ± 3.8 b	72.5 ± 6.6 b	129 ± 11.4 b	329 ± 18.5 b	273 ± 13.7 c
75	Control	31.4 ± 4.3 b	52.4 ± 4.7 b	75.0 ± 5.8 b	133 ± 18.2 b	287 ± 22.1 c	316 ± 21.9 b
	A3R3	38.9 ± 3.7 a	60.4 ± 8.4 a	92.0 ± 6.0 a	147 ± 9.4 ab	344 ± 16.0 ab	379 ± 20.3 a
	SRS8	40.5 ± 15.2 a	61.7 ± 8.2 a	93.9 ± 10.8 a	159 ± 9.6 a	358 ± 13.7 a	393 ± 12.0 a

Average ± standard deviation from three samples. Each value is the mean of triplicates.Data of columns indexed by the same letter are not significantly different according to Fisher' s protected LSD test (p< 0.05).

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Assessment of students' attitudes and environmental behavior at School of Allied Health Sciences of Polytechnic Institute of Porto

Authors: MafaldaNunes[1], MafaldaAguiar[1]

1 *Research Center on Health and Environment, School of Allied Health Sciences of Polytechnic Institute of Porto, Portugal*

Presenting Author: Email: mmn@estsp.ipp.pt | Tel.: +351 222 092 130 | Fax: +351 222 061 001

INTRODUCTION:

In recent decades, the sustainability in higher education institutions has become an important issue for these institutions, as they realised their impacts in the environment and became more aware of their responsibilities to the society. Sustainability also presents an opportunity to promote new approaches in education such as problem based learning, more interdisciplinary and applied learning (Corcoran et al., 2004).

As one of the key elements in these institutions, students play an important role, and their attitudes and environmental behaviour have a strong impact in the sustainability of the institutions and, as stated by Zsóka et al. (2013),students who behave in a consistently pro-environmental manner are drivers of a more sustainable future. It is crucial to know the students attitudes and environmental behaviour in order to promote, for example, effective environmental education.

OBJECTIVES:

Assess the attitudes and environmental behaviour of the students of Allied Health Sciences School of Polytechnic Institute of Porto and to verify if there were changes after the implementation of an environmental education program; to evaluate if there are differences between the attitudes and environmental behaviour of the students attending Environmental Health´s course degree and the remaining students of the institution.

MATERIALS AND METHODS:

The questionnaire was applied to 327 students, attending six courses degrees. These were chosen randomly with the exception of Environmental Health (EH) course. The same questionnaire was delivered to the same students in June 2013 and March 2014. The identification of the students was required but the confidentiality of the answers was ensured. In the period of time between the first and second administration of the questionnaire, in the School of Allied Health Sciences of Polytechnic Institute of Porto, took place several initiatives of environmental education, directed to the students. The data obtained by the first administration of the questionnaire were used to compare the Environmental Health students with the students of other course degrees.

The questionnaire was based on the audit of Eco-Schools Portuguese program of the Foundation for Environmental Education and consisted of thirty-eight questions, concerning attitudes and behaviours related with four environmental issues: waste, water, energy and transportation.Respondents were offered a choice of five pre-coded responses (Likert scale). For each respondent, a score was created with the sum of the values of each selected option and it was converted to a percentage (100%corresponding to the most environmental sustainable attitudes and behaviors).

Statistical analysis of the data was performed using SPSS Statistics 21 software. To verify if there were differences in the attitudes and environmental behaviours between the first and second administration of the questionnaire, it was used the paired sample t-test. The differences between the students of Environmental

Health course and the remaining 5 courses in analysis were checked by t-test for independent samples. For all tests it was assumed a significance level of 95% (α = 0.05).

RESULTS AND DISCUSSION:

The mean results, of the globality of the students, before and after the development of the environmental education program, are presented in Table 1.

Table 1 - Evolution of the sustainability of student's attitudes and behaviours

	Waste	Water	Energy	Transportation
June 2013	71%	76%	76%	53%
March 2014	72%	81%	83%	61%

The student's attitudes and behaviours were especially suitable in the matters water and energy and lower, although they can still be considered good, on the theme waste. Regarding the results concerning transportation, the deficit of public transports or unsuitable schedules could explain the lower percentages, namely the preference by the use of private vehicles. Comparing the results obtained before and after the implementation of an environmental education program, although there were improves in student's performance in all of the issues analysed, the modifications between the first and the second responses were not statistically significant.

The difference of attitudes and behaviours of the students of Environmental Health course and the students attending other courses is presented in Table 2.

Table 2 - Comparison of sustainability of EH student's attitudes and behaviours with students of other courses

	Waste	Water	Energy	Transport
EH	75%	76%	75%	50%
Other courses	69%	78%	77%	55%

The differences obtained were not statistically significant. This result was unexpected, since the Environmental Health course has the sustainability issues integrated in the curriculum and it was predictable a better performance from these students. But, as stated by Vicente-Molina et al. (20013), informal environmental education through the media, the Internet or social interaction with the family and friends can be determinant for students from other courses to acquire pro-environmental attitudes.

CONCLUSION:

The students' attitudes and environmental behavior at the School of Allied Health Sciences of Polytechnic Institute of Porto, in general, present a high valuation. However, the statistical analysis revealed there were no significant statistical differences before and after the implementation of the environmental education program and the between the students attending the course of Environmental Health and the remaining courses in analysis. These features lead to a challenge: to create an environmental education program that further compels the students to behave in a more sustainable way.

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Biodiversity of microorganisms in activated sludge in relation to some abiotic environmental factors

Authors: Ana-Marija Ladiš[1], Lana Feher-Turković[1], Ana Mojsović Ćuić[1]
Translated by: Martina Klanjčić[1]

1 *University of Applied Health Studies, Mlinarska cesta 38, Zagreb, Croatia*

Presenting Author: Email: ana.marija.ladis@gmail.com | Tel.: +385 91 769 3988

INTRODUCTION:

In the process of wastewater treatment the key factor is the effectiveness of activated sludge. Microorganism biodiversity in the activated sludge increases its efficacy which is a changing variable due to the constantly changing organic load of wastewater. The constant injection of atmospheric air into the wastewater creates equilibrium aerobic conditions for the process of digestion in the second stage of purification. Abiotic environmental factors, which are also changing variables, are an important component of the environment in which activated sludge and its biological diversity operate.

OBJECTIVES:

The aim of the study was to determine to what extent the O_2 and temperature, affects the biological diversity of activated sludge and it' s endurance due to constantly changing income water and organic load.

MATERIALS AND METHODS:

The study was conducted on the wastewater treatment unit in Zagrebačke otpadne vode d.o.o. (Zagreb Wastewater Treatment Plant, Ltd.), in the period of 2012 by monthly collection and microscopic analysis (Figure 1) of the samples of activated sludge, daily monitoring of temperature, O_2 of incoming and outcoming water.

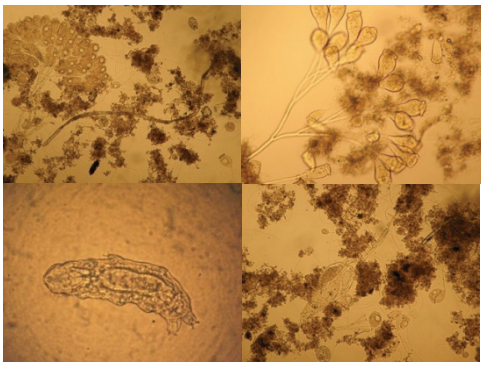


Figure 1 - Microscopic preparation of activated sludge, 400 X ZOV u. i p.

RESULTS AND DISCUSSION:

Average monthly temperatures in 2012 from January to December ranged from the lowest -1.7 ° C in February, up to a maximum of 23.95 ° C in August, also the lowest O_2 of incoming and outcoming water were in August. The highest O_2 of incoming water was in December, while the highest O_2 outcomig of water was in January (Table1).

Table 1 - Average monthly air temperature (MHS, 2012) , O2 and flow rate for incoming and outcoming water 2012

Year 2012			
Month	Average monthly temperatures of air ° C	O_2 mg/L	
		Incoming water	Outcoming water
January	2,75	2,8	9,81
February	-1,7	3,7	9,62
March	9,8	1,5	9,64
April	12,7	1,5	9,47
May	16,5	0,8	9,38
June	21,65	0,7	9,34
July	23,75	0,6	8,41
August	23,95	0,4	8,32
September	18,4	1,0	8,54
October	12,4	1,9	8,65
November	9,45	2,5	8,73
December	1,5	3,9	8,91

The records of microorganism were taken during identification of the microorganisms present in activated sludge in 2012, and the graph (Figure 2) shows the biodiversity of all recorded microorganisms in relation to the average monthly mean temperature of incoming and outcoming water, O_2 of incoming and outcoming water and atmospheric air for 2012. The graph shows the increase of biodiversity with the increase of temperature; therefore the increase in biodiversity can clearly be seen in July and August compared to the previous month of June, and unexpectedly high levels of biodiversity can be noted in January and February of the same year. According to previous research, the effect of temperature on the sludge volume index reveals that with the temperature increase from 15 °C to 35 °C, the index increased from 40 ml/g to 130 ml/g (Ghanizadeh 2001). High levels of O_2 in January and February can be explained with the increase of O_2 in incoming water, which were the highest that year. In January the level of O_2 was 9.81 mg/L and in February was 9.62 mg/L, compared to the months of July and August, when the level was 8,32 mg/L in August and 8,41 in July. It is important to note that there isn't a single method that lead to the microbiological target; combination of different methods can reach the target from different directions and can be very useful (Chalasani 2007). In one of the studies it was found that the absorption of oxygen in the activated sludge increases with temperature, and the results indicate that the absorption of oxygen reflects the activity of activated sludge microorganisms (Mathur 2007).

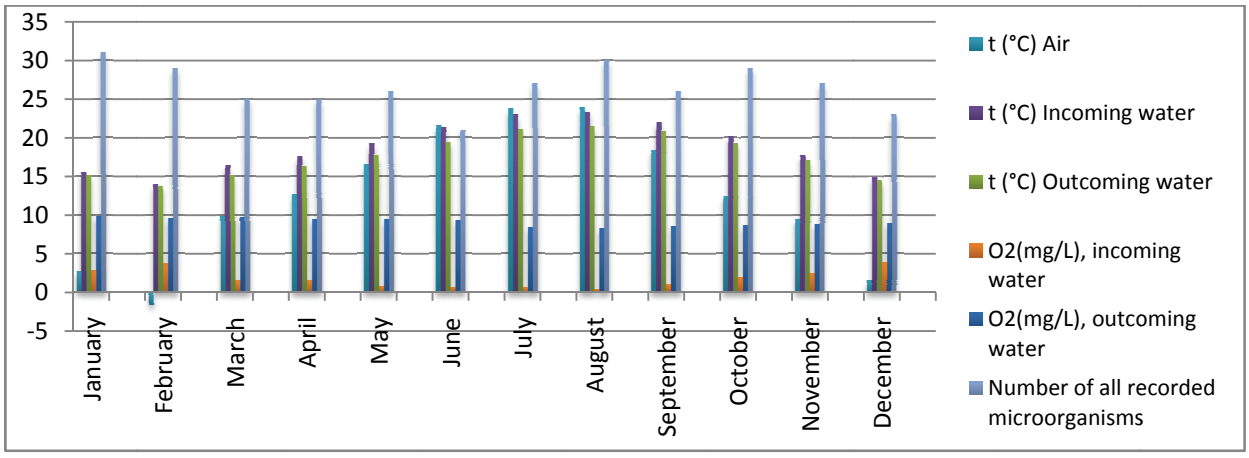


Figure 2 - Biodiversity of microorganisms in activated sludge in relation to the average monthly mean temperature of incoming and outcoming water, O_2 of incoming and outcoming water and atmospheric air, 2012

CONCLUSION:

The results suggest a correlation between temperature, O_2 of incoming and outcoming water and atmospheric air as an abiotic environmental factors and biodiversity of activated sludge organisms, as key factors in the effectiveness and usability of the sludge in the wastewater treatment systems. Research has also revealed

an unexpectedly high biodiversity in January and February, suggesting the need for further investigation of activated sludge organisms and the possible impact on the biodiversity.

ACKNOWLEDGMENTS:
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Application of different extraction methods on the determination of pharmaceutical contaminants in soil and plant samples

Authors: Carmen Lidia Chitescu[*1], AncaIoana Nicolau[1], Alida Adriana Maria (Linda) Stolker[2]

1 *University Dunarea de Jos of Galați, Faculty of Food Science and Engineering, Str. Domnească 47, 800008 Galați, Romania*
2 *RIKILT - Wageningen University and Research, P.O. Box 230; 6700 AE Wageningen, The Netherlands*

Presenting Author: Email: carmen.chitescu@ugal.ro | Tel.: +40 728 040 255 | Fax: +40 236 460 165

INTRODUCTION:
Different classes of pharmaceutical compounds are used in agricultural livestock. Manure application has been recognized as a source of veterinary drug contamination of soils and water (Kemper, 2008).Soil contamination can lead to resistance of bacteria and fungi, to qualitative and quantitative effects on resident microbial soil populations (Anderson, 2005), disturbances in soil functioning (Vaclavik, 2004), or to plants growth inhibition (Liu, 2009).

In order to establish post-marketing control mechanisms, and risk management, monitoring programs for environment contamination including pharmaceuticals and fungicides are needed. Therefore, there is a need for sensitive and selective analytical methods applicable to test for a broad range of compounds.

OBJECTIVES:
The present study focuses on developing a multimethod for the simultaneous determination of forty two compounds, including pharmaceuticals, antifungal drugs, biocides and fungicides, in soil and plant material samples.

MATERIALS AND METHODS:
Different extraction condition and different solvents were tested comparing ultrasonic assisted method against accelerating solvent extraction (ASE). An SPE procedure was applied in order to purify and concentrate the samples extract. The analyses were performed in one single full scan MS method, use U-HPLC-ExactiveOrbitrap MS at 50,000 (FWHM - full width at half maximum) resolution. The recovery, detection limit and matrix effects were evaluated together to determine the performance of the method. Detection was based on calculated exact mass and on retention time of target compounds. The optimized method was validated and applied to a set of ten different soil samples and ten plant material samples (grass and corn crop) collected from agricultural areas within the Netherlands.

RESULTS AND DISCUSSION:
Although both extraction approaches are applicable, the optimal extraction efficiency was obtained by applying accelerated solvent extraction (ASE). Organic solvents in combination with 0.2M citric acid/ACN, acetone, MeOH (50:50) were tested on soil and fresh plant sample, in different extraction conditions (temperature, pressure, duration). Please see Figure 1.

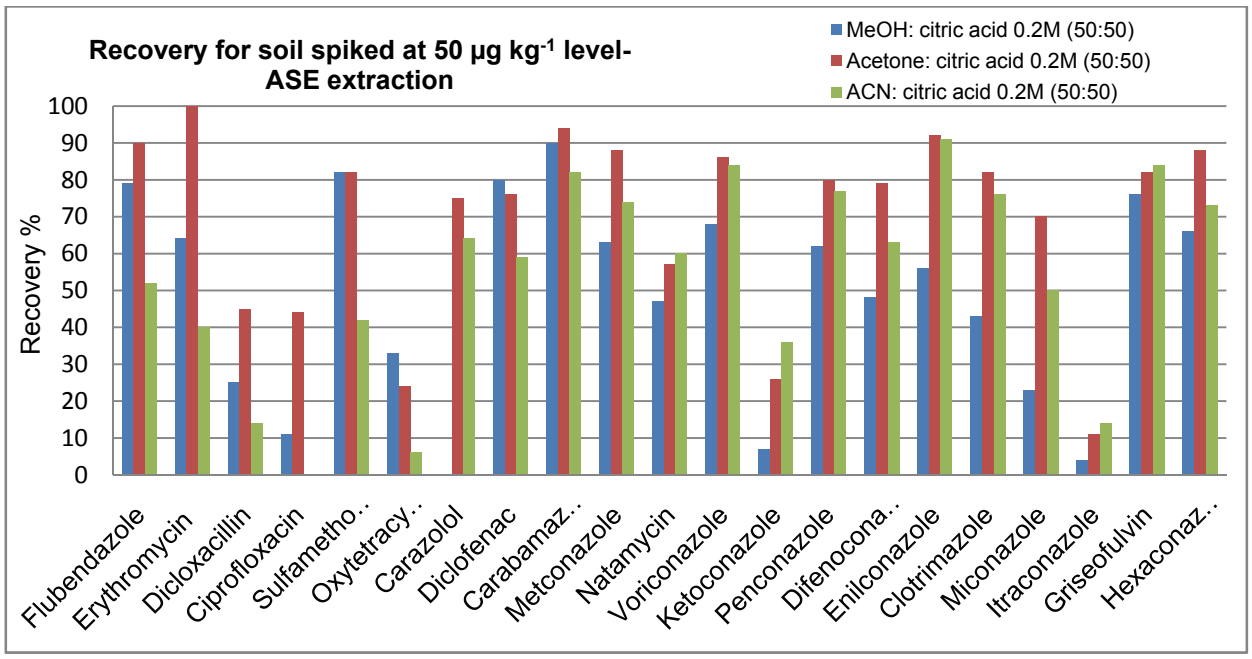


Figure 1 - Recovery for twenty-one of forty-two analysed compounds in case of different organic solvents (ACN, acetone, MeOH) mixtures with citric acid 0.2M, in soil samples, by ASE.

The recovery of analytes for soil matrix in the acetone mixture was between 70-100% for 68% of the compound, between 40-70% for 21% of the compounds and < 40% for 11% of the compounds. Low recovery was obtained for oxytetracycline: 33% in methanol, 24% in acetone and 6% in acetonitrile. All compounds were detected: 93% of them at 10 µg kg^{-1} level and 7% at 50 µg kg^{-1} level.

For plant samples, methanol proved to be the best choice. The recoveries were >70% for 64% of the compounds tested. Oxytetracycline recovery was at an acceptable value of 53%. A proportion of 66% of the compounds were detected at 10 µg kg^{-1}, and 28% of the compounds at 50 µg kg^{-1} level.

Among the extraction solvents tested, it appeared that higher extraction efficiencies were achieved for the mixtures containing acetone/citric acid 0.2M (50:50) in case of soil sample, and methanol/citric acid 0.2M (50:50) in case of grass sample.

The validation procedures were based on the approach of Guideline of Screening Methods of Residues of Veterinary Medicines, for Community Reference Laboratories Residues 20/1/2010.

New tendency in mass spectrometry analysis, especially with regard to environmental monitoring programs, is post target and untargeted analysis, which offer the possibility of retrospective analysis of full-scan data, enabling laboratories to search for “unknown” contaminants after data recording. In this experiment analyses were carried out by full-scan-mass spectrometric detection using ExacteOrbitrap technology (FWHM 50,000). Powerful resolution of the Exactive was a very important parameter, in combination with mass accuracy, for significant sensitivity and selectivity improvement. Superior chromatographic resolution was provided by UHPLC system.

Average mass accuracy in this experiment ranged within 0.6-3 ppm with a standard deviation that ranged within 0.3 -3.5.The detection capability CCβ was lower than 50 µg l^{-1} (for β error 5%) for all analyzed compounds.

CONCLUSION:

The method was applied to real-world sample material. Oxytetracycline was detected in soil samples, indicates that farming activities affect the contamination of the area by pharmaceutical compounds.

ACKNOWLEDGMENTS:

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Isolation of Amino Acids after High-Risk Bio-Waste Processing by Alkaline Hydrolysis

Authors: Sanja Kalambura[1], Aleksandar Racz[2], Darko KIŠ[3]

1 *University of Applied Sciences Velika Gorica, Velika Gorica, Croatia*
2 *University of Applied Health Studies, Zagreb, Croatia*
3 *Faculty of Agriculture, J. J. Strossmayer University of Osijek, Osijek, Croatia*

Presenting Author: Email: sanja.kalambura@vvg.hr | Tel.: +385 915 107 400

INTRODUCTION:

Increasing waste volumes are a grave burden to modern civilisation and the reduction of their quantity must be set as a priority. The volume of generated slaughterhouse waste is also alarmingly increasing. Each year, millions of tons of high-risk slaughterhouse waste are disposed of or treated worldwide (Garcia & Rosentrater, 2006). Greater re-usage and recycling, among other things, requires the application of new technologies (Gwythner, Williams, Golyshin, Edwards-Jones & Jones, 2011). The development of new technologies for an efficient use of high-risk materials has therefore become an important aspect for integrated waste management. As an alternative method for the management of animal by-products, alkaline hydrolysis offers itself as a solution for the disposal of high-risk biodegradable waste and opens up new horizons regarding highly profitable and environmentally acceptable technologies. Alkaline hydrolysis is particularly applied in the treatment of contaminated K1 and K2 category tissues.

OBJECTIVES:

The objective of this paper was to determine the amino acid content in hydrolysed material following the alkaline hydrolysis of high risk slaughterhouse waste in the form of animal remains.

MATERIALS AND METHODS:

Brain samples from oxen younger than 30 months were used. The laboratory reactor case was made of stainless steel, 175 mm in height and 165 mm in diameter and hydrolysis was conducted under the following selected testing conditions: Sodium hydroxide (NaOH) and Potassium hydroxide (KOH), hydrolysation duration t_1=2 h, t_2=3 h, and t_3=6 h; temperature ϑ_1=135 °C, ϑ_2=150 °C, and ϑ_3=153 °C; and pressure p_1=2.75 x 10^3 hPa , p_2=4.78 x 10^3 hPa, and p_3=5.20 x 10^3 hPa. For each treatment, measurements were carried out in three repetitions. Hydrolysis was carried out in closed ampules under nitrogen atmosphere at 110 °C for 24 h (Imai, Fukushima, Homma, Homase, Sakai & Kato, 2006) . Ionic exchange chromatography analyses were carried out on an amino acid analyser. In accordance with the randomly selected experimental design (three repetitions), ANOVA and Student t-test (LSD) were carried out (three repetitions) in order to establish the significance of the differences among the studied factors (temperature, time, and base), and also within their combination (after the proven importance of interaction), using commercial software SAS 9.1® (SAS Institute Inc., Cary, NC, USA). The values are expressed as arithmetic mean±SD of three replications. *P*-values lower than 0.05 obtained by F-test in ANOVA or LSD were considered statistically significant.

RESULTS AND DISCUSSION:

As a result of the treatments, the concentration of individual amino acids in samples increased compared to the initial materials. The reason for this lay in the fact that, during the treatments, the samples lost a certain amount of solvent thus becoming more concentrated. Protein content in the hydrolysates was relatively low. Comparing the values obtained by the joint activity of all the factors: temperature, alkali, and time the protein content was not significant and in reaction with NaOH the highest value of protein was achieved at 135 °C and 2 h, whereas with KOH the highest obtained value was achieved at 150 °C and 3 h. Dry matter amino acid content in the analysed samples was generally very low. The results of this study suggest an identical effect even at lower temperatures than the standard one (150 °C), which is visible from the values of the Amino

acids such as asparagine, glutamic acid, glycine, alanine and leucine achieved higher concentrations. Regarding the contents of other amino acids; arginine has experienced certain degradation; threonine appears only in traces, whereas serine was present in a very low concentration. The concentrations of cysteine, methionine and tyrosine were also low. Regarding interactions between the duration of hydrolysis and temperature the presented results suggest also the achievement of the same effect even at lower temperatures than the standard one. It should be noted that the isolation of some amino acids was possible because they showed higher concentrations in the hydrolysates. The occurrence of D-iso leucine in the analysed samples indicated that the largest amount of amino acids was most likely racemizated during alkaline hydrolysis (Loki, Kalambura, Mandoki, Varga-Vasi, Albert & Csapo, 2010). Results showed that lysine had higher resistance to the alkali medium than other amino acids.

CONCLUSION:

With these investigations we can open new possibilities for using high risk material for amino acid production after the alkaline process. Investigate environmentally suitable and publicly acceptable options for hydrolysates disposal is one of biggest problems for this treatment. This investigation gives as an answer that is possible to use this material for amino acid isolation. Based on the present investigation we can conclude that the analysed amino acid composition of the hydrolysed material at different testing conditions showed successful hydrolysis for all of the samples. Amino acids appear in small amounts and they are mostly racemizated but still we can focus on some specific one. These results will also help in terms how to use alkaline hydrolysis technology to accommodate large amounts of hydrolysates.

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Integrated use of mineral and bio fertilizers for increasing profitability of wheat and reduce environmental pollution.

Authors: Essam A. Abd El-Lattief

Agronomy Department, Faculty of Agriculture, South Valley University, Qena, Egypt

Presenting Author: Email: essamelhady2@gmail.com

INTRODUCTION:

The high cost of chemical fertilizers and the low purchasing power of most of the farmers restrict its use in proper amounts, hampering crop production. Besides, a substantial amount of the nitrogen is lost through different mechanisms including ammonia volatilisation, denitrification and leaching losses, causing environmental pollution problems (Choudhury and Kennedy, 2005). Now, increased attention is being paid to develop an integrated plant nutrition system that maintains and enhances soil productivity through balanced use of different sources of nutrients, including chemical fertilizers, organic fertilizers and biofertilizers. Also, microbial inoculants could be used as an economic input to increase crop productivity, lowering fertilizer doses and more nutrients increasing harvested from the soil (Jen-Hshuan, 2006). Biofertilizers may help in improving crop productivity by increasing the biological nitrogen fixation, the availability and uptake of nutrients and release of natural hormones (Kannaiyan, 2002). Free-living nitrogen-fixing bacteria *eg Azotobacter chroococcum* and *Azospirillum lipoferum,* were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis (Fayez *et al,* 1985). Bahrani *et al,* (2010) found that a remarkable stimulation on wheat growth and grain yield with biofertilization.

OBJECTIVES:

The present work was aimed to use biofertilizer (*Azotobacter)* application as an alternative to reduce chemical fertilizers and to maximize the productivity and profitability of wheat at newly reclaimed sandy soils as well as reduce environmental pollution.

MATERIALS AND METHODS:

The investigation was carried out at the experimental farm of the Faculty of Agriculture, South Valley University at Qena Governorate, Egypt, during two seasons (2010-11 and 2011-12). The farm is located at an altitude of 79 m above mean sea level and is intersected by 26°10′ N latitude and 32°43′ E longitude. The soil of the experimental site is sandy throughout its profile (74.7% coarse sand, 15.8% fine sand, 5.3% silt and 4.2% clay). Its pH value of 7.72, 2.75 EC (dSm^{-1}), 0.48% organic matter content, 0.27% total N, and available P and K of 7.62 and 165 ppm, respectively. The dose of mineral NPK (220: 52: 120 kg N ha^{-1}) was manipulated at various levels in combination with different biofertilizers as per the treatment schedule. The different treatment combination as follows:

T_1- 100% mineral NPK (MNPK) and uninoculated, T_2- *Azotobacter* (AZO) alone, T_3- 25 % MNPK + AZO, T_4- 50 % MNPK + AZO, T_5- 75 % MNPK + AZO, T_6- Control (without MNPK and uninoculated). The seeds were inoculated by liquid culture of locally isolated strains of *Azotobacter chroococcum* (≈10^9 CFU/ml) which obtained from Biofertilizers Production Unit of Faculty of Agriculture, South Valley University. 1% of carboxy methyl cellulose (CMC) was added to the culture to increase its viscosity to gel form to act as adhesive biostabilizer, the addition of CMC was made just before using. The experiment was carried out in a randomized block design with three replications. Experimental unit measured 3.0 m in width and 4 m in length. At harvest time, grain and straw yields were estimated at plot basis. The data were analyzed by analysis of variance (ANOVA) using MSTAT-C statistical software. Treatment means were compared using Duncan's multiple tests. Since data followed the homogeneity test, pooling was carried out over the seasons and mean data are given.

RESULTS AND DISCUSSION:

The effect of treatments on grain and straw yields were significant (Table 1). Means in Table 1 indicates that superiority of grain and straw yields were achieved by application of T_4 (50% NPK + biofertilizer with *Azotobacter*) or T_5 (75% NPK + biofertilizer with *Azotobacter*) compared with other treatments. There is no significant difference between T_4 and T_5 on grain and straw yields. The highest grain yield (5.240 and 5.265 tons / ha) and straw yield (6.700 and 6.850 tons / ha) were obtained from T_4 and T_5, respectively. The minimum grain and straw yields of 3.144 and 4.200 tons / ha, respectively, were obtained from the treatment of T_6 (control). Such increase in yields (grain and straw), due to application of T_4 or T_5, might be due to the role of biofertilizer (*Azotobacter*) in enhancing soil biological activity, which improved nutrient mobilization from organic and chemical sources. Also, the biofertilizer plays a significant role in regulating the dynamics of organic matter decomposition and the availability of plant nutrients and in increasing nitrogen fixer. These results are in concordance with most similar previous studies (Jen-Hshuan, 2006, Bahrani *et al.,* 2010).

It is evident from the results in Table2 that the maximum net profit per ha of 8842 L.E. was obtained from treatment T_4, followed by T_5 of 8581 L.E. Also, the highest value of return-cost ratio (2.01) was obtained by the application of T_4. The highest return and net profit values observed in the T_4 or T_5 treatments can be attributed to the increases in grain and straw yields produced per unit area under these treatments (Table 1).

CONCLUSION:

The use of biofertilizers became inescapable to minimize the environmental pollution, caused by the chemical ones, and to improve the yield quality of various crops needed at the time being. Although 25 or 50 % of mineral NPK was replaced by biofertilizers (*Azotobacter*), the yield as well as return and net profit values per ha of wheat increased compared to that obtained with the recommended dose of mineral NPK.

Table 1 - Effect of integrated fertilization between mineral NPK and biofertilizers on grain yield and straw yieldof wheat (data over two seasons).

Treatments	Grain yield (ton/ha)	Straw yield (ton/ha)
T_1- Recommended NPK (MNPK)	5.006 b	6.445 b
T_2- Biofertilizer (*Azotobacter*) (AZO)	4.150 d	5.550 d
T_3- 25% MNPK + ASO	4.775 c	6.145 c
T_4- 50% MNPK + ASO	5.240 a	6.700 a
T_5- 75% MNPK + ASO	5.265 a	6.850 a
T_6- Control (without NPK and Uninoculated	3.144 e	4.220 e

The same letters within columns means not significant differences at 5% level.

Table 3 - Some economics of wheat productivity per ha at various fertilization treatments (data over two seasons).

Treatments	Total costs (L.E*/ha)	Return (L.E/ha)			Net profit (L.E/ha)	Return-cost ratio
		Grain	Straw	Total		
T_1- Recommended NPK (MNPK)	9500	11679	5156	16844	7344	1.77
T_2- Biofertilizer (*Azotobacter*) (AZO)	7865	9682	4440	14122	6257	1.80
T_3- 25% MNPK + ASO	8303	11140	4916	16056	7753	1.93
T_4- 50% MNPK + ASO	8743	12225	5360	17585	8842	2.01
T_5- 75% MNPK + ASO	9182	12283	5480	17763	8581	1.93
T_6- Control (without NPK and Uninoculated	7745	7335	3376	10711	2966	1.38

*1 L.E. (One Egyptian pound) = $ 0.164

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Effects of copper oxychloride on the histology of earthworm Coelomocytes

Authors: Volkan Kılıç[1], Gözde Aydoğan Kılıç[1]

1 *Anadolu University, Faculty of Science, Department of Biology, 26470, Eskişehir, Turkey*

Presenting Author: Email: vkilic1@anadolu.edu.tr | Tel.: +90 222 3350580 4712 | Fax: +90 222 3204910

INTRODUCTION:

Environmental contamination of soils by copper containing agrochemicals is a current problem. Copper oxychloride-based fungicides are the most widely used agents as foliar sprays against fungal diseases in a number of crops. Earthworms are keystone species within ecosystems since they play a major role in physical, chemical and biological processes in the soil [Helling et al., 2000].The coelomic fluid of earthworms has functions in pollutant disposition and tissue distribution while coelomocytes are responsible for the immunedefense of the animal. Any impairment of coelomocyte functioning can compromise the health of the entire organism.

OBJECTIVES:

Coelomocytes can give selective responses to certain environmental contaminants. However there are very limited studies on the use of histological features of earthworm coelomocytes as biomarker of exposure to the environmental contaminants [Calisi et al., 2009]. Therefore the present study aims to investigate the cellular responses of the earthworm (*Aporrectodea caliginosa*) coelomocytes as a result of copper oxychloride exposure at light microscopic level.

MATERIALS AND METHODS:

Samples consisted of control and experimental group animals. They were exposed to 35 and 100 mg/kg (artificial soil) doses of copper oxychloride during 7 days. Healthy worms weighing between 300-400 mg each were left on filter paper in petri dishes for 24 h in order to reduce the soil content in their gut. Non-invasive extrusion technique of Eyambe et al., (1991) was used with slight modifications for collecting the earthworm coelomocytes. Freshly isolated *cell suspension* was then *dropped onto* specimen *slides*. Samples were stained with toluidin blue after ethanol (95 %) fixation.

RESULTS AND DISCUSSION:

Granulocytes and ameobocytes did not show any significant alterations while the most significant changes were observed in eleocytes such as swelling and deformation of cells at the dose of 100 mg/kg. This response of eleocytes was thought to be a result of their putative function which is to synthesize extracellular respiratory pigments responsible of attracting exogenous particles such as heavy metals including Cu [Adamowicz, 2005].

CONCLUSION:

The results manifested that eleocytes give selective responses to copper oxychloride toxicity. Besides observation of isolated earthworm coelomocytes with simple histological staining can be a primary and effective way for the evaluation of structural changes as a result of environmental exposure.

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Histopathological alterations in the body wall and alimentary canal of earthworms as biomarker of copper oxychloride toxicity

Authors: Gözde Aydoğan Kılıç[1], Volkan Kılıç[1]

1 *Anadolu University, Faculty of Science, Department of Biology, 26470, Eskişehir, Turkey*

Presenting Author: Email: gozdea@anadolu.edu.tr | Tel.: +90 222 3350580 4712 | Fax: +90 222 3204910

INTRODUCTION:

Copper oxychloride is a broad-spectrum fungucide applied to the foliage of a wide variety of fruits and vegetables. [Synman et al. 2005]. It is sprayed directly on crops and may contaminate soils. Since its general toxicity is considered to be low, there are only a few studies about the toxicity of this substance on soil fauna [Helling et al. 2000]. However it has been shown to reduce populations of some earthworm species in field trials [Maebota et al. 2003].Earthworms are continuously exposed to soil chemicals through their alimentary surfaces and skins. Because of the capacity to accumulate and concentrate large quantities of inorganic and organic pollutants, they are widely recognized as suitable organisms for biomonitoring the effects of heavy metals and other chemicals in contaminated soils. Histopathological alterations in earthworms have been reported as valuable markers of heavy metal toxicity in previous studies which may signal a prior or ongoing damaging effect on these organisms [Reddy and Rao 2008, Kılıç 2011].

OBJECTIVES:

The present study focused on the histopathological responses in body wall and alimentary canal of earthworm (*Aporrectodea caliginosa*) resulted from copper oxychloride exposure.

MATERIALS AND METHODS:

Samples consisted of control and experimental group animals which were exposed to different doses of copper oxychloride (35, 100, 350 mg/kg artificial soil) during 2,7 and 14 days of periods. Healthy worms weighing between 300-400 mg each were left on filter paper in petri dishes for 24 h in order to reduce the soil content in their gut. Worms were cut into 0.5 cm pieces and were fixed in paraformaldehyde(4%) in phosphate buffer pH 7.2. They were dehydrated in a graded series of ethanol and treated with a mixture of London Resin (LR) White and ethanol (2:1) (v:v) for 1 h at room temperature. Samples were then embedded in LR White, sectioned at 700 nm thickness, stained with toluidin blue and observed under a Leica DM 750 bright field microscope.

RESULTS AND DISCUSSION:

Earthworm skin is extremely permeable to water and it represents a main route for contaminant uptake. Thus, primary lesions were observed in earthworm skin as changes which were observed at the doses of 100 and 350 mg/kg and after the periods of 7 and 14 days. The intensity of pathology was increasing depending on exposure time. The lesions observed after 7 days of exposure at 100 mg/kg were enlargement and torsion of epithelial cell lining, hyperplasia of glandular cells and increased mucus production which are characterized as an early result of contact with pollutants. Deformation of muscular structure were accompanying these lesions at 350 mg/ kg at the end of 7 days. After 14 days of exposure, intestinal epithelium and chloragogenous cells lost their intact nature both at the doses of 100 and 350 mg/kg. Disruption of cellular compartmentation and atrophy of the intestinal epithelium were also observed. There was an increase in intestinal mucus production which may be a result of its function to play role in breakdown of organic macromolecules. Necrosis was observed at the dose of 350 mg/kg after 14 days in epidermal and intestinal tissues of animals.

CONCLUSION:

The study showed that copper oxychloride exposure may result in a crucial toxicity inbioindicator organism *Aporrectodea calliginosa* depending on the dose and duration of exposure leading to significant deformation of tissues in vital parts of their body.

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Arbuscular mycorrhizal fungi can improve the growth of aromatic plants and prevent soil erosion after forest fires

Authors: Beatriz Duarte[1], Sara Soares[1], Rui S. Oliveira[1,2,3]

1 *Department of Environmental Health, School of Allied Health Sciences of Polytechnic Institute of Porto, Portugal*
2 *Research Centre on Health and Environment, School of Allied Health Sciences, Polytechnic Institute of Porto, Portugal*
3 *Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Portugal*

Presenting Author: Email: bia19_01@hotmail.com / saracrs_4906@hotmail.com | Tel.: +351 912 848 313

INTRODUCTION:

Forest fires are regarded as one of the major environmental problems in Portugal. Among their negative impacts is loss of green area and soil structure damage, which increase soil erodibility. Adequate soil structure is essential to support plant and animal life and to moderate environmental quality with particular emphasis on soil carbon sequestration and water quality (Certini, 2005). After a forest fire the ecosystem has the capacity to recover through ecological succession. This is, however, a slow process and there is the need for human intervention in order to mitigate the negative impacts of forest fires. Aromatic plants, such as rosemary, are among the first to grow in post-fire Mediterranean forest ecosystems (Luis et al., 2006). This together with their commercial value makes these plants attractive candidates to be used in the first stages of restoration of burned forest soils. Arbuscular mycorrhizal fungi (AMF) are a group of soil microorganisms that forms mutualistic symbioses that can benefit plants by improving the uptake of mineral nutrients, mineralising organic nutrients and conferring drought resistance (Oliveira et al., 2005).AMF have also the capacity to promote soil aggregation by several mechanisms including the release of soil binding agents.

OBJECTIVES:

The aim of this study was to assess the effect of inoculation with an arbuscular mycorrhizal fungus, *Glomusclaroideum*, on the growth of rosemary (*Rosmarinusofficinalis* L.), an aromatic plant species native to Mediterranean ecosystems, and on soil aggregation after forest fires.

MATERIALS AND METHODS:

Rosemary plants were propagated through cuttings in trays with 70 cm^3 cells. Half the cells received 5 g of inoculum of *G. claroideum* consisting of spores, mycelia and colonised root fragments. After rooting the plants were transplanted to 1 L pots containing either burned or unburned forest soil. The burned soil was prepared by heating samples in a muffle furnace for 20 min at 500 ºC. The experiment comprised four treatments: (i) non-inoculated control plants in burned soil, (ii) non-inoculated control plants in unburned soil, (iii) plants inoculated with *G. claroideum* in burned soil and (iv) plants inoculated with *G. claroideum* in unburned soil. After a growth period of 4 months, plant height was measured in order to compare the growth of the rosemary plants within the different treatments. Leaf chlorophyll a+b concentration was determined by spectrophotometry. The number of trichomes per mm^2 of leaf was determined by microscopy and was used as an indication of the potential of the rosemary plants to produce essential oils. The soil aggregation was assessed in soil samples collected from the pots by determining the mean weight diameter (MWD) according to the dry method of Kemper and Rosenau (1986). Fresh root samples were collected and stained as described in Oliveira et al. (2005). Root colonisation by mycorrhizal fungi was then assessed by microscopy.

RESULTS AND DISCUSSION:

A significant increase in plant height was obtained in plants inoculated with *G. claroideum* and grown in burned soil (Table 1). This may have been due to the increased soil nutrient availability that is usually associated with forest fires and their subsequent uptake by the inoculated AMF. The leaf chlorophyll a+b concentration was also significantly higher in plants inoculated with *G. claroideum*, suggesting improved

nutrition of these plants. The number of trichomes in the leaves of rosemary was found to be significantly higher in plants inoculated with *G. claroideum* and grown in burned soil, indicating that these plants can potentially produce larger quantities of essential oils.

Table 1 - Effect of inoculation of *Rosmarinusofficinalis* with *Glomusclaroideum* in burned and unburned forest soil

AMF	Soil	Plant height (cm)	Number of trichomes / mm^2 of leaf	Concentration of chlorophyll a+b (mg/g)	MWD (mm/g)	AMF colonisation
Control	Burned	16.83 ± 2.33b	12.74 ± 3.80b	0.53 ± 0.10a	2.94 ± 0.23a	Negative
Control	Unburned	13.80 ± 1.69a	7.92 ± 2.00a	0.48 ± 0.18a	3.32 ± 0.03b	Negative
Glomusclaroideum	Burned	19.24 ± 2.25c	26.52 ± 7.34d	0.74 ± 0.10b	3.17 ± 0.07b	Positive
Glomusclaroideum	Unburned	13.30 ± 1.29a	16.50 ± 4.99c	0.65 ± 0.07b	3.54 ± 0.12c	Positive

Values are means ± SD. Means followed by the same letters within each column are not significantly different according to Duncan's multiple range test at the level of $P < 0.05$; AMF, arbuscular mycorrhizal fungi; MWD, Mean weight diameter.

The burned soil had lower values of soil aggregation (determined by MWD) when compared with unburned soil. This may be explained by the negative impact on the soil structure caused by forest fires. However, soils inoculated with *G. claroideum* had significantly greater values of aggregation than their respective non-inoculated controls (burned and unburned). These results show that the soils inoculated with *G. claroideum* were less prone to erosion. Typical structures of arbuscular mycorrhizas here observed in the roots of inoculated plants grown in both burned and unburned soils, while no colonisation was observed in non-inoculated control plants. This indicates that the inoculated *G. claroideum* was capable of colonising the roots of rosemary in both burned and unburned soils and may have contributed to the observed improved plant growth and soil aggregation.

CONCLUSION:

This study demonstrates that the relationship between rosemary plants and AMF is an asset for future restoration of burned soil, contributing to the improvement of soil aggregation and plant growth, thereby reducing the effects of forest fires, and possibly enhancing the production of compounds of interest with economic benefits.

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Microbial degradation of Sodium Trifluoroacetate under aerobic and anaerobic conditions

Authors: Diogo A. M. Alexandrino[1], Rui S. Oliveira[1,2,3], M. Fátima Carvalho[4]

1 *Department of Environmental Health, School of Allied Health Sciences of Polytechnic of Porto, Portugal*
2 *Research Centre on Health and Environment, School of Allied Health Sciences, Polytechnic Institute of Porto, Portugal*
3 *Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Portugal*
4 *CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal*

Presenting Author: Email: diogomalexandrino@gmail.com | Tel.: +351 910 092 456

INTRODUCTION:
The extensive use of hydrofluorocarbons (HFCs) and hydrochlorofluorocarbons (HCFCs) as environmental acceptable alternatives to chlorofluorocarbons (CFCs) has been responsible for the introduction in the environment of significant amounts of Trifluoroacetate (TFA). Specifically, TFA is produced by the atmospheric breakdown of HCFC-123, HCFC-124, HFC-134a and HFO-1234yf, which undergo oxidative, photolytic and hydrolytic reactions to generate the compound. TFA is a highly persistent, water soluble and extremely stable fluorinated compound, that tends to accumulate in low-streamed aquatic environments, with marine ecosystems as its ultimate environmental sink. Although it does not exert considerable toxicological effects neither in microbial communities, nor in aquatic organisms, it reveals a mild toxicity regarding plants (Boutonnet*et al.*, 1999). There are no reports on the aerobic biodegradation of TFA, but the mineralisation of the compound by anaerobic methanogenic bacteria was demonstrated in few studies (Visscher, *et al.*, 1994; Kim *et al.,* 2000).

OBJECTIVES:
This study aimed to investigate the biodegradation of sodium Trifluoroacetate (NaTFA) by microbial cultures obtained from an industrial contaminated site, microorganisms pre-exposed to other fluorinated compounds and aerobic activated sludge, under aerobic and anaerobic conditions. Biodegradation of NaTFA was studied as a sole carbon source or in co-metabolism with sodium acetate, the latter being only investigated in aerobic conditions. Under anaerobic conditions, this study assessed the TFA biodegradation potential of methanogenic, sulphate-reducing and denitrifying bacteria.

MATERIALS AND METHODS:
A variety of soil (wet and dry sediments), rhizosphere and water samples were collected from a contaminated drain in northern Portugal (Estarreja, Aveiro) and used as an environmental microbial source for the experiments. Two microbial cultures capable of degrading fluorinated compounds, samples of aerobic activated sludge from a local wastewater treatment plant were also used as inoculum.

The aerobic experiment was divided in two experimental setups: in setupA, biodegradation of NaTFA as a sole carbon source (20 ppm NaTFA) was investigated, while in the setup Bbiodegradation of NaTFA was studied in co-metabolism with sodium acetate (5 ppm NaTFA + 500 ppm sodium acetate fed daily). In the anaerobic experiments, biodegradation of TFA was only tested as a sole carbon source (0.7 ppm). Sterile controls supplemented with NaTFA were also included (5 ppm NaTFA).

The aerobic experiments were conducted in 250 mL Erlenmeyer flasks, containing 70 mL of a sterile minimal salts medium supplemented with NaTFA in concentrations according to the experimental setup, and sodium acetate for the co-metabolic experiments. Half of the culture volume was removed and replaced with fresh medium at a 10 days interval. Anaerobic experiments were performed in 100 mL serum vials, containing 50 mL of a culture medium which composition varied according to the type of the anaerobic bacteria to select. To ensure that cultures were free of oxygen, cysteine and sodium sulphide were added to reduce all the

oxygen remaining in the cultures, and resazurin (1 mg l^{-1}) was used as an oxygen indicator. Prior to incubation, anaerobic cultures were flushed with a N_2 stream and sealed with aluminum capsules.
All cultures were incubated on a rotary shaker at 100 rpm and 25ºC. Samples were taken every 10 days for the aerobic cultures and after a period of 15 days for the anaerobic cultures.

Defluorination of NaTFA was detected through the measurement of fluoride anions in the cultures' supernatants, with a fluoride-selective electrode. For every measurement, calibration curves were established by using freshly prepared standard solutions of sodium fluoride with concentrations ranging from 0.001 to 1 mM sodium fluoride.

RESULTS AND DISCUSSION:
Significant defluorination was not verified in any of the aerobic cultures, with the most prominent defluorination rates being well below 10% throughout the 118 days of the experiment. The aerobic cultures fed with NaTFA as a sole carbon source revealed non-existing or insignificant biodegradation patterns, and supplementation with sodium acetate as a co-metabolite was also found not to exert a significant effect in the aerobic biodegradation of this compound.

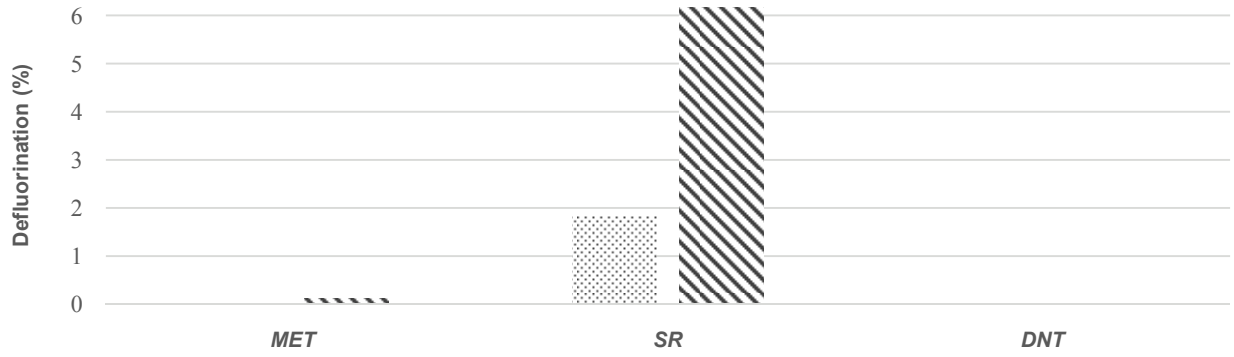


Figure 3 - Defluorination data, regarding the anaerobic cultures, after 15 days. MET, SR and DNT stand for methanogenic, sulphate-reducing and denitrifying bacteria, respectively. The striped bars correspond to the rhizosphere inoculum, while the dotted bar corresponds to the wet sediment inoculum.

Such results could be explained by the lack of a proper bacterial acclimation to the compound, since it has been suggested that a considerable pre-exposure period is needed before the microbial communities can develop and improve suitable catalytic mechanisms (Bott&Standley, 1999). This hypothesis is also supported by the fact that the microbial cultures capable of degrading fluorinated compounds, revealed higher microbial densities and slightly better defluorination rates in both experimental setups (data not shown).

Regarding the anaerobic experiments, from the three groups of bacteria tested, only sulphate-reducing bacteria showed some (although low) defluorination of NaTFA along a 15 days period, which is a much shorter period than the one tested in the aerobic conditions (Fig. 1). Both methanogenic and denitrifying bacteria revealed insignificant biodegradation patterns (Fig. 1). These results are not in agreement with the ones reported in the literature, which revealed a more active role of methanogenic bacteria on the biodegradation of NaTFA (Visscher*et al.*, 1994; Kim *et al.*, 2000). This incongruence may be due to the concentrations of NaTFA used in the anaerobic experiment, which both Visscher*et al.* (1994) and Kim *et al.* (2000) reported to be inhibitory to methanogenic activity. Although, the same authors also suggested that sulphate-reducing bacteria are only capable of TFA biodegradation in concentrations bellow 0.925 M TFA, which we found not to be the case.

CONCLUSION:
From the results obtained it is possible to state that biodegradation of NaTFA is not a direct mechanism. This compound seems to be highly recalcitrant under aerobic conditions, indicating that environmental occurring

aerobic bacteria do not possess efficient catalytic mechanisms necessary to attack the molecular structure of TFA. Under anaerobic conditions, the results obtained after a two weeks period indicate that biodegradation may occur, although at a very low rate.

Although TFA does not represent a major threat to the environment, its persistence and natural ability to accumulate in the environment may lead in the future to environmental hazardous concentrations. Such increase over time may be however balanced if the strong driving force posed by the presence of this compound in the environment is able to develop better biodegradation mechanisms.

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Arbuscular mycorrhizal fungi are more efficient than chemical fertilisers in the production of essential oils of common thyme (*Thymus vulgaris*)

Authors: Rui S. Oliveira[1,2,3,4], Vanessa Moreira[3], Mafalda Nunes[2,3], Inês Rocha[1], M. Fátima Carvalho[5], Miroslav Vosátka[6], Carlos Cavaleiro[7], Lígia Salgueiro[7], Helena Freitas[1]

1 *Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Portugal*
2 *Research Centre on Health and Environment, School of Allied Health Sciences, Polytechnic Institute of Porto, Portugal*
3 *Department of Environmental Health, School of Allied Health Sciences of Polytechnic Institute of Porto, Portugal*
4 *CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Portugal*
5 *CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal*
6 *Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice, Czech Republic*
7 *Centre of Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Portugal*

Presenting Author: Email: rso@estsp.ipp.pt | Tel.: +351 222 092 130

INTRODUCTION:
Common thyme is an aromatic plant with medicinal, cosmetics and culinary uses. Due to its antimicrobial, antioxidant/antiradical and antitumor activity the essential oils of common thyme are in great demand (Nikolić et al., 2014). A significant part of the production of aromatic plants relies on the use of chemical fertilisers, which when applied in large scale can have significant environmental consequences (Malik et al., 2011). Arbuscular mycorrhizal fungi (AMF) are a group of soil microorganisms that forms mutualistic symbioses that can benefit plants by improving the uptake of mineral nutrients, mineralising organic nutrients and conferring drought resistance (Oliveira et al., 2005).AMF have the potential to be inoculatedin the production of common thyme as an ecotechnological tool to reduce the input of chemicals fertilisers.

OBJECTIVES:
The aim of this study was to assess the effect of inoculation with an arbuscular mycorrhizal fungus, *Glomusintraradices*, and the application of chemical fertilisers on the composition of essential oils produced by common thyme (*Thymus vulgaris* L.).

MATERIALS AND METHODS:
Common thyme plants were propagated through cuttings in trays with 70 cm^3 cells. Half the cells received 5 g of inoculum of *G. intraradices*consisting of spores, mycelia and colonised root fragments. After rooting the plants were transplanted to 1 L pots containing growth substrate. Plants were watered with either deionised water, a low dosage of a soluble chemical fertiliser or a high dosage of a soluble chemical fertiliser. The experiment comprised six treatments: (i) non-inoculated control plants without chemical fertiliser, (ii) non-inoculated control plants with a low dosage of chemical fertiliser, (iii) non-inoculated control plants with a high dosage of chemical fertiliser, (iv) plants inoculated with *G. intraradices*without chemical fertiliser, (v) plants inoculated with *G. intraradices* and a low dosage of chemical fertiliser and (vi) plants inoculated with *G. intraradices* and a high dosage of chemical fertiliser.

After a growth period of 4 months, the essential oils of the leaves of common thymewere isolatedby water distillation using a Clevenger-type apparatus, according to theprocedure described in the European Pharmacopoeia.

Analysis was carried out by gas chromatography (GC) and by gas chromatography-mass spectroscopy (GC/MS). Analytical GC was carried out in a Hewlett-Packard 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph with a HP GC ChemStation Rev. A.05.04 data handling system, equipped with a single injector and two flame ionization detection (FID) systems. A graphpak divider (Agilent Technologies,

part no. 5021-7148) was used for simultaneous sampling to two Supelco (Supelco, Bellefonte, PA, USA) fused silica capillary columns with different stationary phases: SPB-1 (polydimethylsiloxane 30 m × 0.2 0mm i.d., film thickness 0.20 μm), and SupelcoWax-10 (polyethyleneglycol 30 m × 0.20 mm i.d., film thickness 0.20 μm). Oven temperature program: 70-220 ºC (3 ºC.min^{-1}), 220 ºC (15 min); injector temperature: 250 ºC; carrier gas: helium, adjusted to a linear velocity of 30 cm.s^{-1}; splitting ratio 1:40; detectors temperature: 250 ºC. GC-MS was carried out in a Hewlett-Packard 6890 gas chromatograph fitted with a HP1 fused silica column (polydimethylsiloxane 30 m × 0.25 mm i.d., film thickness 0.25 μm), interfaced with an Hewlett-Packard mass selective detector 5973 (Agilent Technologies) operated by HP Enhanced ChemStation software, version A.03.00. GC parameters as described above; interface temperature: 250 ºC; MS source temperature: 230 ºC; MS quadrupole temperature: 150 ºC; ionization energy: 70 eV; ionization current: 60 μA; scan range: 35-350 units; scans.s^{-1}: 4.51.

Essential oil components were identified by their retention indices on both SPB-1 and SupelcoWax-10 columns and from their mass spectra. Retention indices, calculated by linear interpolation relative to retention times of C8-C23 of n-alkanes, were compared with those of reference samples included in the Faculty of Pharmacy, University of Coimbra laboratory database. Acquired mass spectra were compared with reference spectra from the laboratory database, Wiley / NIST library and literature data (Adams, 2004). Relative amounts of individual components were calculated based on GC raw data areas without FID response factor correction.

Fresh root samples were collected and stained as described in Oliveira et al. (2005). Root colonisation by arbuscular mycorrhizal fungi was then assessed by microscopy.

RESULTS AND DISCUSSION:

Both the inoculation with *G. intraradices* and the application of chemical fertilisers influenced the composition of the essential oils produced by common thyme. The application of low and high dosages of chemical fertiliser in non-inoculated plants increased the production of thymol when compared with the plants that did not receive any chemical fertiliser. However, the highest production of thymol was achieved in plants inoculated with *G. Intraradices* without the application of chemical fertiliser, with a 208% increase when compared with the non-inoculated treatment. The highest production of carvacrol was also obtained in plants inoculated with *G. Intraradices* without the application of chemical fertiliser. Thymol and carvacrol are two of the major constituents of the essential oils of common thyme and are known for their antimicrobial and antimutagenic properties (Nikolić et al., 2014). Typical structures of arbuscular mycorrhizas here observed in the roots of inoculated plants, while no colonisation was observed in non-inoculated control plants. The mycorrhizal colonisation was, however, higher in plants without chemical fertiliser. This indicates that the inoculated *G. Intraradices* was capable of colonising the roots of common thyme and that the chemical fertiliser reduced the mycorrhizal colonisation. We hypothesise that the observed increased production of important essential oil components such as thymol and carvacrol was due to the higher efficiency of *G. intraradices* to uptake mineral nutrients in the plants without chemical fertiliser.

CONCLUSION:

The highest contents of thymol and carvacrol, two economically important components of the essential oils of common thyme, were obtained in plants inoculated with *G. Intraradices* without the application of chemical fertiliser. Inoculation with AMF can, therefore, be regarded as an eco-friendly alternative to the application of chemical fertilisers in the production of essential oils of common thyme.

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EXPOSURE TO NANOPARTICLES ABSTRACTS

R&D of Polymer Composite Materials Modified With Nano-Oxides and Phosphinates: Related Risk Assessment

Authors: S.P.B. Sousa[1], M.C.S. Ribeiro[1], J.S. Baptista[2]

1 *Composite Materials and Structures Research Unit (UMEC), Institute of Mechanical Engineering and Industrial Management (INEGI), Porto, Portugal*

2 *Research Laboratory on Prevention of Occupational and Environmental Risks (PROA / LABIOMEP), Faculty of Engineering, University of Porto, Porto, Portugal*

Presenting Author: Email: ssousa@inegi.up.pt | Tel.: +351 229 578 710 | Fax: +351 229 537 352

INTRODUCTION:

A new amazing and exponentially growing field of knowledge to the scientific community is nanotechnology. It is expected that related industries will have an annual turnover by 2015 over 1.5 trillion euros (Savolainen et al., 2010). However, new technologies are usually related to new risk factors and researchers are most of the times the first ones to be exposed to them (e.g., the revolutionary research on radioactivity made by Marie Curie and how her discovery led to her death). Recent inquiries have shown that many researchers do not have internal occupational safety regulations relative to nanomaterials (Groso, Petri-Fink, Magrez, Riediker, & Meyer, 2010). This lack of knowledge about nanomaterials (NM) safety came under the attention when, some years ago, seventy seven persons complained of severe respiratory problems and some of them were even hospitalised with fluid in the lungs after using "Magic Nano", a bathroom cleaning product (Miles, 2006). Another dramatic case occurred in a paint factory in China. Nanoparticles (NP) were found in the lungs of seven female workers who become sick, and two of them died later. These tragic events could have been avoided had there been implemented preventive measures and adequate monitoring in occupational safety health (Groso et al., 2010).

OBJECTIVES:

This study aims to evaluate the risk in research and development (R&D) of polymeric composite materials modified with nano-oxides and phosphinates, focusing on exposure to NP in a known research laboratory.

MATERIALS AND METHODS:

This study has been developed under a national Portuguese project (PTDC/ECM/110162/2009) at the Institute of Mechanical Engineering and Industrial Management (INEGI). The production of different polymer composites used a commercially available unsaturated polyester resin, with trade name Aropol® FS3992 (Quimidroga Portugal-Produtos Químicos Unipessoal Lda) as matrix. The fillers, aphosphinate based flame retardant(FR) and alumina (Al_2O_3) NP, were used with different contents as specified in Table 1.The FR was Exolit® OP 1240 (Clariant-Químicos, Lda., Portugal) which consists of a fine white grain-based organic phosphonate powder (37.50 μm average size) with high phosphorus content. The NanoDur® (99.5% purity, Alfa Aesar®) alumina NP(45.0 ηm average size and a 36.0 $m^2.g^{-1}$ specific surface area) were purchased from Cymit Quimica S.L. (Spain). The production process of polymer composites and sequent analysis were conducted under the same conditions as usually observed in the industry (general exhaustion).In order to obtain exposure data control, air particle measurements before the production or testing are essential. To measure the particles concentrations in the workplace air, the Dust-TrakTM Aerosol Monitor (model 8520) was used, which is a reference equipment for sampling and measuring indoor air quality under buildings' HVAC systems regulation. This equipment, measures the fine and coarse particles concentration by weight between 1-10μm, and for this study the 1 μm nozzle was used, to try detecting particles with lower dimension than those of the used FR. The established maximum concentration limit in Portugal is 0.15 $mg.m^{-3}$ for particles smaller than 10 μm.(M.O.P.T.C., 2006).

RESULTS AND DISCUSSION:

During the production and testing of polymer composite materials (Figure1),it was observed that particles go through different "states":in the pre-production phase, particles are at powder state; throughout production, particles are dispersed in a resin (solution); and finally, in the post-production and testing phases the particles are embedded in the solidified resin matrix. In order to analyze the mechanical properties of the different composite materials under study, it was necessary to cut the samples according to the required standard specimen sizes. It was found that both the cutting and testing operations led to dust release into the work environment, which potentially contains NP. Additionally, during all the steps involved in the production process, the handling of a variety of chemicals is required which can also expose the workers to potentially dangerous substances. Usually, occupational disorders are the result of cumulative exposures and not the consequence of a single severe incident.There is still no adequate legislation applicable to NM; however, some standards appropriate to these materials have begun to emerge. In the traditional risk assessment, the exposure doses are compared with the occupational exposure limit values (OELs). However, there are no specific OELs for NP; so, these new standards propose a pragmatic orientation, referring that for insoluble or poorly soluble NM not included in the fibrous or carcinogenic, mutagenic and reprotoxic chemicals category, the final OEL should be reduced 15 times (Schulte, Murashov, Zumwalde, Kuempel, & Geraci, 2010). It was found by observation that many tasks involved in samples' production show different risk levels of exposure to NP. Considering the statutory limit values for the used NM, they were not exceeded or even reached (OEL for nanoAl_2O_3=10mg.m^{-3}). However, considering the proposed reduction and that all the dust could potentially be NP, it was verified that the value (adjusted OEL for nano Al_2O_3 = 0.67 mg.m^{-3}) is higher than the suggested for the cutting task (Table 1). The same is observed relative to the recommended maximum limit values for air quality, for the same task. One point must however be stressed: the used equipment is standardized and it is only appropriate to detect particles lower than 1.0 μm and not specifically NP. With these results, some precautionary and prevention measures should be made and enforced. Even for situations within the exposure limits, there is still lack of specific toxicological data for NPs. Therefore, the potential presence of NPs in the workplace should require that workers always use collective and individual safety equipment.

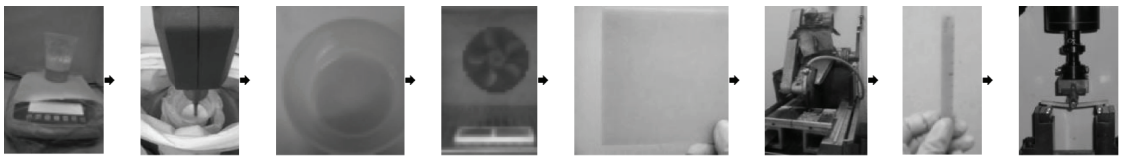


Figure 1 - Main phases in the in R&D of polymer composites

Table 1 - Measurements obtained with the dust track during different tasks

Aerosol Concentrations	Production with:									
	Control	15%FR	20%FR	2.5%NP +15%FR	2.5NP+2 0%FR	5%NP+1 5%FR	5%NP+2 0%FR	Cutting	Measuring	Testing
Mean(mg/m^3)	0.015	0.049	0.044	0.019	0.037	0.010	0.007	0.173	0.021	0.112
Minimum (mg/m^3)	0.009	0.024	0.038	0.005	0.013	0.007	0.003	0.009	0.008	0.057
Maximum (mg/m^3)	0.045	0.118	0.054	0.034	0.068	0.013	0.019	0.707	0.052	0.136

CONCLUSION:

Recommendation 2011/696/EU (definition of NM) and the Commission Communication "Regulatory Aspects of Nanomaterials" indicate that the legal basis exists; however, there is still a long way to go through in terms of safety in handling and use of NM and derivative products. Current knowledge about the NP toxicity is insufficient and preliminary scientific assessments show there is enough evidence to suspect that, at least some NP can be hazardous to human health. Still, with the present and impending legislation applicable to NM, it is possible to implement prevention and protection based on generic technical (e.g., adequate

ventilation, manipulation under fume hood or glove box and access restriction),organizational (e.g.,education and training), personal (e.g.,eye protection, laboratory mask for respiratory protection - at least a ffp3 mask, body protection - non-woven lab coat and overshoes and hand protection - more than 1 pair of adapted gloves) and cleaning management safety measures (e.g.,wet cleaning only).

These measures should be undertaken to ensure safety during R&D of materials with NP. Future challenges relative to NP monitoring and health risk assessment should focus on the development and use of more sensitive instruments specific for NP with realistic potential for real-time measurement, with capacity for shape identification, and determining other important properties that could influence the NP hazard.

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Application of a Control Banding Tool for Risk Level Assessment and Control of Nanoparticles Exposure in Welding Operations

Authors: João Gomes[1,2], Catarina Pereira[1], Paula Albuquerque[3], Rosa Miranda[4]

1 *Área Departamental de Engenharia Química, ISEL - Instituto Superior de Engenharia de Lisboa, R. Conselheiro Emídio Navarro, 1959-007 Lisboa, Portugal*

2 *IBB - Instituto de Biotecnologia e Bioengenharia / Instituto Superior Técnico - Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal*

3 *ESTESL, Escola Superior de Tecnologia da Saúde de Lisboa, Av. D. João II, Lote 4.69.01, 1990-096 Lisboa, Portugal*

4 *UNIDEMI, Departamento de Engenharia Mecânica e Industrial, Faculdade de Ciências e Tecnologia, FCT, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal*

Presenting Author: Email: jgomes@deq.isel.ipl.pt | Tel.: +351.963 902 456 | Fax: +351 218 317 267

INTRODUCTION:

It is well known that the traditional industrial hygiene approach for controlling exposures to harmful particles in the workplace is to measure the air concentrations of the said particles in the worker's breathing zone and then compare those concentrations to existing exposure limits determined for those particles[1]. From those comparisons, protection control measures can be derived in order to reduce concentrations below the exposure limits. This implies that: i) the sampled concentrations are representative of what the worker is actually breathing, ii) the adequate exposure limit is known, iii) appropriate analytical methods are available to quantify the exposure level, and iv) the exposure levels at which those particles can produce adverse health effects are also known. If any of these factors is not well characterized, any measurements performed may have limited value as it would be difficult to obtain a valid risk assessment. Consequently, the derived protection measures are also questionable as they cannot be ascertained as effective. This situation tends to be rather complicated when addressing exposure to nanomaterials, both engineered nanoparticles and/or accidentally emitted nanoparticles. In fact, when worker exposures to nanoparticles are concerned, it is not easy to observe the previously mentioned requirements. Control Banding (CB) strategies[2] seem to offer a simplified control of worker exposures when there is an absence of firm toxicological and exposure information. Control Banding was developed in the pharmaceutical industry as a pragmatic tool to manage the risk resulting from exposure to a wide variety of potentially hazardous substances in the absence of the above mentioned data. Basically, it is a risk assessment approach using the generally accepted risk paradigm, where risk can be measured as a function of the severity of impact (also known as hazard) and the anticipated probability of that impact (exposure). Both hazard and exposure are then classified into two to five different levels, usually referred to as bands. The two sets of bands are combined in a matrix, resulting into control or risk bands.

OBJECTIVES:

The objective of this study is to contribute to perform risk assessment of welding processes, considering the characteristics of ultrafine and nanoparticles emitted during operation, by use of a Control Banding tool.

MATERIALS AND METHODS:

This study consisted in the application of the CB Nanotool 2.0[3] in order to assess the welders' exposure to nanoscale particles emitted in common industry used welding processes, as follows:a) Metal Active Gas (MAG) for mild steel, using three different shielding gas mixtures (Ar+10% CO_2 ; Ar+18% CO_2, and 100% CO_2);b) MAG for austenitic stainless steel, using three different shielding gas mixtures (Ar+5% CO_2 ; 81% Ar+18% He+1% CO_2 ; 91% Ar+5% He+2% CO_2+ 2% N_2).

Regarding these conditions, monitoring tests were made in real welding situations described elsewhere[4], which comprised the determination of Alveolar Deposited Surface Area of emitted particles in the nano scale range, using a Nanoparticle Surface Area Monitor (NSAM, TSI, model 3550), the determination of size distribution of those particles by means of a Scanning Mobility Particle Sizer (SMPS, TSI, model 3034), the collection of emitted particles using a Nanometer Aerosol Sampler (NAS, TSI, model 3089), and further morphology analysis by Transmission Electronic Microscopy (TEM, Hitachi, model H-8100 II), equipped with an Energy Dispersive X-ray Spectroscopy (EDS) probe for determination of elemental chemical composition, as described by Gomes et al.[5]. The CB matrix is presented in figure 1.

RESULTS AND DISCUSSION:

The results were, then obtained for each welding process. Risk assessment was mainly based of the following criteria:i) chemical composition of the filler material;ii) chemical composition of the shielding gas;iii) chemical composition of the material to be welded (mild steel and stainless steel). As, an example, the obtained results for the application of the CB matrix are presented in figure 2, for the case of MAG welding of carbon steel using a shielding gas of 100% CO_2, which comprises 3 activities.

		Probability			
		ExtremelyUnlikely (0-25)	LessLikely (26-50)	Likely (51-75)	Probable (76-100)
Severity	VeryHigh (76-100)	RL3	RL3	RL4	RL4
	High (51-75)	RL2	RL2	RL3	RL4
	Medium (26-50)	RL1	RL1	RL2	RL3
	Low (0-25)	RL1	RL1	RL1	RL2

Control bands: RL 1 - General Ventilation; RL 2 - Fume hoods or local exhaust ventilation; RL 3 - Containment; RL4 - Seek specialist advice

Figure 1 – RL matrix as a function of severity and probability

Activity	Severity	Probability	Total	ControlBand
1	Medium (35)	Likely (57,5)	RL2 (125)	Fume hoods or local exhaust ventilation
2	Medium (30,5)	Likely (57,5)	RL2 (125)	Fume hoods or local exhaust ventilation
3	High (62,5)	Likely (55)	RL3 (150)	Containment

Figure 2 – Determination of the risk level for MAG welding of carbon steel, using a shielding gas of 100% CO_2

CONCLUSION:

From the obtained results it can be said that the nature of the base material to be welded (as by its chemical composition) seems to result in a higher risk level when evaluating the derived control measures in order to protect exposed workers. However, no final marked differences were observed when considering separately the cases of mild steel and stainless steel, which call for still some improvements to be done in the use of this tool. Nevertheless, this is considered as a preliminary approach to the risk assessment resulting from welding operations.

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Effects of Nanosilver in Medical and Consumer Products – A critical Review on Impacts to Health and Environment

Authors: Erik Petersen

Ecological Physicians Society - ÖkologischerÄrztebunde.V., www.oekologischer-aerztebund.de, Bremen, Germany

Presenting Author: Email: Oekologischer.aerztebund@t-online.de | Tel.: +49 421 498 42 51 | Fax: +49 421 498 42 52

INTRODUCTION:

Nanosilver is the most used nanoscaled material in consumer products in Germany, whereas most consumers are less aware of which products contain nanosilver. Due to the absence of any obligation for marking the products and the fact that the term „nano“ was unprotected by legal regulations, there were - and probably still are - products in the market which are faulty advertised as „nano“ for reasons of selling better.

The new Biocidal Product Regulation (BPR) contains an obligation for marking such products both as “biocidal” and as “nano” from 1.9.2013 on (European Commission, 2013).

Uses of nanosilver are still increasing. More and more products are created to protect against real or suggested risks of infections or even unpleasant odours. On the other side metallic silver is acting like most biocides by killing various germs without differentiating between good and bad ones. Furthermore, biocides are contributing considerably to the increasing antibiotic resistance of pathogens. Therefore the medical use of silver coatings or for example nanosilver in wound dressings is recommended in some special cases.

OBJECTIVES:

The main effect of nanosilver is biocidal. The potential risks for the environment and human health are not clear yet.

Nanoparticles used in clothing, paint or fillings of washing machines are washed out into the waste water and finally into the environment. There are increasing scientific efforts to determinate the risk estimates for the different compartments of the environment as there are: sewage plants, ground or rivers, especially for the plankton and microbes.

On the other side it is still uncertain, whether the use of antibacterial products containing nanosilver for example is creating positive health effects. Moreover, recent research shows that the effective toxic concentration of nanosilver is almost in the same range towards bacteria and human cells (Greulich, Braun, Peetsch, Diendorf, Siebers, Epple, & Köller, 2012). Research into the probability to develop more resistances against aggressive pathogens is already recognized to be lacking.

The precautionary principle, as advocated by the World Health Organization and the European Environmental Agency allows to face the possible benefits and hazards of a new technology and to act without definitive proof if there is objective evidence of serious problems. So far the current state of the discussion is summarized with special reference to the precautionary principle and the NGO perspective.

MATERIALS AND METHODS:

This overview concerns new products providing nanotechnological protection and highlights the discussion in the European Union and especially in Germany regarding the environmental and health implications caused by the use of nanosilver in particular. Resulting demands from the governmental and non-governmental point of view are referenced. Finally, we summarize the latest research results as published and were recently presented at international conferences.he abstract should be typed in the A4 page layout (210x297mm) with 20 mm margins (top, bottom, left and right). They should be organized in the following

order: Introduction, Objectives, Materials and Methods, Results and Discussion, Conclusion, Acknowledgments (optional) and References.

RESULTS AND DISCUSSION:

The majority of the scientific community is convinced that the use of nanosilver in consumer products is needless and probably more harmful than benefitting or useful. The worst effect seems to be that nanosilver contributes to a high degree to increase antibiotic resistance of pathogens and at the same time being toxic to human cells.

Most of the Non-Governmental Organisations (NGO) on the European and national level lend strong support to the restriction of any use of biocides in consumer products and to the use of nanoparticles for medical indications only (for example: Healthcare without Harm, 2013), similar to the Federal Institute of Risk Assessment (BfR) in Germany (Federal Institute of Risk Assessment, 2012). The NGOs are in favour of an inventory and an effective marking of all kinds of products with nanoscaled material. Only France has so far established a governmental inventory of all products which contain nanoparticles. There are existing a few web-based inventories, which are hosted by NGOs, for example in Germany (BUND-Friends of the Earth Germany, 2014).

The first steps have been taken under the new Biocidal Product Regulation (BPR) with an obligatory labelling of nanomaterial. Although there are still some gaps to close: **the use of biocides in general must become more specific and more binding.** An inventory of all kinds of nanoscaled materials, which is still missing, is under discussion on the European level.

CONCLUSION:

The new Biocidal Product Regulation BPR is one step in the right direction to assess the risks of nanosilver in consumer products both for environment and human health.

ACKNOWLEDGMENTS:

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Permeable Reactive Barriers using nanoparticles to remediate nitrate pollution

Authors: Rui Araújo[1], Ana Meira Castro[2], António Fiúza[3]

1 *Universidade do Porto, Portugal*
2 *Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Portugal*
3 *Faculdade de Engenharia, Universidade do Porto, Portugal*

Presenting Author: Email: rms.araujo@gmail.com | Tel.: +351 225 081 929

INTRODUCTION:

Nitrate (NO_3^-) is pervasive in the biosphere [1, 2]. Contemporary agricultural practices are among the major anthropogenic sources of reactive nitrogen species, where nitrate is the most abundant of these [2]. Excessive amounts of reactive nitrogen in soils and groundwater are creating significant threats to human health and safety [3] as well as a host of undesirable environmental impacts [2]; it is currently considered the second most relevant environmental issue, after carbon dioxide emissions. Nowadays, among the most relevant and promising approaches to reduce nitrate concentration in water, namely groundwater, are denitrification-based processes [4]. Permeable reactive barriers (PRB) have been proven effective in reducing various contaminants in copious amounts, particularly in shallow groundwater [5]. However the possible added effectiveness of using nanoparticles in these structures to obtain nitrogen gas from nitrates requires further investigation.

OBJECTIVES:

This work explores the possibility of employing permeable reactive barriers comprising nanoparticles as an effective end-of-pipe technology to tackle nitrate pollution in groundwater. By nitrate pollution it is considered a nitrate concentration in groundwater above 50mg dm^{-3} NO_3^- (11,3 mg dm^{-3} NO_3^--N) as limited by Nitrates Directive - Directive 91/676/EEC from 12th December 1991.

MATERIALS AND METHODS:

A literature review was conducted in 34 databases, following a four steps methodological approach. First, a set of pertinent terms was used in the initial searches. Second, synonyms from the first set of terms were used to perform further searches. Third, from the selected papers, other relevant terms were identified to perform a third set of searches. In the forth step, the bibliography of the selected papers was researched for further relevant papers. The set of studies selected for in-depth analysis were exclusively laboratorial ones. Each study was assessed by set of relevant characterization variables.

RESULTS AND DISCUSSION:

The degradation of nitrate from soil and groundwater can occur by physical adsorption, chemical reduction or biological denitrification. Physical adsorption is a surface mediated process where nitrate bonds to the surface of an adsorbent, e.g. biocharcoal, ion exchange resins, bamboo powder charcoal.

With chemical reduction, nitrate is chemically reduced by, e.g. nanoscale zero valent iron (NZVI), bimetallic Fe/Cu nanoparticles, copper-coated zero valent iron nanoparticles, bimetallic Fe/Ni nanoparticles, nanosized zero valent iron-polymeric anion exchanger composites, nanosized zero-valent iron supported by polystyrene resins, iron oxide/magnetite (Fe_3O_4), palladium-copper catalysts supported on cationic resin, Poly[β-(1→4)-2-amino-2-deoxy-D-glucopyranose] based zero valent nickel nanocomposite. Nitrate chemical reduction processes yield mostly ammonium, a soil and groundwater contaminant. Moreover, NZVI and zero valent iron (ZVI) particles have been proven effective remediation agents for several pesticides, e.g. atrazine and achalor for the former, and flutriafol for the latter.

Whereas with physical and chemical processes the reactive nitrogen species are kept in the terrestrial or aquatic system, in biological denitrification, nitrate is transformed in a hypoxic media by bacteria into N_2, thus closing the natural nitrogen cycle. While autotrophic denitrificantion uses hydrogen gas from the oxidation of, e.g. iron as an electro donor, heterotrophic denitrification is reliant on source of organic carbon and electron donor for the denitrifying microorganisms. The organic carbon sources used for the promotion of heterotrophic denitrification encompass wood byproducts, mulch, compost and leaves. Autotrophic sulfur-oxidizers were also used to remove nitrate. Moreover, it has also been employed a two-layer permeable reactive biobarrier for the biotic reduction of nitrogen, where the first layer of the barrier addressed dissolved oxygen by ZVI, in order to create a down-gradient anaerobic environment for the heterotrophic denitrification to take place in the second layer of the barrier. NZVI also has affinity to react with dissolved oxygen, a characteristic which has to be accounted for when using such materials. Tests with different media by combining cotton burr compost, a sediment low in organic carbon and NVZI have proven that media made of cotton burr compost removed nitrate at a faster rate than did the combination of cotton burr mixed with NZVI and/or sediment. Nitrate reduction processes can depart from ammonia; in such cases a prior microbial aerobic nitrification stage must be ensured so that the resulting nitrate can be then reduced into nitrogen gas.

The primary controls for the denitrification process are the amount of available NO_3^-, available carbon, the energy source, and the absence of gaseous oxygen. Other relevant factors in the denitrification process encompass pH, ion composition in the media and the redox potential.

CONCLUSION:

Nitrate is considered to be the most ubiquitous groundwater contaminant in the world, being the agricultural runoff the major source of groundwater contamination by nitrate. The denitrification processes are complex and subject to an intricate interplay of several variables in the soil. Nevertheless, PRB have been proven effective to ensure denitrification, mostly by heterotrophic pathways. However how the denitrification effectiveness varies with changing particle size of the electron donor, for different electron donor substances, is not yet clear. Thus though there is a substantive body of knowledge on PRB, the use of nanoparticles in these structures is relatively novel and emergent for soil and groundwater remediation, so further research and advancements are required for a clear understanding of the principles that govern their effectiveness.

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HEALTHY AND SAFE SCHOOLS ABSTRACTS

Biological Air Assessment in Primary Schools – The ARIA Project

Authors: Lívia Aguiar[1], Cristiana Pereira[1], Ana Mendes[1], Paula Neves[1,2], João Paulo Teixeira[1,3]

1 *Environmental Health Department, Portuguese National Health Institute Doutor Ricardo Jorge, Porto, Portugal*
2 *Research Centre of Health, School of Allied Health Sciences of Polytechnic of Porto, Portugal*
3 *Institute of Public Health (ISPUP), Porto University, Porto, Portugal*

Presenting Author: Email: livia7_aguiar@hotmail.com | Tel.: +351 912 720 263

INTRODUCTION:

Exposure and sensitization to indoor allergens are important risk factors for asthma and allergic respiratory diseases, playing a key role in triggering and exacerbating allergy and asthma symptoms in children (Salo *et al.*, 2009). While children's greatest exposure to indoor allergens is at home, other public places where they spend a large amount of time, such as primary schools, may also be sources of significant allergen encounters (Abramson *et al.*, 2006), such as bacteria and fungi. Children are considered a susceptible group because they are particularly vulnerable to the development of respiratory diseases, such as asthma, and also spend much of their time inside classrooms, in a confined atmosphere, reasons why they should deserve priority attention in indoor air quality (IAQ) studies. In Portugal, this is an increasing and important subject, proved by the development of studies in this area, more sorely since the publication of the national legislation, Ordinance no. 353-A/2013 of December 4th, that establish reference values of maximum concentration for selected indoor air pollutants. The results of this project are part of an ongoing project (ARIA Project), that intend to study the exposure of children to indoor air in 20 public primary schools and investigate the associated respiratory and allergic related health effects in 1600 children's.

OBJECTIVES:

The main goals of this study are 1) characterize IAQ in primary schools, during the winter season, by the evaluation of total bacteria and fungi concentrations in classrooms, in Porto, and compare these results with the current revised national standards; 2) identify the main fungi species found in the evaluated areas; 3) analyze the health impacts caused by biological pollutants on children.

MATERIALS AND METHODS:

Biological assessment took place during winter season, as this period provides the worst case scenario of exposure due to the enclosed environments. From January to February 2014, out of a predicted total of 20 primary schools to be study, 6 schools were assessed. A total of 22 classrooms (average of 4 rooms per school) were evaluated regarding their biological contamination during their normal occupancy, through the analysis of total bacteria count, fungi count and identification. Air sampling was carried out with a microbiological air sampler (Merck Air Sampler MAS-100) and using *Tryptic Soy Agar* (TSA) for total bacteria and *Malt Extract Agar* (MEA) for fungi. Outdoor air samples were also collected for comparison to the indoor measurements. Quantification of bacteria and fungi was performed by naked eye count and the identification of fungal colonies was based upon phenotypic characteristics and followed standard mycological procedures, Results were expressed as colony-forming units per cubic meter of air (CFU/m^3) and compared with the recently revised Portuguese standards. Classical statistical methods were used to estimate means, medians and frequencies (percentages).

RESULTS AND DISCUSSION:

Mean bacteria concentration is above the reference value for all schools evaluated, from 6 to 548 times higher (Table 1). Regarding primary schools mean fungi concentrations, only in one school the value is according with the reference value, nonetheless being very close to the established limit (Table 1). If the previous Portuguese legislation was still ruling (Decree-Law no 79/2006 of April 4th: Bacteria and Fungi

reference value = 500 CFU/m^3), concerning fungi concentrations found indoors, only 2 of the 6 primary schools were above the reference value. The revised legislation changed the limit: for fungi, concentrations indoors shouldn't be higher than outdoors; for bacteria, concentration found outdoors plus 350 CFU/m^3 is the reference value.

Cladosporium sp. and *Penicilium* sp. were the prevalent species found in 5 primary schools, being *Penicillium* sp. the more common species in 4 of these 5 schools. In another school, *Aspergillus fumigatus*, a known potential pathogenic/toxigenic species, was the prevalent specie identified (40%), followed by *Cladosporium* sp. (20%) and *Penicillium* sp. (20%). Nevertheless, this specie was identified in all evaluated primary schools. The presence of toxin-producing fungi like *Aspergillus fumigatus* indoors should be a cause for concern considering the potential risk of mycotoxicosis (Ayanbimpe *et al.*, 2010). Another species from *Aspergillus* genus were found in 2 schools: *Aspergillus flavus* (2% in both schools) and *Aspergillus niger* (2% in both schools).

Table 1 - Biological Air Assessment Descriptive Statistics

	Bacteria	**Fungi**
	Mean ± Standard Deviation (CFU/m^3)	
PTE01	2466 ± 984	323 ± 58
Outdoor	398	394
Reference *	748	394
PTE02	1182 ± 407	242 ± 138
Outdoor	98	170
Reference *	448	170
PTE03	1442 ± 308	542 ± 313
Outdoor	44	122
Reference *	394	122
PTE04	3855 ± 3241	6393 ± 2830
Outdoor	96	160
Reference *	446	160
PTE05	2836 ± 1781	363 ± 80
Outdoor	174	192
Reference *	524	192
PTE06	2194 ± 1036	253 ± 131
Outdoor	4	118
Reference *	354	118

* Ordinance no. 353-A/2013 of December 4th: Bacteria _ Indoor < Outdoor + 350 CFU/m^3; Fungi _ Indoor < Outdoor CFU/m^3.

CONCLUSION:

The presented results show that 30% of the targeted primary schools raise concern due to the high concentrations of bacteria and fungi in all evaluated areas. Toxin-producing fungi *Aspergillus fumigatus*, identified in all primary schools and in considerable concentrations, is another warning sign of the poor IAQ that exists in classrooms full of young children. This factor, large number of occupants, along with low ventilation rates may be the cause of the results found in this ongoing study. Thus, to promote the air exchange, classrooms should be ventilated, by opening the doors and windows, during the classes' breaks or, when that is not possible, through the implementation of a mechanical ventilation system allowing a minimum flow of fresh air of 24 m^3/hour.person. All the results obtained will contribute to more knowledge on how children response to indoor environmental conditions and how needs and requirements of individuals can be translated to requirements of the indoor environment, both in home and in school.

ACKNOWLEDGMENTS:

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Sound exposure of music students during the classes

Authors: Matilde A. Rodriques[1], Marta Amorim[1], Manuela V. Silva[1], Cristina Rodrigues[1], Lívia Aguiar[2], Paula Neves[2], AidaSousa[3], Octávio Inácio[4]

1 *Research Centre on Environment and Health, Allied Health Sciences School of Polytechnic Institute of Porto, Portugal*
2 *Department of Environmental Health, National Health Institute, Dr. Ricardo Jorge, Porto, Portugal*
3 *Department of Audiology, Allied Health Sciences School of Polytechnic Institute of Porto, Portugal*
4 *Laboratory of Musical Acoustics, School of Music and Performing Arts of Polytechnic Institute of Porto, Portugal*

Presenting Author: Email: mar@estsp.ipp.pt | Tel.: +351 918 043 393 | Fax: +351 222 061 001

INTRODUCTION:

It is broadly recognized that professional musicians are at risk of developing ear pathologies due to the exposition to loud music in the course of rehearsals and performances (MacDonald *et al.*, 2008; O'Brien *et al.*, 2008; Jansen *et al.*, 2009). However, while much has been published regarding the sound exposure of professional musicians, particularly of orchestral musicians, little is known of the sound exposure of the music students. In fact, it is important to recognize that the musicians' noise exposure may start very early, in the course of their training as students, in the school classrooms and at homes.

Studies about sound exposure of music students and about their hearing pathologies are very scarce and do not contemplate all the important variables to analyze, particularly the influence of the class room, music style and instrument, as well as the class typology. However, they pointed that students can be exposed to high sound levels and Noise Induced Hearing Loss can appear (Phillips *et al.* 2010; Stewart *et al.* 2013). Therefore, this study is an attempt to better characterize this problem. However, it is important to note that the results present in this work are only referent to the first results of a bigger project, where different factors that can have influence on students' sound exposure are being studied, as well as the hearing effects analyzed.

OBJECTIVES:

This study aimed to characterise the noise exposition level in Jazz and Classic music students.

MATERIALS AND METHODS:

Measurements of the noise level were performed at a Conservatory of Music and in a Higher Music School. Evaluations of two groups of Jazz music students and one group of classic music students were performed independently on each school. Twelve students of jazz music style and thirteen students of classic music style were selected based on their chosen instrument and their place on the group/orchestral rehearsal. All the classes were evaluated with an acoustic dosimeter for a two-week period.

RESULTS AND DISCUSSION:

The results showed that the studied students are exposed to high noise levels in the curse of individual instrument classes and group rehearsals. In the classes without instruments practice the noise levels were lower. Other important finding is related with the differences between music styles. In general, the jazz music students are exposed to high noise levels than the classic music students, even in the group classes, were the number of instruments in the classroom was higher in classic music. These results can be related with the use of amplifiers in the course of rehearsals and to the encouragement to play high. The sound levels found for the jazz students in the classes with instrumental practice range between 66.6-99.2 dB(A) for percussion, 72.3-93.9dB(A) for contrabass, 87.9-96.4 dB(A) for vibraphone,82.4-96.2dB(A) for piano, 73.1-96.2 dB(A) for guitar, 88.4-101.6 dB(A) for trombone and finally between98.8-99.1 dB(A) for saxophone. For the classic students musicians, the sound levels range between 86.3-96.5dB(A) for percussion, 73.9-77.0 dB(A) for contrabass, 76.3-88.2 dB(A) for violin, 74.3-87.4 dB(A) for viola, 75.8-85,9 dB(A) for cello, 80.7-88.0 dB(A) for clarinet, 78.6-91.1 for bassoon and 92.0-93.0 dB(A) for French horn. We

also have found high values for the L_{Cpico} for specific instruments. Concerning, the saxophone it was exceeded the higher legal action level and for the piano and percussion it was surpassed the value for the exposition limit. Relatively to the theoretical lessons, the findings obtained showed values between 48,7 e 88,9 dB(A) exceeding once more the recommendation of 35 dB (A) proposed by the World Health Organisation.

CONCLUSION:

The findings of this particular study showed the need of an implementation of risk reduction measurements that would allow a reduction of the noise levels that the students are exposed in their own schools.

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Association of indoor particles of classrooms with prevalence of Rhinithis' symptoms among students

Authors: Nuno Canha[1,2], Susana Marta Almeida[1], Maria do Carmo Freitas[1], Hubert Th. Wolterbeek[2]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa,Polo de Loures - Campus Tecnológico e Nuclear, E.N. 10 ao km 139.7, 2695-066 Bobadela LRS, Portugal*

2 *Department of Radiation, Radionuclides and Reactors, Faculty of Applied Sciences, Delft University of Technology, Mekelweg 15, 2629 JB Delft, The Netherlands*

Presenting Author: Email: nunocanha@ctn.ist.utl.pt | Tel.: +351 219 946 155

INTRODUCTION:

Children's exposure to particulate matter in scholar environments has been assessed in last decades due to the increasing concern and awareness of the negative impact that a bad indoor air quality can have in the children's health, scholar performance and attendance (Mendell and Heath, 2005; Almeida et al., 2011, Canha et al., 2013). One of the air pollutants that gathered special focus is particulate matter due to its complexity in terms of chemical composition and also due to its impact in human health, such as respiratory and cardiovascular diseases. In October 2013, International Agency for Research on Cancer (WHO, 2013) classified outdoor air pollution and PM as carcinogenic to human beings.

OBJECTIVES:

A survey about health issues was conducted in urban and rural primary schools of Portugal, following the standard ISAAC type of questionnaire. Indoor air quality parameters, such as chemical and water soluble ions composition of indoor particles, were assessed at the classrooms where the questionnaires were applied to the students, for different seasons. The main goal of this work is to assess associations between particulate matter of classrooms' indoor with student's health indicators, such as monthly rhinitis' occurrences.

MATERIALS AND METHODS:

A passive sampling methodology was applied to collect particulate matter (PM) in classrooms of urban and rural primary schools of Portugal (Figure 1). Shortly, particles were collected by passive deposition in polycarbonate filters exposed inside the classrooms for a period of 2-3 months. The sampling was carried out for 1 year, which allowed studying the seasonal variability of the particles. Mass concentration of the particulate matter was assessed by gravimetry, its chemical content by Instrumental Neutron Activation Analysis and its water-soluble ions content by Ionic Chromatography and Atomic Absorption Spectroscopy.

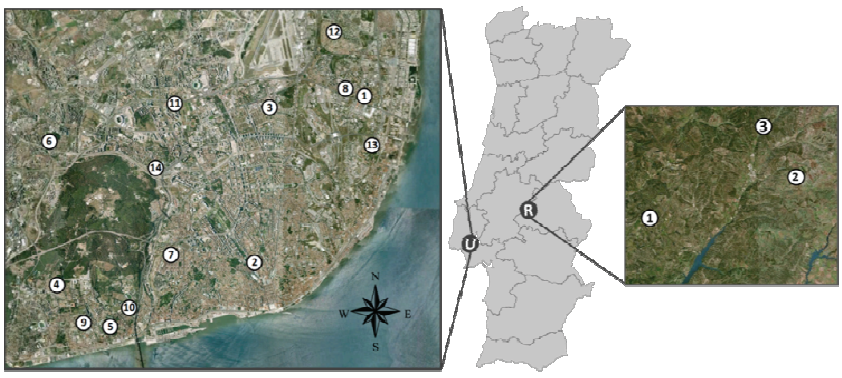


Figure 1 - Location of the studied primary schools at urban and rural clusters.

A questionnaire was built based on the ISAAC - International Study of Asthma and Allergies in Childhood Program and it was applied to all students attending the studied classrooms, in order to identify children with respiratory diseases (wheeze, asthma and rhinitis) as well their physical, nutritional and house living habits. A total of 446 viable questionnaires were obtained (67% of the total number of questionnaires given to be fulfilled). The monthly rhinitis' occurrences in each classroom were grouped by season.

RESULTS AND DISCUSSION:

Figure 2 shows the seasonal variability of the prevalence of rhinitis symptoms among the students attending the studied classrooms.

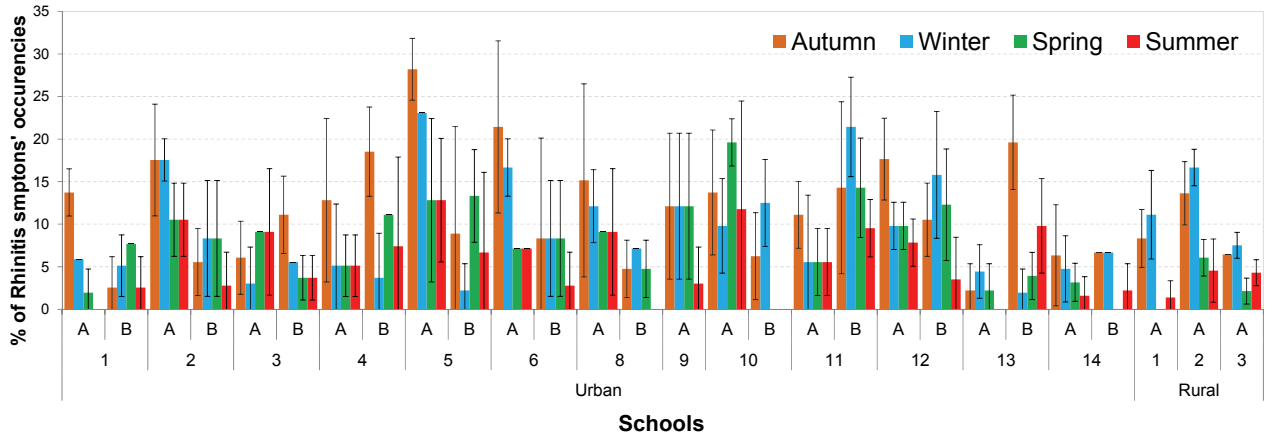


Figure 2 - Seasonal rhinitis symptoms (%) per classrooms (A and B).

Autumn was the season with higher prevalence of rhinitis symptoms among the children, with a mean value of 11.6 ± 6.0 %, followed by winter, spring and summer with, respectively, 9.4 ± 5.7 %, 7.3 ± 4.8 % and 5.2 ± 3.7 %.Figure 3 shows the seasonal variability of the collected mass particles in the classrooms indoors. Characterization of the chemical composition of the collected particles (regarding chemical elements and water soluble ions) along with their seasonal variability study, was already fully describe elsewhere (Canha et al., 2014).

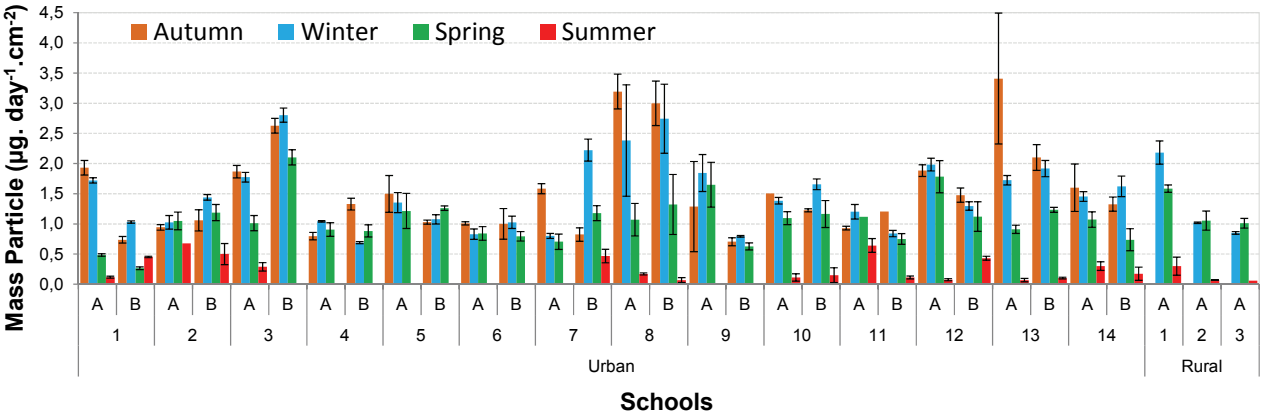


Figure 3 - Seasonal variation of collected particle mass per school and its classrooms (A and B).

On-going statistical studies are being conducted to evaluate potential associations between the percentage of rhinithis'symptoms among the children attending the classrooms and the indoor levels of particles, regarding their masses and chemical composition (chemical elements and water-solube ions).

CONCLUSION:

The present study revealed that some classrooms have high prevalence of rhinithis' symptoms along with high mass particles, with autumn showing the higher values when comparing with other seasons. The follow-up of this work is to understand eventual associations between the assessed health indicator (prevalence of rhinithis' symptoms) and indoor particles (regarding concentrations and composition), using multivariate statistical methods.

ACKNOWLEDGMENTS:

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Exposure of 3-5-years-old children to indoor ultrafine particles: assessment of homes and schools

Authors: Klara Slezakova[1, 2], Catia Teixeira[1], Jimmy Fonseca[1], Simone Morais[2], Maria do Carmo Pereira[1]

1 *LEPABE, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Porto, Portugal*
2 *REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Porto, Portugal*

Presenting Author: Email: slezakok@fe.up.pt | Tel.: +351 220 414 946 | Fax: +351 225 081 449

INTRODUCTION:

Throughout the entire lives, humans are exposed to the particulates omnipresent in indoor air. Considering that most people spend the majority of their time indoors, the consequences of this exposure can range from insignificant to fatal and depend on many factors (type of particulates present, type of indoor environment, duration, and etc.). Whereas in the last two decades the scientific attention focused on health risks caused by exposure to particulate matter such as PM_{10} and $PM_{2.5}$, in the last years it has shifted towards ultrafine particles (UFP; i.e. particles smaller than 100 nm). UFP contribute very little to the overall particle mass but they dominate the number concentration. When compared to larger particles, UFP have higher surface area and larger concentrations of adsorbed (or condensed) toxic pollutants per unit mass (Diapouli et al., 2007). In comparison to larger particles, UFP also have higher deposition rates in the lower respiratory tract. The available epidemiological studies provide (though not consistently) evidence of adverse effects due to short-term exposure to ambient UFP, while information on long-term exposure is not yet available (Beko¨ et al., 2013). In addition, the combined effect of UFP high surface area and potentially toxic composition may promote both physical and chemical reactions inside the organisms that can too result in adverse health outcomes. UFP particles penetrate to indoor environments from outdoors. They also originate from various indoor sources such as cooking, tobacco smoking, candle and incense burning, the use of gas and electric appliances, hair spray, cleaning products, and furniture polish; chemical reactions such as those between ozone and terpenes can also generate UFP indoors (Morawska et al., 2013). The complexity of UFP exposure (spatial variability, different indoor sources, infiltration of UPF from various outdoor emission sources and seasonal variability in concentrations and composition; Sioutas et al., 2005) indicates the need to further study this pollutant in order to fully comprehend its impacts on human health. This is especially relevant for sensitive groups. Young children in particular are very susceptible population because they receive a higher dose of airborne particles relative to lung size compared with adults while at the same time their physiological and immunological systems are still developing (Sioutas et al., 2005). Children spend up to 23 h per day indoors moving between specific microenvironments, such as homes and schools where the levels of air pollution can differ significantly. Therefore, quantification of UFP in these specific microenvironments is important in order to correctly assess child overall exposure to UFP.

OBJECTIVES:

The aim of this work was to assess the exposure dose rate of pre-school children to UFP in indoor environments, namely in schools and homes.

MATERIALS AND METHODS:

The exposure dose to UFP particles were assessed in Portuguese 3-5-years-old children considering three different pre-schools (urban, rural), and five homes over the period of 70 days. UFP number concentrations in size range 0.02-1 m were measured by condensation particle counters - TSI P-Trak™ using an intake flow of 0.7 L min^{-1} and UFP logging interval of 60 s. Various indoor school microenvironments (classrooms situated on ground and first floor; canteens; gymnasium, playrooms) and homes were evaluated. The characteristics of each studied micro-environment as well construction properties were registered. In order to better understand the impacts of outdoor UFP emissions to indoor, the levels of UFP were concurrently measured in ambient air (i.e. outdoors). During sample collection, a detailed record of room's occupancy, ventilation

systems (door and window positions) and potential sources was kept. The daily activity patterns of children were analyzed throughout each day. In addition, school staff and parents were daily inquired regarding the occurrence of additional sources/activities. Locations in which the different activities happened during the day were identified. Total daily residence time of children spent in each micro-environment and the types of performed activities were registered. Each activity was characterized in terms of intensity level in order to assess the corresponding breathing rates (USEPA, 2011).

RESULTS AND DISCUSSION:

At two urban pre-schools the inhalation exposure dose rate of UFP for 3-5-years-old children ranged from 4.60-7.52×10^9 and 2.94-7.52×10^9 particles $kg^{-1}day^{-1}$. The exposure doses of UFP were approximately 1.5 times for older children (5 years) than for younger ones (3-4 years) as they spent approximately twice more time outdoors; older children also performed more frequently physical activities such as exercising, running, and playing (both indoors and outdoors) which were associated with the highest breathing rates and consequently led to higher inhalation doses of UFP. The estimated dose rates of UFP in children at the rural school were higher than expected (in a view of much lower indoor UFP concentrations at this pre-school) probably due to the considerably longer period spent outdoors. At rural pre-school, children spent approximately 40% of their school times outdoors (whereas it was 7-25% at urban schools) and the UFP dose rates due outdoor exposure accounted for 60% of the total school exposure, thus being at the highest proportion of all three pre-schools. These findings show that daily activity patterns influenced significantly the overall child exposure dose rates to UFP. Concerning the homes, high exposure dose rates were caused especially by contributions of UFP originating from indoor sources. Cooking, namely grilling, boiling, use of an electric stove and a toaster were identified among the most significant exposure sources in homes.

CONCLUSION:

The results demonstrated that the levels of exposure to UFP in various indoor microenvironments differed significantly. Therefore, future population-based studies focusing on the health effects of airborne pollutants need to account for the exposures occurring in these different microenvironments in order to obtain a representation of child's overall exposure profiles.

ACKNOWLEDGMENTS:

This work was supported by Fundação para Ciência e Tecnologia through fellowship SFRH/BPD/65722/2009 and by the IJUP project PP_IJUP2011 121. It also received financial support from the European Union (FEDER funds through COMPETE) and National Funds (Fundação para a Ciência e Tecnologia) through projects PEst-C/EQB/UI0511/2013 and Pest-C/EQB/LA0006/2013.

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Health risk assessment of children's exposure to gaseous pollutants in urban nurseries' indoor environments

Authors: Pedro TBS Branco[1], Maria CM Alvim-Ferraz[1], Fernando G. Martins[1], Sofia IV Sousa[1]

1 *LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal*

Presenting Author: Email: p.branco@fe.up.pt | Tel.: +351 225 082 262 | Fax: +351 225 081 449

INTRODUCTION:

Children are more vulnerable to air pollution than adults, mainly due to their not fully developed immune and respiratory systems and their relative higher amount of air inhalation per body weight, being considered a risk group; furthermore they spend more time in school microenvironments (MEs) (namely in nurseries in the case of younger ones) than in any other indoor MEs besides home (Sousa et al., 2012). In nursery indoor MEs children are exposed to several air compounds which are likely to cause adverse health effects, namely formaldehyde (CH_2O), carbon monoxide (CO), nitrogen dioxide(NO_2) and ozone(O_3) considered as high priority chemicals (Autrup et al., 2007; Kotzias et al., 2005). In Portugal there are 91200 infants (< 3 years old) and 276125 pre-school children (3-5 years old) attending a total of 9405 nurseries (PORDATA, 2013). However, and as far as known there are no studies focusing on the health risk assessment of children's exposure to indoor air pollution in Portuguese nurseries.

OBJECTIVES:

This study aimed to: i) evaluate the concentrations of four gaseous pollutants (CO, CH_2O, NO_2, O_3) in different indoor MEs of urban nurseries; ii) analyze those concentrations according to guidelines and standards; and iii) assess health risks of children's exposure to indoor air in nurseries.

MATERIALS AND METHODS:

This study was carried out on four different nurseries (N_URB1, N_URB2, N_URB3 and N_URB4) located at urban sites influenced by traffic emissions in Porto, Portugal. Indoor air quality measurements of CO, CH_2O, NO_2 and O_3were performed in 3 classrooms in N_URB1 and N_URB2, and 2 classrooms in N_URB3 and N_URB4, as well as in the lunch rooms (LR) of the four nurseries. Continuous sampling was performed using electrochemical detection with the Haz-Scanner IEMS (Environmental Devices Corporation, USA), from 2 to 9 days in each room (both weekdays and weekends), from February to November 2013 (with a break during summer holidays - June to September). Hourly means were calculated. Outdoor NO_2 and O_3concentrations were obtained from the ambient air quality monitoring network managed by *Comissão de Coordenação e Desenvolvimento Regional do Norte* (CCDR-N).

A health risk assessment methodology was used to estimate lifetime cancer health risks from children's indoor exposure to CH_2O and non-cancer health risks posed by exposure to CH_2O, CO, NO_2, and O_3in the studied nurseries.Lifetime cancer risk (LCR) was used for assessing the risk posed by CH_2O as a carcinogenic compound and it was given by the formula in Eq. (1):

$$LCR = EC.IUR \quad (1)$$

where EC is the exposure concentration (μg m^{-3}) and IUR is the Inhalation Unit Risk factor (μg $m^{-3})^{-1}$. The EC was determined by using the equation Eq. (2):

$$EC = CA \times ET \times EF \times ED / AT \quad (2)$$

where CA is the contaminant concentration in the air (μg m^{-3}), ET is exposure time per day (hours), EF is the exposure frequency per year (days), ED is the exposure duration (years), and T_t is the total time of the period considered (hours).The Hazard Quotient (HQ) was used for assessing inhalation effects of non-carcinogen effects of CH_2O, CO, NO_2 and O_3, being estimated by using Eq. (3):

$$HQ = EC / RfC \quad (3)$$

where EC is the exposure concentration (μg m^{-3}), and RfC is the reference concentration (μg m^{-3}) for inhalation pathway. To estimate HQ for short-term exposure (1 hour), it was assumed that EC was the maximum hourly mean value registered, while for long-term exposure Eq. (2) was used.

RESULTS AND DISCUSSION:

The presence of children (occupation) and their routines, building characteristics and ventilation habits were found to be the main determinants of indoor air quality in nurseries. The absence of indoor sources, supported by the calculated I/O ratios lower than 1, indicated that the influence of outdoor air was the main determinant of CO, NO_2 and O_3 indoor concentrations, which never exceeded Portuguese legislation and WHO guidelines. The observed CH_2O concentrations indicated the presence of specific indoor sources for this pollutant, namely from the emission of some materials like furnishing.

LCR was estimated for the exposure of children to CH_2Owhile attending nurseries. Children were assumed to be exposed to the average concentrations during occupation periods in the nursery indoor MEs, considering ED: i) 2 years in the case of the infants (< 3 years old); and ii) 3 years in the case of pre-schoolers (3-5). Nursery global scenario was also evaluated, i.e., the case of children attending the nursery from the first year of life until 5/6 years old (before going to primary school). For this scenario, EC was estimated based on the cumulative exposure, accounting for both infancy and pre-school age periods of exposure. IUR value (6.0 x 10^{-6} (μg m^{-3})$^{-1}$) was obtained from OEHHA database (OEHHA, 2014). LCR for exposure to CH_2O during pre-school age was higher than for exposure during infancy, as in the latter children were exposed to lower concentrations (Table 1).

Table 1 - Summary of the EC, CR and HQ estimates for long-term exposure to CH_2O by children's age

Children's age during exposure	CA (μg m^{-3})	ET (hours)	EF (days)	ED (years)	AT (hours)	EC (μg m^{-3})	LCR	HQ
Infancy	1.52	9	220	2	122.5	0.34	2.1×10^{-6}	0.04
Pre-school age	14.73	5	183	3	178.9	1.54	9.2×10^{-6}	0.17
Nursery global	-	-	-	-	129.5	1.88	1.1×10^{-5}	0.21

For non-carcinogenic risk of long-term exposure to CH_2O, chronic toxicity was evaluated (RfC - 9 μg m^{-3}; target organ - respiratory system), and children were assumed to be exposed to these pollutants by inhalation for the whole period attending the nursery, like in the case of LCR. HQ values were found higher for exposure during pre-school age than for exposure during infancy, but both below 1 - the same as in the nursery global scenario (Table 1).There were not found RfC for long-term exposure for CO, NO_2 and O_3, neither studies focusing on their chronic effects. Acute toxicity due to short-term exposure (1 hour) was evaluated for CH_2O, CO, NO_2 and O_3 (RfC - 55 μg m^{-3}, 23000 μg m^{-3}, 470 μg m^{-3}, and 180 μg m^{-3} respectively; target organs - respiratory system).HQ values were estimated both per indoor ME and per children's age (Figure 1 a) and b), respectively).

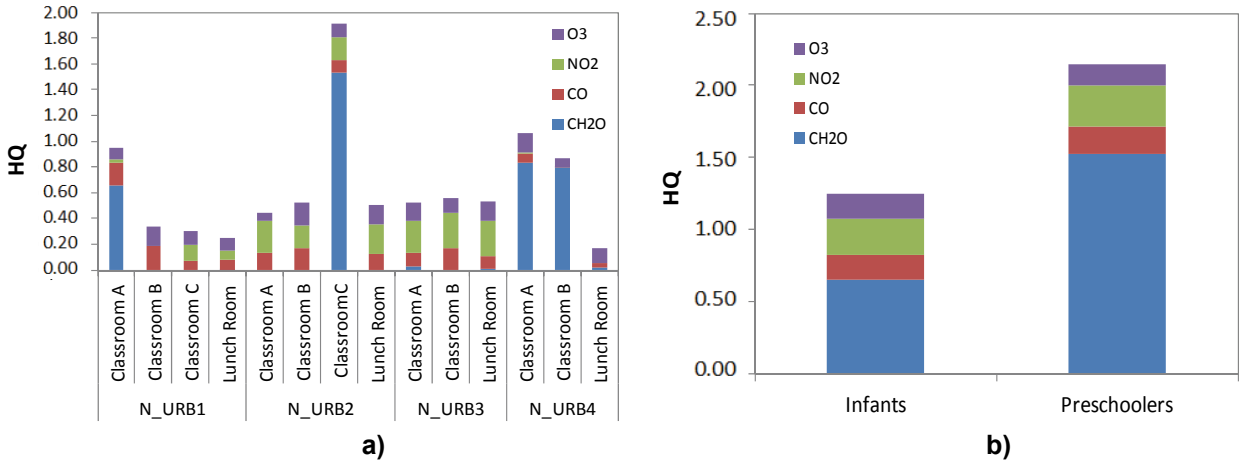


Figure 1 - HQ for exposure to CH_2O, CO, NO_2 and O_3: a) in the microenvironments, and b) by children's age

HQ values were usually found higher for exposure during pre-school age than for exposure during infancy, with the highest difference observed for CH_2O. Both during infancy and pre-school age, exposure to CH_2O resulted in the highest hazard, followed by exposures to NO_2, CO and O_3. Hazard due to pre-schoolers' exposure to CH_2O was found higher than 1.

CONCLUSION:

Although for CO, NO_2 and O_3 the health risk assessment did not show concerning situations, children's exposure to CH_2O in the indoor air of nurseries (namely during pre-school age) is likely to cause concerning risk situations, as an estimated excess of 2.5 new cases of cancer during lifetime in the Portuguese population attending nurseries, and mild or moderate eye irritation (acute non-carcinogenic effect) due to short-term exposure. Similar situations are expected to be found in other nurseries. So, measures to improve IAQ and reduce associated children's health risks should be applied. Changing materials emitting CH_2O is a good measure to reach this goal. Health risk assessment of other compounds (namely volatile organic compounds and particles) could also be important for future studies.

ACKNOWLEDGMENTS:

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Schoolchildren's exposure to indoor ultrafine particles in urban and rural environments

Authors: João Cavaleiro-Rufo[1], Joana Madureira[1], Inês Paciência[1], Eduardo de Oliveira Fernandes[1]

1 *Institute of Mechanical Engineering of Faculty of Engineering of University of Porto, Porto, Portugal*

Presenting Author: Email: jcrufo@gmail.com | Tel.: +351 225 574 585

INTRODUCTION:

Scientific studies concerning the effects of ultrafine particles (UFPs) on the human respiratory system have shown their potential adversity and toxicity, suggesting an apparent relation between particulate air pollution and increased morbidity and mortality (Englert, 2004; Weichenthal, Dufresne, & Infante-Rivard, 2007). Schoolchildren tend to be more susceptible to UFPs pollution particularly due to their immature respiratory systems and reduced lung function, and since they spend a significant part of their daytime at school, the assessment of the exposure to indoor pollution levels becomes fundamental. However, since available information regarding the levels of UFPs inside school classrooms remains limited, as well as the evidence related to their possible sources, further research in this area is essential.

OBJECTIVES:

The purpose of this study is to assess the children indoor exposure to UFPs number concentrations in urban and rural Portuguese primary schools and to estimate their indoor and outdoor potential sources.

MATERIALS AND METHODS:

Ultrafine particles were sampled, between January and March 2014, in 6 public primary schools located in the urban area of Porto (US1 to US6) and 2 primary schools from a rural area of Trofa (RS1 and RS2) situated 20km north of Porto. Indoor and outdoor UFPs number concentrations (pt/cc) were simultaneously measured daily throughout teaching hours (between 9:00 am to 5.30 pm) by TSI P-Track^{TM} (model 8525, TSI Inc., MN, USA) condensation particle counters at logging intervals of 1 minute. Information regarding outdoor environment, building characteristics, occupants' behaviour, indoor activities and occupation periods, as well as others potential indoor sources of UFPs was also recorded using a standardized and validated checklist.

Statistical analysis was performed using IBM SPSS Statistics v20. The one-sample *Kolmogorov-Smirnov* test was used to verify the data normality. The hypothesis of UFPs number concentrations being normally distributed was excluded and thus the *Mann-Whitney U* test was used to analyse the data. Statistical significance was considered with a $p<0.05$.

RESULTS AND DISCUSSION:

The mean indoor UFPs number concentrations for urban and rural environments were respectively 1.0×10^4 (±150) pt/cc and 5.7×10^3 (±93) pt/cc. Daily profiles of indoor UFPs number concentrations in classrooms were similar among each school (data not shown).

Considering the schools located in urban areas, a significant difference between indoor and outdoor UFPs number concentrations was found, being indoor levels higher than outdoors ($p<0.001$). Contrarily, indoor UFPs number concentrations in the rural environment were significantly lower than outdoors ($p<0.001$). Moreover, after analysing each school individually, the results showed that 4 schools located in urban environment (US1, US4, US5 and US6) and 1 rural school (RS1) displayed higher UFPs number concentrations indoors than outdoors (Figure 1).

Urban schools presented significantly higher indoor and outdoor UFPs concentrations when compared to rural schools (p<0.001, for both situations). This difference is probably associated with traffic density, which is considerably higher in the city of Porto. However, the I/O ratios were also increased in urban schools when compared to rural schools (I/O=1.16 *vs.* I/O=0.97), suggesting a larger contribution of UFPs from indoor sources, thus the augmented amounts of particulate in the urban schools cannot be entirely attributed to outdoor sources such as traffic density. As a matter of fact, classroom characteristics may also influence UFPs concentrations (Zhang & Zhu, 2012). For instance, it was observed that UFPs number concentrations in classrooms with chalk boards were significantly increased when compared to those without chalk boards (1.3 x 10^4 *vs.* 8.7 x 10^3, respectively, p<0.001), and the classrooms located on the ground floor presented increased particle counts when compared to those at least one storey above ground (1.2 x 10^4 *vs.* 8.6 x 10^3, respectively, p<0.001).

Regarding occupational periods, no significant differences in UFPs number concentrations were observed between lecture and recess periods (9.4 x 10^3 *vs.* 1.1 x 10^4, respectively, p=0.556). A possible explanation for these results may be related with the permanence of children within the classrooms during recess times due to the bad weather conditions at the time.

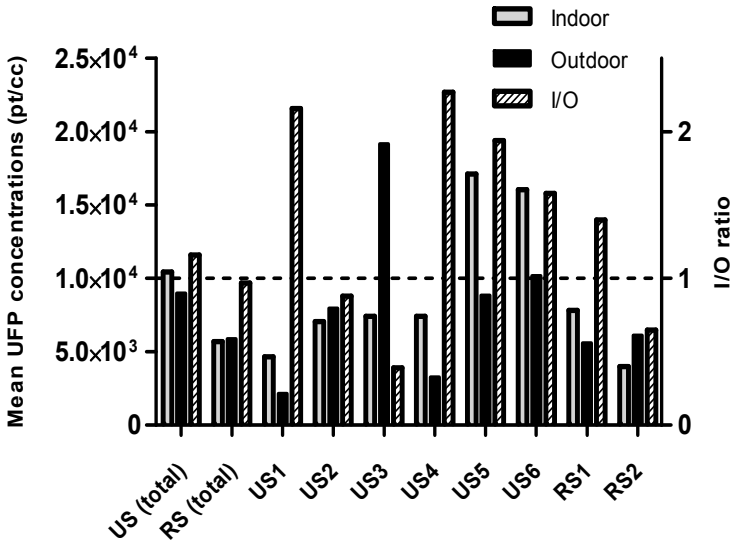


Figure 1 - Daily mean number concentrations of UFPs in 8 schools among urban (US) and rural (RS) environments during teaching hours.

CONCLUSION:

The outcomes of this preliminary study show that UFPs pollution is present in augmented concentrations in indoor and in urban environments when compared to outdoor and rural settings, respectively, corroborating the results from previously published studies (Yoon, Lee, & Park, 2011). Following the I/O ratio analysis, it is possible to conclude that the contribution of indoor sources of UFPs is higher in urban than in rural primary schools. Several classroom characteristics, such as the presence of chalk boards or the storey level, may be associated with elevated indoor UFPs concentrations, although additional research concerning these sources appears to be necessary. Nevertheless, taking into account the exposure levels as well as the potential causes and sources, the results presented in the current study may be useful to the understanding on how to reduce indoor air pollution in primary schools caused by ultrafine particulate matter.

ACKNOWLEDGMENTS:

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Radon Levels on Nurseries and Primary Schools at Mogadouro

Authors: Sofia IV Sousa[1], Pedro TBS Branco[1], Maria CM Alvim-Ferraz[1], Fernando G. Martins[1]

1 *LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal*

Presenting Author: Email: sofia.sousa@fe.up.pt | Tel.: +351 225 082 262 | Fax: + 351 225 081 449

INTRODUCTION:

Epidemiological studies all over the world have been providing evidence of an association between indoor radon exposure and lung cancer, even at the relatively low radon levels commonly found in residential buildings (smokers are at higher risk due to synergistic effects). Radon is considered the leading cause of lung cancer among non-smokers and after tobacco is the second cause of lung cancer in the general population and there is no known threshold concentration below which radon exposure presents no risk; the dose-response relation is linear (USEPA, 2010; WHO, 2009).Several studies have been reported on radon levels at granitic regions in countries as Spain, Romania, Iran, Norway (Sainz et al., 2009). Levels of radon have been also measured in Central Portugal (Coimbra, Viseu, Castelo Branco, Guarda) and were reported as being very variable and several times higher than 200 Bq m^{-3}(1-3 months measurements, being the concentrations at ground floor rooms higher than 400 Bq m^{-3}). The worldwide average indoor radon concentration has been estimated at 39 Bqm^{-3} (WHO, 2009).Considering the latest scientific data, WHO proposes a reference level of 100 Bq m^{-3}indoor radon to minimize health hazards. However, if this level cannot be reached under the prevailing country-specific conditions, the reference level is 300 Bq m^{-3}. The European Union recommends annual reference levels of400 Bq m^{-3} for existing buildings and 200 Bqm^{-3} for the new ones (EU, 90/143/Euratom). Portugal legislation defines a limit of 400 Bqm^{-3} and mandatory measurements in Braga, Vila Real, Porto, Guarda, Viseu and Castelo Branco, granitic areas, which may present higher risk

OBJECTIVES:

This study aimed to: i) determine diurnal variations; ii) compare the standards for human health protection with radon levels observed on 3 nurseries and 1 primary school at *Mogadouro* (North of Portugal); and iii) compare with legislated standards and analyse the legislated procedures.

MATERIALS AND METHODS:

Measurements were performed in 3 nurseries (1 with children from 0 to 2 years old - N_RUR_2 and 2 with children from 3 to 5 years old - N_RUR_1 and N_RUR_3) and 1 primary school - PRIM_RUR_1 - at the village of *Mogadouro*, *Bragança* district, in the North of Portugal. These sites are considered rural and with very low traffic influence. Table 1 shows the main characteristics of each studied microenvironment and sampling periods.

Table 1 - Main characteristics of each studied microenvironment and sampling periods

Site	Construction Year	Room (children age)	Area (m^2)	Occupation (# children)	Occupation period	Sampling period (week+weekend)
N_RUR_1	2011	CR[1] (4-6Y)	46.9	24	9h-12h, 13h30-18h30	2+2
N_RUR_2	2010	CR (0Y)	17.1	9	7h45-20h	2+0
		CR (2Y)	20.6	15	9h-19h	2+2
N_RUR_3	1999 (with recent renovations)	CR (4Y)	50.2	23	9h-17h30	2+0
		CR (5Y)		17	9h-17h30	2+2
PRIM_RUR_1	2011	CR (6-1st grade)	46.9	19	9h-12h, 13h30-17h30	2+0
		CR (8-3rd grade)		26		2+2
		LR[2]		160	12h-13h30	2+0

[1] CR - classroom; [2] LR -lunch room

A prior inspection to the studied nurseries and rooms (observations and staff interviews) was developed to capture relevant information on activities, ventilation habits and building characteristics. N_RUR_1 and PRIM_RUR_1 are located in the same building. All the studied buildings were single floor. Only N_RUR_2 had a mechanical ventilation system (HVAC); the other ones had natural ventilation. During occupation periods, electric/oil heating systems were usually turned on, and windows to outdoor as well as doors to inner corridors were always closed to avoid heat loss. Daily cleaning activities were performed at the end of the occupation period. All buildings were completely closed at night, dawn and weekends.

Measurements were carried out continuously (hourly means), during 2 to 4 days in several rooms of the nurseries and primary school, including weekdays and weekends. Radon levels were measured with Radim 5Bmonitor, which measures the α-activity of radon decay products (^{218}Po and ^{214}Po) collected from the detection chamber on the surface of a semiconductor detector by an electric field. The monitor was placed inside each room at the approximate breath height of the children. Temperature and relative humidity indoor (T_{int} and RH_{int}) were measured with electrochemical sensors (Haz-Scanner, EDS/SKC).

RESULTS AND DISCUSSION:

T_{int} and RH_{int} varied, respectively, between: i) 19 and 22 ºC (mean: 20 ºC) and 36 and 54% (mean: 43%) in N_RUR_1; ii) 20 and 26 ºC (mean: 23 ºC) and 23 and 47% (mean: 30%) in N_RUR_2; iii) 16 and 25 ºC (mean: 20 ºC) and 37 and 59% (mean: 45%) in N_RUR_3; and iv) 16 and 24 ºC (mean: 20 ºC) and 34 and 62% (mean: 62%) in PRIM_RUR_1. T_{int} was several times out of the ASHRAE proposed range (20-23.1ºC) and H_{int} was generally within the ASHRAE proposed range (30-60%). The daily mean profiles of radon for weekdays and weekends can be found in Figure1 a) and b), respectively.

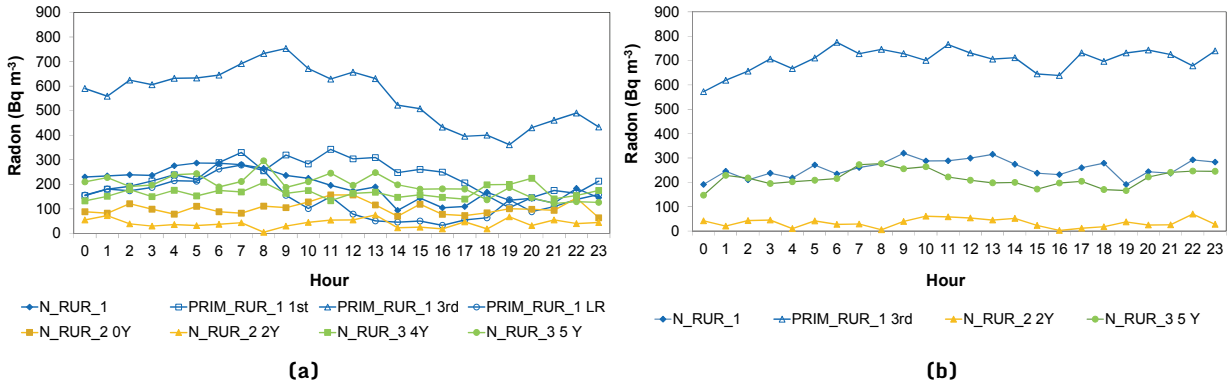


Figure 1 - Hourly mean concentrations of radon on the measured sites for a) weekdays and for b) weekends

Radon concentrations in N_RUR_1 varied between 58 and 359 Bq m^{-3}, in N_RUR_2 between 0 and 157 Bq m^{-3}, in N_RUR_3 between 67 and 385 Bq m^{-3} and in PRIM_RUR_1 between 2 and 888 Bq $m^{-3}$and were generally higher in weekends than in weekdays, and in some cases in weekdays non-occupied periods due lack of ventilation in those periods, leading to radon accumulation. The highest concentrations were observed at the primary school-3rd grade classroom. It should be enhanced that N_RUR_1 and PRIM_RUR_1 are located at the same floor (same building), with similar ventilation patterns and, even so, different rooms had very different concentrations (mean differences reaching 490 Bq m^{-3}), which can be due to the position of a crack or other type of hole in the building foundations. Radon daily variability seemed to be dependent on the ventilation profiles. In some classrooms there was a tendency for the concentrations to lower during occupation, namely on RUR_1 nursery and primary school, due to the higher air renovation with the children transit.N_RUR_2 was the location with lower radon levels, both on weekdays and weekend, which might also be due to the use of HVAC. This variability enhances the importance of radon measurements in all occupied rooms and for larger periods of time. Regarding the Portuguese legislated standard only the 3rd grade classroom of PRIM_RUR_1 presented exceedances (94%) during the occupation periods. Nevertheless, considering the European standard and the WHO guideline (100 Bq m^{-3}) almost all the classroom presented exceedances to both (between 27 and 100%).

CONCLUSION:

It was not possible to find a well-defined daily pattern for radon concentrations, although they seemed to be higher in the early morning. Radon concentrations found in the nurseries and in the primary school surpassed several times the recommended guidelines and thresholds (WHO-100 Bq m^{-3}; Portaria nº 353-A/2013-400 Bq m^{-3}). The variability found between classrooms and in some cases between periods of the day might calls into question the possibility of complying with the legislated standard through a discrete measurement, once these measurements may mask radon concentrations. Additionally, *Bragança* is not a mandatory area of measurement, but still has worrying concentrations, thus there might be other areas following the same profile. Considering the results obtained, measures to reduce exposure are essential, such as the simple implementation of active ventilation reducing concentrations from 30 to 70% (WHO, 2009).

ACKNOWLEDGMENTS:

The authors are grateful to the nurseries and primary school, namely children, professors and staff involved in this study. The authors are also grateful to *Fundação para a Ciência e a Tecnologia* (FCT), COMPETE, QREN and EU for PTDC/SAU-SAP/121827/2010 funding. PTBS Branco and SIV Sousa are also grateful to FCT, POPH/QREN and European Social Fund (ESF) for the financial support of grants SFRH/BD/97104/2013 and SFRD/BPD/91918/2012, respectively.

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Exposure to metals and allergens in settled dust in nursery and elementary French schools

Authors: Nuno Canha[1,2], Corinne Mandin[2], Olivier Ramalho[2], Guillaume Wyart[2], Jacques Ribéron[2], Claire Dassonville[2], Mickäel Derbez[2]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, Km 139.7, 2695-066 Bobadela LRS, Portugal*

2 *Université Paris-Est, CSTB (Scientific and Technical Building Centre), OQAI (French Indoor Air Quality Observatory), 84 avenue Jean Jaurès, Champs sur Marne, 77447 Marne la Vallée cedex 2, France*

Presenting Author: Email: nunocanha@ctn.ist.utl.pt | Tel.: +351 219 946 155

INTRODUCTION:

Indoor environment of school buildings is a subject that has been gathering a concern within the scientific community due to scientific evidence associating a poor indoor air quality(IAQ) to negative impacts upon health, performance and attendance of students (Mendell and Heath, 2005). In order to prepare its national survey in schools, the French IAQ observatory carried out a pilot study to test and to optimize sampling and analysis procedures in a set of 51 classrooms from 17 schools.

OBJECTIVES:

The main goal of this work is to describe the exposure levels of children concerning two groups of pollutants in the national survey, namely metal sand allergens in settled dust.

MATERIALS AND METHODS:

A total of 17 schools were chosen on a voluntary basis to perform this study, namely 7 nursery schools and 10 elementary schools, from the Clermont-Ferrand area, France (Figure 1).

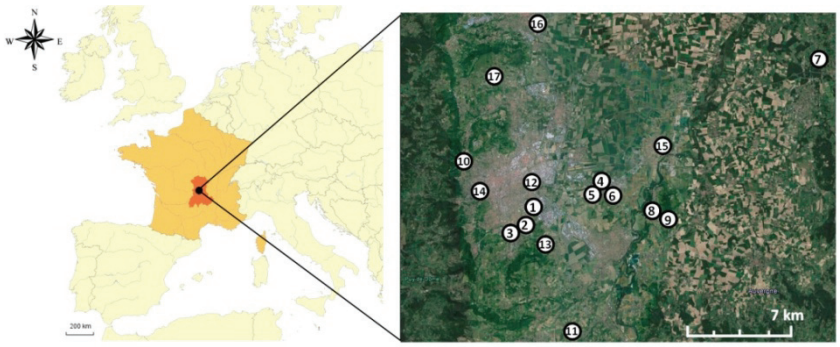


Figure 1 - Location of the 17 studied schools in Clermont-Ferrand area (right) and its location in Auvergne region (dark orange), in center of France (left).

Regarding the allergens, a total of 61 dust samples were collected with a vacuum cleaner equipped with a Mitest collector in the studied 51 classrooms. The mean surface sampled on a smooth floor was 4 m^2 with an average amount of collected dust of 130 ± 150 mg. For carpets and rugs, the sampled surface area was 1.3 m^2 with an average amount of collected dust of 870 ± 630mg. Samples were analyzed by Paris Laboratory of Hygiene and the following allergens were quantified by Elisa test: *Felisdomesticus* catallergen (Fel d 1), *Canisfamiliaris* dog allergen (Can f 1), *Dermatophagoidesfarinae* (Der f 1), and *Dermatophagoidespteronyssinus* (Der p 1) dust mite allergens.

Simultaneously, a wipe sampling method was used to collect dust on the hard surfaces of the classrooms in accordance to the AFNOR NF X46032 standard (AFNOR, 2008). Settled dust was sampled in each classroom on three 32cm x 32cm areas (i.e. 0.1m^2) by trained surveyors as described elsewhere (Le Bot et al., 2010).

After the sampling, wipes were stored in polyethylene tubes and sent to the laboratory until analysis. Bioaccessible and quasi total dust concentrations of Pb, As, Cd, Cr, Cu, Mn, Sb, Sr and V analyses were made by the French School of Public Health Laboratory as previously described by Le Bot et al (2010). A gastric digestion using hydrochloric acid was performed to determine metalbioaccessibility and metal analysis was carried out using inductively coupled plasma mass spectrometer (ICP/MS) with a quadrupole mass filter and an octopole reaction system. Results were supplied in µg of metal per square meter area.

RESULTS AND DISCUSSION:

Dog and cat allergens were detected in 60% of the collected samples. Higher detection percentages for both allergens were found in samples from carpet/rug when comparing with results of samples from smooth floor. For carpet/rug, the detection percentage was 80% for cat allergens and 70% for dog allergens. Levels of cat and dog allergens measured in the settled dust in the 51 classrooms ranged from 0.01 to0.39 µg/g and 0.01 to 0.94 µg/g, respectively. Regarding both allergens, none of the samples exceed the limit values for allergic sensitization (1 µg/g for Fel d 1 and 2 µg/g for Can f 1) as defined by Platt-Mills et al. (1997).

Mite allergens were evaluated only in dust samples from carpet/rug floors. Der p mite allergen was not detected in any of the 10 studied samples and Der f was only measured in one sample with a concentration of 1.69 µg/g, which is below the allergic sensitization limit of 2 µg/g established by Platt-Mills et al. (1997). This result is similar to the ones observed in Swedish and Asian classrooms, but much lower than the ones observed in classrooms of United States (Salo et al., 2009). The low mite allergens concentrations can be due to values of relative humidity lower than 51% that were measured inside the classrooms (median over the sampling week: 34%). Relative humidity of 51% is the minimum relative humidity required by mites since water vapour is their only water source (Arlian et al., 2001).

Regarding metal concentrations in settled dust, Pb results in the studied schools have already been described elsewhere (Derbez et al., 2011). Pb was detected in almost all analyzed samples. Table 1 shows the concentrations of selected metals measured in classrooms indoors.

Table 1 - Concentrations of selected metals ($\mu g.m^{-2}$) in settled dust in the 51 studied classrooms. (n - number of samples; LOQ - Limit of Quantification; SD - standard deviation; P - percentiles)

Metals ($\mu g/m^2$)	n	LOQ	% >LOQ	Mean	SD	Min	P5	P25	Median	P75	P95	Max
As_{total}	151	0.6	97	3.5	2.6	<LOQ	0.7	1.7	2.7	4.3	8.9	15.4
$As_{leachable}$	152	0.2	96	1.3	1.9	<LOQ	0.3	0.6	0.9	1.3	4.2	17.3
Cr_{total}	151	10	46	12	11	<LOQ	<LOQ	<LOQ	<LOQ	16	30	83
$Cr_{leachable}$	152	4	26	<LOQ	--	<LOQ	<LOQ	<LOQ	<LOQ	4	7	16
Cu_{total}	151	32	27	43	195	<LOQ	<LOQ	<LOQ	<LOQ	33	73	2399
$Cu_{leachable}$	152	15	27	23	26	<LOQ	<LOQ	<LOQ	<LOQ	26	62	193
Mn_{total}	151	64	37	65	72	<LOQ	<LOQ	<LOQ	<LOQ	78	164	643
$Mn_{leachable}$	152	30	37	36	25	<LOQ	<LOQ	<LOQ	<LOQ	45	74	169
Sb_{total}	151	0.8	73	4.2	14.2	<LOQ	<LOQ	<LOQ	1.2	2.3	14.5	117.9
$Sb_{leachable}$	152	0.4	41	0.9	2.4	<LOQ	<LOQ	<LOQ	<LOQ	0.6	4.3	18.5

Arsenic (total and leachable) was present in almost all the analyzed samples. Regarding the other elements, only total Sb was quantified 73% of samples, while the remaining of metals were only quantified in less than 2/3 of the samples.

CONCLUSION:

This study allowed establishing sampling and analysis procedures to evaluate metals and allergens in settled dust of classrooms indoor. Moreover, it made it possible to provide a first overview of children exposure to these parameters. Low levels of allergens in settled dust were observed and only some of the selected metals, such as As and Sb, were quantified in a high percentage of samples, in addition to Pb.

ACKNOWLEDGMENTS:

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Mobile phone use among children: an emerging child public health issue?

Authors: Hans-Peter Hutter[1], Peter Wallner[1,2], Janie F.Shelton[1], Michael Kundi[1]

1 *Institute for Environmental Health, Center of Public Health, Medical University Vienna, Austria*
2 *Medicine and Environmental Protection, Vienna, Austria*

Presenting Author: Email: hans-peter.hutter@meduniwien.ac.at | Tel.: +43 1 40160-34930

INTRODUCTION:
During the last decade use of mobile phones (MP) has increased in children and spread even among the very young (< 6 years of age). Public concern has been raised regarding adverse health effects related to radiofrequency (RF) electromagnetic fields (EMF).

OBJECTIVES:
To report on the results of a review concerning the use of mobile phones and health effects with children resp. adolescents.

MATERIALS AND METHODS:
A systematic literature search was conducted using Pubmed. Peer-reviewed publications of exposure to RF-EMF in children with relevance to health were included.

RESULTS:
Cross-sectional and cohort studies of MP use in children have focused on wellbeing, behavior, maternal use of MP during pregnancy, and cancer. Few experimental studies (juvenile volunteers >10 years) have focused on cognitive effects of short-term exposure to RF-EMF. Experimental studies, although limited with respect to age and scope, do not indicate large differences between children and adults. Mothers' use of MP during pregnancy or early childhood may adversely affect later psychosocial development of the child. The only epidemiological investigation on brain tumors published so far demonstrated a significant trend of increasing risk with increasing time since first use based on operator data.

DISCUSSION:
Despite concerns about the long-term health effects of RF-EMF exposure, there is very limited scientific research on the issue of mobile phone use by children. Childhood RF-EMF exposure is a concern for several reasons such as: deeper RF penetration into the head, developing central nervous system, and longer lifetime exposure. RF-fields from cell phones are classified as a possible human carcinogen by the International Agency for Research on Cancer (2013) and there is evidence for greater risk if MP use starts early in life.

CONCLUSION:
The popularity of MP use even among very young children is growing and there are even applications for use at infant age. Health authorities should prohibit advertisement of MP for children and selling of "apps" specifically designed for the very young and out of an abundance of caution, should discourage parents to provide MPs to infants and young children. Children and parents should be informed about prudent use of a MP. Research should be promoted including aspects of addictive behavior in MP use.

From a child public health standpoint a more prudent use of MPs is needed. Pediatricians as well as public health specialists are called for action, as the children using MP are becoming very young.

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Nitrate concentration in exhaled breath condensate and indoor air quality - relationship in preschool-age children

Authors: João Marques[1,2], Pedro Martins[1,2], Joana Belo[1], Iolanda Caires[2], Maria Guarino[3], Catarina Pedro[2], Manuela Cano[3], Paula Leiria-Pinto[1,2], Nuno Neuparth[1,2]

1 *Immunoallergy Department, Hospital de Dona Estefânia - CHLC, Lisboa, Portugal*
2 *CEDOC, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Lisboa, Portugal*
3 *Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal*

Presenting Author: Email: gasparmarques@yahoo.com.br | Tel.: +351 213 126 600 | Fax: +351 213 126 667

INTRODUCTION:

In modern society, children spend most of their time indoors (up to 85%), at home or in children day care centers (DCC), and they are particularly vulnerable to the effects of indoor air quality (IAQ)[1]. Different studies have shown that IAQ and ventilation are associated with respiratory symptoms, allergy and infections. The evaluation of metabolites of nitric oxide (NO), namely nitrates (NO3-) in exhaled breath condensate (EBC), is a good biomarker of airway inflammation. Studies evaluating the relationship between individual exposure to indoor pollutants and airway inflammation are scarce, particularly in preschool-age children.

OBJECTIVES:

Evaluate the relationship between airway inflammation parameters in EBC of children attending children day care centers and indoor air quality (IAQ) in these institutions.

MATERIALS AND METHODS:

In the 2nd phase of ENVIRH project, EBC samples were collected, using a disposable device (RTube - Respiratory Research Inc., Austin, TX, USA), from a group of preschool-age children. Exhaled breath is saturated with water vapor, which can be condensed by cooling and used to sample a wide range of mediators. Children aged between 4 and 5 years were invited to participate but a limit of 100 EBC collections was established due to budgetary constraints. EBC was collected over a 15 minute period and samples were frozen at -40°C. Airway inflammation biomarkers (pH and total nitrites/nitrates) were determined. pH was measured through a glass microelectrode (Hi 9025, Hanna Instruments, Italy) after sample deaerating with argon. Nitrate levels were determined through a chemoluminescence method by reaction with vanadium trichloride and release of nitrous oxide with a Sievers 280i NOATM analyser (Sievers Instruments, Inc., CO, USA). From the several IAQ analyzed parameters, main ones were:PM10, VOCs, house dust mites, fungi, bacteria, temperature and relative humidity.Evaluation was carried out under typical occupation conditions, during everyday activities.Chosen methods to evaluate chemical parameters were based on the Technical Note NT-SCE-02 document that establishes the proper methodologies to perform indoor air quality audits in buildings, in accordance with published legislation and reference methods were used for PM10 and VOCs. Thermal comfort, and bacteria and fungi quantifications followed accredited methods that comply with ISO and EN standards. No standards or reference methods are described to quantify house dust mite antigens. Dust samples for house dust mite evaluation were collected on filters using a vacuum cleaner with a Dustream™ collector (INDOOR Biotechnologies LTD, Cardiff, Wales). Dust samples were analyzed using an ELISA test to quantify Derp1 and Der f1 mite allergens. For data analysis, two logistic random-intercept models were used. The level of significance considered was $\alpha = 0.05$.

RESULTS AND DISCUSSION:

We collected 100 samples of EBC in preschool-age children, with a mean age of 5.0±0.6 years. 57% of the subjects were male. Schoolroom average occupancy was 19.73±3.97 children. EBC NO3- mean values were 4.42±1.60 µmol and VOCs median values were 200 µg/m3 (P25-P75: 82-436 µg/m3). When analyzing the

association between IAQ parameters and airway inflammation, it was only found an association between VOCs and NO3-: regression coefficient ($\hat{\beta}$) = 0.32(CI95%: 0.11 to 0.53; p=0.003).No significant association between PM10, house dust mites, fungi, bacteria, temperature and relative humidity was found. EBC pH had no association with any of the main IAQ parameters analysed. The relationship between EBC analyses can be a useful non-invasive and easy-to-collect technique for airway inflammation monitoring if proper parameters are defined. Different studies showed that nitrogen products and in EBC were associated with asthma and FEV_1deterioration.

CONCLUSION:

At this study, VOC were the only IAQ parameter significantly associated with EBC airway inflammation biomarkers, namely nitrates. VOCs monitoring may constitute a parameter to consider in order to minimize the impact of IAQ in airway inflammation of preschool-age children.

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Noise induced hearing loss among gym teachers

Authors: Aida Sousa[1], Matilde Rodrigues[1], Raquel Alves[1], Inês Moreira[1]

1 *Research Centre on Environment and Health, Allied Health Sciences School of Polytechnic Institute of Porto, Portugal*

Presenting Author: Email: aidas@eu.ipp.pt | Tel.: +351 222 063 232 | Fax: +351 222 063 232

INTRODUCTION:

Noise is one of the most common workplace hazards, being the source of several problems to the workers' health and to the organization. In fact, noise-induced hearing loss (NIHL) has been listed as one of the most prevalent self-reported illness in Europe (Eurostat, 2004), being subject of great concern at occupational settings. The development process of the NIHL is along the years of exposition, being the initial damage caused by loud sounds often small and the source of slight hearing problems like temporary hearing threshold shift. However, with repeated exposure to loud sounds, hearing disturbances increase and NIHL will be developed. For other side, peak sound levels can be the source of an acoustic trauma. Despite the importance of NIHL, people exposed to high noise levels can also experience other health effects as tinnitus, hyperacusis, diplacusis and balance dysfunction. These health problems related with noise exposition are largely described for industrial settings; however, other professional groups can also be exposed to high noise levels from different sources than noisy machines, as is the case of loud music, which can be also the source of NIHL. In fact, several studies had showed that occupational exposure to music at a high sound pressure level could be the source of NIHL (Sataloff and Sataloff, 1998). Indoor gymnasiums are one of the occupational environments where professionals can be exposed to loud music. Different gym classes can be identified at gymnasiums, which are accompanied by music at high sound pressure levels that can exceeding the legal recommendations. Accordingly, professors can be exposed to high noise levels in the course of classes, being important to assess the risk of NIHL.

OBJECTIVES:

This work aims to evaluate the prevalence of loud music induced hearing disturbances among gym teachers.

MATERIALS AND METHODS:

A sample of 10 gym teachers, ages between 28 and 43 years old, took part in this study. Sound pressure levels exposure has been evaluated with acoustic dosimeters during the different gym classes - cycle, dance kids, push power and bodybuilding. Audiological evaluation took place including pure tone audiometry, tympanometry, stapedial reflex threshold and transient otoacoustic emitions.

RESULTS AND DISCUSSION:

Subjects worked more than 10 years, 6 days per week and between 3 to 8 hours per day. They had a normal hearing but a slight acoustic trauma at 3 to 6 KHz. Tympanometry, stapedial reflex threshold and transient otoacoustic emissions were normal. None complaint about tinnitus was identified and only one reported to balance dysfunction. These results were related to the high noise levels found in the course of the classes. Gym teachers were exposed to values of average equivalent continuous sound pressure levels (L_{Aeq}) between 79,2 dB(A) and 102,6 dB(A). Lower L_{Aeq} values were found at bodybuilding classes and higher levels at Push Power. In relation to the peak sound pressure level (L_{Cpeak}), the obtained values ranged between 120,5 dB(C) and 133,6 dB(C), being lower than the actual lower exposure action value presented in the Portuguese legislation. According the results, it is expected that in the course of the years of exposition the audiological damages from getting worse, being important the implementation of risk reduction measures.

Table 1 - Noise exposure

Gym classes	$L_{Aeq\ (dB)}$	L_{Cpiak}
Cycle	100,9	133.2
Dance kids	98,8	133.6
Push power	102,6 79,2	132.5
Bodybuilding		120.5

CONCLUSION:

High sound pressure level exposure is real among gym teachers. They may be at risk for hearing loss. In order to prevent it, is necessary to raise awareness to the problem and develop hearing conservation programs to this population.

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Adolescents' attitudes towards anorexia nervosa and bulimia nervosa

Authors: Dorota Żołnierczuk-Kieliszek, Teresa B Kulik, Mariola Janiszewska, Ewelina Piwko

Chair of Public Heath, Medical University of Lublin, Poland

Presenting Author: Email: dorota.zolnierczuk@gmail.com | Tel.: +48 794 984 699 | Fax: +48 817 423 714

INTRODUCTION:
Eating disorders are more common in the Western World, where almost everybody has easy access to large amount of food. The problem affects mainly women, especially of Caucasian race. Incidence rates of anorexia and bulimia are higher in the upper social classes. In other ethnic groups incidence rates are definitely lower.

OBJECTIVES:
The aim of the study was to analyze the attitudes of high school students to anorexia and bulimia.

MATERIALS AND METHODS:
The study was conducted from December 2012 to March 2013 and involved 150 3rd grade students in two public high schools in Hrubieszów (a town in southeastern Poland, Lublin Province, with a population of 18,661). All participants gave their informed consent. The research was realized by means of a survey method, a poll auditorium technique. An original questionnaire consisting of 39 questions was used. The examined pupils were filling the questionnaire in the presence of the researcher. Explanations were given by her to any respondents' doubts or questions.

The attitudes of high class students towards anorexia and bulimia were treated as dependant variables, whereas the pupil's gender, permanent place of residence, family situation (being raised by both parents or in incomplete family) and the respondents' body mass were treated as independent variables.

To identify distinctions and dependences between variables chi^2 test was used. For a small number, Yates correction was applied. Values with probability <0.05 were taken as statistically significant. Statistical analysis was conducted using STATISTICA v 9.0 software (StatSoft, Poland).

The students' age varied between 18 and 20 years, mean 19.08; standard deviation 0.47. Most of the polled subjects were female teenagers (64.0%). Over a half of the respondents were living in the rural areas (55.3%), the rest were town and city dwellers. Over three fourths of the pupils (77.0%) declared that they were brought up by both parents (complete family), 22.7% of them were raised by only one parent or by other relatives (incomplete family).

The pupils were asked to give their body mass (in kg) and their height (in cm). The body mass index of every pupil was calculated. It turned out that the body mass of 75.0% of the students was normal, 16.7% of the students were overweight, 2.7% were obese and 5.3% teenagers were underweight. Statistical differences were observed according to gender: obesity and overweight were more common among males, whereas underweight was more common among females ($p<0.05$).

RESULTS AND DISCUSSION:
95.0% respondents considered anorexia and bulimia to be human illnesses. None of the examined expressed positive attitude towards anorexia. 1.3% of the respondents declared positive attitude towards bulimia. Less than a half of the examined pupils were satisfied with their appearance, the satisfaction was more prevalent among men. 39.3% adolescents wanted to lose their weight, the desire was significantly more common

among women. Our results coincide in this respect with those obtained by Chwałczyńska and Bembenek (2010), who proved that 36.0% of 16-years old girls from middle school in Tarnów (PodkarpackieVoivodeship) were not satisfied with their appearance and used different methods of weight reduction, mainly starvation.

People suffering from anorexia collect information on food energy very often. They are often experts in this matter. 64.0% of the polled adolescents revealed that they had never counted calories of eaten food, 29.3% pupils declared counting calories of their meals occasionally, 4.7% of respondents often count calories during their meals, and 2.0% of the students always count calories during their meals. So in our study 36.0% of respondents declared counting calories during meals. The findings of Ziora, Pilarz, Sztyc, and Oświęcimska (2009) confirm the observation.

45.3% respondents were on a weight loss diet in the past or at the moment of conducting the survey (36.0% and 9,3% respectively), girls more often than boys. According to Ziora, Pilarz, Sztyc, and Oświęcimska (2009) total 49.9% of secondary and high schools students in Gliwice (Silesian Voivodeship) tried dieting at least once in their life. Our data suggest that 50.0% of female students in comparison with 37.1% of male students have ever been on a weight loss diet. It corresponds with the findings of Sepulveda, Carrobles, and Gandarillas (2010) who claim that Spanish female students aged 18-26 presented unhealthy weight-control behaviours as dieting, laxatives use or self-induced vomiting to lose weight more often than males. It also confirms the conclusions of Forman-Hoffman (2004) that abnormal eating and weight control practices during the past month were reported by over 26% of female students and 10% of male students from 144 different high schools in the United States.

Anorexia nervosa can be diagnosed when a patient's body mass index is lower than 17.5. It is important to know the norms of the index. 72.0% of the polled adolescents knew the proper values of BMI, 20.0% were not able to answer the question, 8.0% gave the false answer.

Anorexia and bulimia are characterized by common features, such as preoccupation with eating problems, with own body mass and own body shape, self-induced vomiting and the use of laxative or diuretic drugs. Both illnesses may lead to metabolic and hormonal disorders. Sometimes the symptoms of anorexia coexist with the symptoms of bulimia. In spite of similar symptoms anorexia and bulimia are different eating disorders. The vast majority of students (84.0%) represented the opinion that anorexia and bulimia constituted different illnesses. 7.3% of the examined claimed that anorexia and bulimia were synonyms, and 8.7% were unable to answer the question. Male students were significantly more often unable to answer the question (20.4% males versus 2.1% females, $p<0.05$). The same conclusion refers to pupils being brought up in incomplete family (20.6% students from incomplete family did not know the answer versus 5.2% students with both parents, $p<0.05$).

Anorexia nervosa starts with being on diet predominantly. The absence of three consecutive menstrual cycles is the elementary symptom of anorexia among women. The sufferers may develop complications among which anemia and muscle hypotrophy appear first. In our study girls more often than boys knew the symptoms and complications of both eating disorders. The differences between genders were statistically significant. The percentage of students claiming that anorexia and bulimia require hospital treatment was significantly higher among women. On the other hand male students more often declared that receiving medication as an out-patient is sufficient to treat anorexia and bulimia and that the illnesses can be cured by one self.

Anorexia can lead to patient's death. Cachexia or suicide is usually the direct cause of death. The answer of 92.0% of adolescents to the question "Can anorexia lead to death?" was „yes", 0.7% said „no" and 7.3% or respondents were unable to answer the question. Similar conclusions were reached by Ziora, Pilarz, Sztyc, and Oświęcimska (2009).

To sum up, the students' knowledge about anorexia and bulimia can be estimated as insufficient, although examined adolescents were aware of main symptoms, complications and management of anorexia and bulimia. The level of knowledge on anorexia and bulimia was significantly higher among female than male respondents.

The mass media turned out to be the primary source of students' knowledge about anorexia and bulimia. The vast majority of the pupils got know about various aspects of the illnesses from the internet (80.0%), television (72.7%) nearly half of the respondents from newspapers and magazines (46.0%).To a lesser extent, students derived knowledge about anorexia and bulimia from scientific literature, from parents, friends, colleagues and lessons at school. Similar outcomes were obtained by Ziora, Pilarz, Sztyc, and Oświęcimska (2009), who claim that 70.5% of secondary and high schools pupils in Gliwice (Silesian Voivodeship) obtained the information about anorexia from the mass media (internet and television), whereas pupils' parents or school lessons gave them the knowledge to a lesser extent. The findings of Lee (1997) confirm that mass media are the main source of Chinese students' information about anorexia.

CONCLUSION:

According to 95.0% of respondents anorexia and bulimia are human illnesses. None of the examined expressed positive attitude towards anorexia. 1.3% of the respondents declared positive attitude towards bulimia.

The students' knowledge about anorexia and bulimia can be estimated as insufficient, although examined adolescents were aware of main symptoms, complications and management of anorexia and bulimia. The level of knowledge on anorexia and bulimia was significantly higher among female than male respondents.
Professional eating disorders educational programs should be implemented in the population of Polish students. It will help to identify anorexia and bulimia in the population in the early stages what will increase the probability of full recovery from the illnesses.

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Natural ventilation as a simple strategy for the improvement of the indoor environmental quality in classrooms

Authors: Ricardo M.S.F. Almeida[1], Manuel Pinto[1], Paulo G. Pinho[1], Luís T. de Lemos[1]

1 *School of Technology & Management, Polytechnic Institute of Viseu, Portugal*

Presenting Author: Email: ralmeida@estv.ipv.pt | Tel.: +351 232 480 500 | Fax: +351 232 424 651

INTRODUCTION:

It now seems clear that the indoor environmental conditions in classrooms, in particular the effect of temperature and indoor air quality (IAQ), influence students' health, attitude and performance. In recent years several studies that evaluate the effects of the classrooms environmental conditions on the learning process were published (Bakó-Biró, Clements-Croome, Kochhar, Awbi, & Williams, 2012; De Giuli, Da Pos, & De Carli, 2012).

In recent years several studies regarding indoor environmental quality (IEQ) were published, covering schools of different levels of education with natural ventilation systems (single façade or cross ventilation), in continuous or purge ventilation. Natural ventilation proved to have great potential, particularly in southern European climate. However, the results, particularly in terms of thermal comfort (air temperature) and ventilation rate or levels of CO_2concentration, have not always been satisfactory.

OBJECTIVES:

Natural ventilation, as other ventilation systems has advantages and disadvantages. However, towards the goals of reducing energy consumption and considering the adaptive possibilities of students, we believe that in Portugal, and in other southern European countries, natural ventilation in schools, both new and refurbished, has great potential for successful implementation. It was on that basis that this study was developed.

MATERIALS AND METHODS:

This paper presents the results of part of a research project of broad scope which aims to assess, in an integrated way, several aspects that contribute to IEQ in classrooms. The project comprises 7 schools of different levels of education (from kindergarten to college) located in urban and peri-urban areas of the city of Viseu (within 5 km), installed in buildings of different types and ages, and a total of 28 classrooms are involved, with different orientations and sun exposure. The classrooms had an approximate average area of 50 m^2 and all have bottom-hung windows on the outside and small openings in the interior with adjoining corridors, allowing for the implementation of a cross ventilation strategy.

This part of the research was performed during September and October of 2013 and included the evaluation of the hygrothermal performance, for 4 consecutive days in each school, with occupied classrooms. Air temperature (T), relative humidity (RH) and carbon dioxide (CO_2) concentration were measured. In each school were selected 2 classrooms where specific conditions for cross ventilation were imposed (ventilation protocol). The other 2 classrooms had no control on the window opening.

The sampling interval was of 1 minute and the existing international recommendations were accomplished, in particular, for the location of the sensor, avoiding proximity to windows and heaters. Generally, sensors were positioned next to the teacher desk (approximate height of 0.70 m). The following equipment were used: 1 indoor air quality measurement device Fluke, ref.: 975, that records T, RH and CO_2 concentration (T accuracy ±0.5 ºC; RH accuracy ±2%; CO_2 concentration accuracy 2.75% + 75 ppm), 3 data loggers Hobo U12

for T and RH (T accuracy ±0.35 ºC; RH accuracy ±2.5%) and 3 infrared dispersive measurement devices Telaire 7001 for CO_2 concentration (±50 ppm or ±5% of the reading, whichever is greater).

RESULTS AND DISCUSSION:

Previous results revealed that IEQ of the classrooms was poor in terms of IAQ, namely CO_2 concentration(R. Almeida & Freitas, 2010; R. M. S. F. Almeida, Pinho, & Lemos, 2013). From those results the importance of improving classrooms ventilation arises. The next step on this investigation was then to improve the ventilation rates by simple adjustments based on a ventilation protocol that must be implemented in such a manner that the comfort conditions of the classrooms are not neglected.

As an example, Figure1 shows the CO_2 concentration in 8 classrooms of 2 school buildings, 4 with ventilation protocol and the others without ventilation protocol. It is included in the graph the mean concentration and respective standard deviation. For the statistical analysis only the occupied periods of the classrooms were used.

Attending the results shown in Figure 1it is clear the importance and the positive impact of the ventilation protocol in the classrooms' IAQ. The improvement is observed both in terms of mean values and lower standard deviation, which indicates minor fluctuations and variability of the results and, consequently, lower peak values of CO_2 concentration.

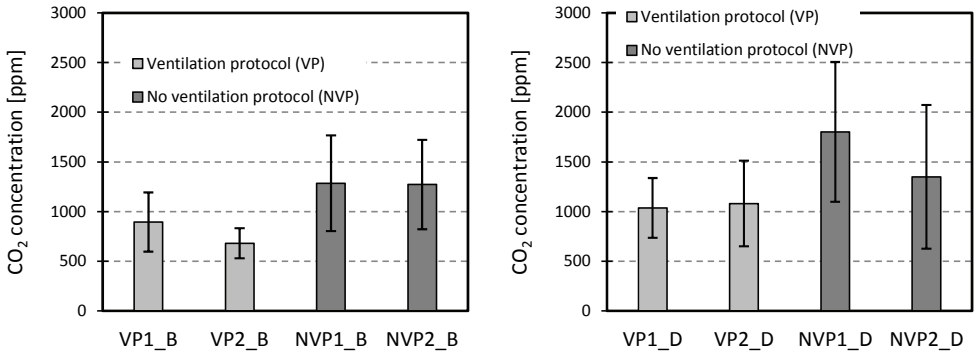


Figure 1 - CO2 concentration mean value and standard deviation in classrooms of 2 school buildings, with and without ventilation protocol

A more detailed analysis is presented in Table 1, including mean values of T, RH and CO_2 concentration separately for classrooms with and without ventilation protocol. The percent improvement in terms of CO_2 concentration is also indicated, with positive values corresponding to a reduction in concentration.

Table 1 - IEQ mean results for classrooms with and without ventilation protocol

School Id.	T (ºC)		RH (%)		CO_2 (ppm)		
	VP	NVP	VP	NVP	VP	NVP	%
A	24.1	24.7	67	70	978	1436	32
B	27.7	26.6	46	53	788	1279	38
C	26.6	27.0	45	45	1611	1222	-32
D	23.0	24.0	67	66	1059	1576	33
E	26.5	26.6	54	57	768	949	20
F	24.4	24.2	48	51	954	1316	28
G	24.3	22.9	52	64	1370	2485	47

The introduction of a ventilation protocol resulted on an improvement of the IAQ in 6 schools. The only exception was school building C, probably because users (teachers) had the possibility to reject the protocol if they felt uncomfortable. A part from this particular situation, the implementation of the ventilation protocol was very positive: the most interesting performance was obtained in school G with a reduction of 47% in the CO_2 concentration and the even for the less efficient scenario (school E) an improvement of 20%

was obtained. Another important result that must be underlined is that the comfort conditions were not neglected with this protocol since no significant difference of temperature between classrooms was found. However, it is important to refer that this results were obtained during autumn, additional measurements must be performed for winter conditions to validate the strategy.

CONCLUSION:

A simple ventilation protocol based on a cross ventilation strategy was implemented in several classrooms of Viseu and the IEQ was assessed. Results were very encouraging and globally a significant improvement on the CO_2 concentration was observed. This strategy should continue to be exploited and validated for winter conditions.

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Daylighting in classrooms –the daylight factor as a performance criterion

Authors: Manuel Pinto[1], Ricardo M.S.F. Almeida[1], Paulo G. Pinho[1], Luís T. de Lemos[1], António Santos[2]

1 *School of Technology & Management, Polytechnic Institute of Viseu, Portugal*
2 *National Laboratory of Civil Engineering - LNEC, Lisboa, Portugal*

Presenting Author: Email: ralmeida@estv.ipv.pt | Tel.: +351 232 480 500 | Fax: +351 232 424 651

INTRODUCTION:
Recent research has shown that environmental conditions in classrooms, and namely daylighting conditions, can influence students health, well-being and performance. In the last years several studies, dealing with the effects of environmental conditions in classrooms in the learning process, have been published (Winterbottom, & Wilkins, 2009; Barret, Zhang, Moffat, & Kobbacy, 2013).

The daylight factor (DF) is the most used parameter in the characterization and quantification of daylight in buildings. The DF at a point of a plane inside a room is defined as the ratio (expressed as a percentage) between the daylight illuminance at that point in the interior of the room and the simultaneous exterior horizontal global illuminance due to a hemisphere of a sky of known or assumed luminance distribution (usually, a CIE overcast sky luminance distribution is considered). The DF reflects the effectiveness of daylight penetration in a particular room or space. The exterior daylight conditions may vary, but the DF remains constant, since the interior illuminances change proportionally to the simultaneous changes in the exterior daylight conditions (Santos, 2006).

OBJECTIVES:
Daylighting, like artificial lighting has advantages and disadvantages. However, taking into account the need for energy consumption reductions, we believe that, in Portugal, and in other Southern European countries, the conscious use of daylight in schools, new or rehabilitated, has a great potential for improving the comfort and the academic performance of users, contributing, simultaneously for the rational use of energy in buildings.

This paper presents the results of part of a research project, of a broader scope, which aims to assess, in an integrated way, several aspects that contribute to indoor environmental quality (IEQ) in classrooms. The project comprises 8 schools of different levels of education (from kindergarten to college level) located in urban and peri-urban areas of the city of Viseu (roughly in the centre of Portugal at approximately 500 m of altitude), installed in buildings of different types and dates of construction. A total of 32 classrooms are involved, with different orientations and sunlight exposures. The classrooms have an approximate average area of 50 m^2.

MATERIALS AND METHODS:
This part of the project was held in December 2013 and January 2014 and included the assessment of the DF at 8 schools, comprising 2 rooms in each school, both located in the top floors. However, due to differences in the nebulosity conditions, there were only considered valid tests in 6 schools. Some of the characteristics of the classrooms used in the study are summarized in Table 1.

Table 1 - School and classroom building characterization

School Id	Level of education	Year of construction	Classroom Id	Window to floor ratio - WFR [%]	Window to wall ratio - WWR [%]
B	Lower secondary	1991	B3	9	20
			B4	12	26
C	Primary	2004	C3	17	31
			C4	17	31
D	Lower secondary	1968	D1	18	38
			D2	18	38
E	Primary and Lower secondary	1996	E3	12	20
			E4	13	27
F	Primary	1958	F2	17	32
			F4	17	33
G	Primary	2011	G3	18	33
			G4	17	32
			Average	15	30

From Table 1, it can be concluded that the window to floor ratio (WFR) and the window to wall ratio (WWR) have high average values. These high glazing areas can lead to overheating problems in the cooling season, which can be avoided if appropriate shading devices (low solar factor with high visible transmittance) are also used. The types of shading devices used are as follows:

- in two of the schools (D and E) only interior shading devices are available;
- among the interior shading devices, the fabric rolling devices prevail (C, F and G);
- four of the schools have both interior and exterior shading devices (C, F, G and H);
- the use of fixed shading devices, horizontal or vertical tabs, is used by schools in various construction periods (schools A, C, and G).

The measurements were obtained using two luxmeters calibrated in the measuring range used (error + expanded uncertainty < 6%). The mesh comprised 25 measurement points spaced approximately 1 m, at a height coinciding with school desks (0.70 m).The measures were made in 5 rows each with 5 points.

RESULTS AND DISCUSSION:

Under the same project, previous results showed that the levels of illuminance, with artificial lighting, in some rooms were not adequate (Pinto, Almeida, Pinho & Lemos, 2013). The present measurement campaign intended to measure the DF in order to assess the daylighting conditions. Figure 1 presents the results obtained in two of the classrooms.

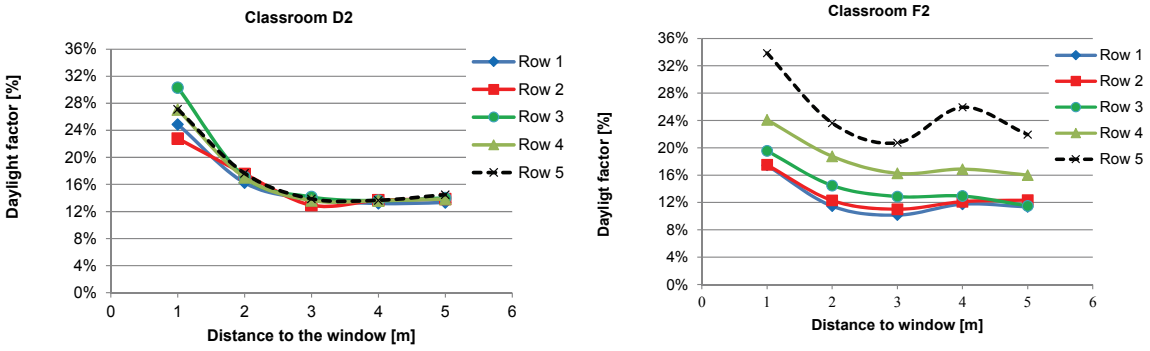


Figure 1 - Daylight factors for the classrooms D2 and F2

In Figure 1, the results in F2 classroom reflect the influence (in points 4 and 5) of large glazing areas facing each other (bilateral daylighting). In accordance with the high values of WWR and WFR, high ADF were to be expected. The values found for both the DF and for the average DF (ADF) are very high, well above the recommended (Santos, 2006).

The measured average daylight factor (ADF) in the different classrooms (without shading devices activated) varied from a maximum of 24 % in classroom B4 and a minimum of 8 % in classroom D1. The mean of the measured ADF were about 18%, which indicates that the quantity of daylight in the classrooms is substantial. As a consequence of these high ADF values, shading activation, particularly in overheating periods is also high. Glare problems due to large glazing areas also contribute to the frequent use of those shading devices, in order to minimize visual discomfort.

CONCLUSION:

In Southern European regions, the DF can be used to define minimum requirements but not average conditions throughout the year. Therefore, the minimum daylighting natural lighting in classrooms studied denoting that are reasonable and the measurements previously performed with artificial lighting was not optimal. This project will serve to set guidelines with regard to natural lighting in architectural designs for new or rehabilitated schools.

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Sensing the Environment with Human Senses and Electronic Sensors in Teachers Education

Authors: Maria João Silva[1], Sara Aboim[1], Alzira Costa[2,] Teresa Ramos[3] Alexandre Pinto[1]

1 *inED (Center for Research and Innovation) School of Education, Polytechnic of Porto, Portugal*
2 *School of Education, Polytechnic of Porto, Portugal*
3 *School of Allied Health Sciences, Polytechnic of Porto, Portugal*

Presenting Author: Email: saraaboim22@gmail.com | Tel.: +351 225 073 480 | Fax: +351 225 073 464

INTRODUCTION:

Senses are the fundamental interface between human beings and the environment and they are part of everyday experiences (Mason, & Davies, 2009). Sensors can be considered extensions of human senses (Magnani, 2004). In the SOS Abstract research project (Using Sensors and Senses in the Environment to Develop Abstract Thinking), sensors are approached as learning mediators (Magnani, 2004), since they can be used by students to explore natural phenomena in a more motivated and engaged way, extending their thinking and creating multiple representations of their understanding (Hug, Krajcik and Marx, 2005). In environmental education, children can use senses and sensors to observe, represent, and control variables in authentic activities that explore complex concepts while developing children's abstract thinking (Silva et al., 2013).

In order to improve environmental health education through the joint use of human senses and electronic sensors, it is necessary to empower future teachers with a set of experimental and didactic competences during pre-service and in-service training.

OBJECTIVES:

In the work here presented, in order to support future elementary school teachers in developing environmental authentic activities, using human senses and electronic sensors, the authors developed a set of resources and validated them through their use in the School of Education of the Polytechnic Institute (a teachers' training school). Two kinds of resources were developed and used:

- Empirical resources: experimental protocols and environmental data resulting from the characterization of a set of variables of indoor air quality and of Douro estuary water quality;
- Theoretical resources: background information on the relevance of the characterized variables to environmental health education.

MATERIALS AND METHODS:

The empirical and theoretical resources were produced in the framework of the SOS Abstract research project, in the context of two environmental health undergraduate traineeships and one undergraduate research project, which also contributed to a PhD project that studies the use of senses and sensors in authentic activities with children.

In the environment of the School of Education of the Polytechnic Institute, a set of variables related to environmental health, namely indoor air quality variables - such as temperature, humidity, illuminance, sound level, and carbon dioxide concentration - and Douro estuary water quality variables - such as conductivity, turbidly and pH - were studied, using senses and sensors (Table1). It was on the basis of this environmental qualitative and quantitative characterization that the empirical and theoretical resources were created and used to support authentic learning activities.

Table 1 - Variables, electronic sensors and human senses used to characterize a set of variables of the School environment

Variables	Electronic Sensors	Human Senses
Temperature	Pasco PS - 2124	Somatic senses[1]
Humidity (absolute and relative)	Pasco PS - 2124	Somatic senses
Sound level	Xplorer GLX para modo sonómetro, Pasco PS - 2002	Auditory sense
Carbon Dioxide Concentration	Pasco PS - 2110	Somatic senses
Illuminance	Pasco PS-2106A	Visual sense
Conductivity	Pasco PS - 2116	Somatic senses, Gustatory (taste) sense
pH	Pasco PS - 2147	Gustatory (taste) sense
Turbidity	Pasco PS - 2122	Visual sense



Figure 1 - Photographs of three sensors used to characterize the School's indoor environment: Humidity and temperature sensor (Pasco PS - 2124); light sensor (Pasco PS - 2106A) and carbon dioxide sensor (Pasco PS - 2110)

RESULTS AND DISCUSSION:

The implemented environmental characterization allowed reporting that:

- The School's indoor environment is heterogeneous regarding the evaluated variables. Some measured values exceeded the reference values, namely in what concerns temperature, and sound level. Suggestions were made to solve the detected problems;
- The analyses of Douro estuary water, performed in four locations, illustrated the complexity of the estuary environment and of the variability of salinity and turbidity;
- In some cases, the sensory evaluation made by the trainees produced estimations that were not in accordance with the values that were subsequently measured by the sensors. Such discordance motivated relevant reflections and informed posterior estimations;
- It was clear that the used sensors were adequate to didactic activities (given their robustness, easy, rapid and regular functioning, as well as their efficient way of making data visible and comparable) and that they were inadequate for analysis of environmental compliance. They allowed the analyses of the variations of the measured values in different locations, but the comparisons with the reference values were not always reliable. This was the case with the light sensor. Therefore, it was necessary to use of a more rigorous luxmeter (MAVOLUX 5032C/B USB) to make comparisons with the reference values.
-

This environmental characterization informed the creation of the empirical and theoretical didactic resources. It is important to highlight that:

- The experimental protocols integrate not only procedural instructions but also background information on each variable and on its environmental behavior and relevance to environmental health education;
- For each environmental variable, a didactic activity was designed and implemented, guiding and supporting future teachers in qualitative multisensory explorations and estimations and in

[1] Somatic senses allow the sensation of pressure, vibration, pain, temperature, as well as noxious sensations.

subsequent comparisons with the quantitative results of the use of sensors. These activities are being implemented in a successful way, engaging future elementary school teachers;

- In those activities, future teachers are challenged: to use their senses in a free, exploratory and meaningful way to characterize the environment and to make a better use of sensors; to discover the qualitative and quantitative variations of each variable in their everyday spaces, as well as the influencing factors and the consequences to the quality of life; to understand the complementarities of senses and sensors.

CONCLUSION:

In this work, a set of empirical and theoretical resources were developed to guide and support future teachers in using human senses and electronic sensors to explore and characterize their everyday spaces, developing awareness and competences related to environmental health education. The interdisciplinary nature of the authors' team facilitated the emergence of meaningful links between environmental health and teachers education.

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Microbiological evaluation of vegetable salads in school canteens

Authors: Mariana Carneiro[1], Manuela Amorim[1,2], Stephanie Ferreira[1,2], Sandra Mota[1,2], Anabela Moreira[1,2]António Augusto[1,2,3]

1 *Department of Biomedical and Public Health Laboratory Sciences, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

2 *Research Centre of Health and Environment, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

3 *Biogerm, SA, Maia, Portugal*

Presenting Author: Email: mas@estsp.ipp.pt | Tel.: +351 222 092 128

INTRODUCTION:

Currently bacteria are the main responsible for infections and food poisoning, being a huge public health problem worldwide. Vegetables are potential vehicles of these microorganisms and their consumption, especially raw, increases contamination risk.

It has been reported an increase in diseases associated with microbiological contamination by vegetables consumption. Vegetable cultivation and growth process makes them vulnerable to contamination sources that may act at any stage from planting to consumption. *Salmonella*, *Escherichiacoli*, *Staphylococcus* and *Listeria* are the microorganisms most commonly isolated from vegetables responsible for food borne diseases.

OBJECTIVES:

To observe microbiological standards of vegetable salads served in school canteens.

MATERIALS AND METHODS:

We conducted a cross-sectional study using the results from the microbiological study of 188 vegetable salads from school canteens in the North of Portugal, performed in a public health private laboratory.The microbiological study consisted in the determination of colony forming units (CFU) of total microorganisms grown at 30 ° C (CFU/g), faecal contamination biomarkers count (total coliforms (CFU/g), *E. coli* (CFU/g), and coagulase-positive *Staphylococci* (CFU/g)),and pathogens screening (*Salmonella* (CFU/25g) and *Listeria monocytogenes* (CFU/25g)), accordingly to the international standard methodsThe results were classified as "Satisfactory", "Acceptable" and "Non-Satisfactory", using national guidelines.

RESULTS AND DISCUSSION:

We analysed 188 salads results for: microorganisms at 30°C, total coliforms, E. coli, coagulase-positive Staphylococci; Salmonella and Listeria monocytogenes. Our results showed a considerable number of “Non-Satisfactory” samples for microorganisms at 30ºC (10%) and total coliform counting (13%). Regarding E. coli and Listeria monocytogenes detection we found a small percentage of "Acceptable" samples (2% and 1%, respectively) and a large number of "Satisfactory" samples (98% and 100% respectively). For Staphylococcus coagulase positive and Salmonella detection we observed a "Satisfactory" result for all samples. Comparing raw salads (n = 113), and mixed salads (n = 78), we observed a higher number of “Non-Satisfactory” salads for total microorganisms at 30°C in raw salads (15%). The same happened for total coliform counting with a frequency of "Not-Satisfactory" of 14% in raw and 12% in the mixed salads.

CONCLUSION:

In conclusion the salads evaluated do not represent risk of serious disease resulting from their ingestion, since, in general, microbiological values are within the acceptable parameters defined by national guidelines. The microorganisms at 30 °C and coliforms found, despite having some health impact, these are not described as potentially dangerous such as the pathogenic microorganisms.

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Indoor air concentrations of endotoxin in primary schools - Preliminary report for exposure and asthma among children

Authors: Cristiana Pereira[1], Ana Mendes[1], Lívia Aguiar[1], Maria Paula Neves[1], Mariana Pinto[2], André Moreira[2], João Paulo Teixeira[1]

1 *Department of Environmental and Occupational Health, National Health Institute, Porto, Portugal*

2 *Department Immunoallergology, HSJ-FMUP, Porto, Portugal*

Presenting Author: Email: cristiana.pereira@insa.min-saude.pt | Tel.: +351 223 401 156

INTRODUCTION:

Children spend most of their daytime in school, mainly indoors. Overlooked and chronic exposure to pollutants in childhood may result in acute respiratory symptoms and future health problems, namely respiratory affections. Endotoxin is a lipopolysaccharide (LPS) molecule found in the outer membrane of Gram-negative bacteria, occurring ubiquitously in indoor environments. Although some inconsistencies are still found in the literature, exposure to endotoxin has been associated with asthma and other respiratory manifestations in several studies (Thorne, Kulhánková, Yin, Cohn, Arbes & Zeldin, 2005) (Rabinovitch et al., 2005) (Gehring et al., 2001).

OBJECTIVES:

This report aims to assess the concentration of endotoxin present in indoor air at schools and verify a possible correlation between the levels found and asthma among children.

MATERIALS AND METHODS:

This project enrolls a total of 20 primary schools in Porto, Portugal, comprising an overall population of 3500 children. This report will discuss the results obtained in selected classrooms of 10 primary schools (n=35), where endotoxin concentrations were assessed using the Limulus Amebocyte Assay (Lonza) as previously described (Duquenne, 2012) and following the European Standard EN 14031:2003. Lung function tests and anthropometry were also performed, after written consent from parents, to 450 pupils of the selected classrooms. Health questionnaires on respiratory and allergy symptoms were applied both to pupils and parents.

RESULTS AND DISCUSSION:

The results will, on one hand, provide insight into the average levels of endotoxin present in ambient air of schools, setting a measure of exposure, and will also enable to establish a possible contribution from exposure to health effects in children.

Using objective measures to estimate exposure and effect, along with the information collected by means of questionnaires will take us a step forward on the evaluation of endotoxin presence effects in indoor environments.

CONCLUSION:

If an abnormal presence of endotoxin in schools' air is proven and if this correlates with respiratory symptoms in children, some measures can be taken. Procedures to improve indoor air quality in schools as well as the elaboration of recommendations and good practices, along with monitoring plans shall be proposed, implemented and evaluated.

ACKNOWLEDGMENTS:

This study is supported by FCT PTDC/DTP-SPA/1522/2012 (How can indoor air quality affect children allergies and asthma - ARIA)

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Musculoskeletal disorders in the use of backpacks – A review

Authors: Gonçalo A. Pereira[1], Mariana R. Batista[1], Matilde A. Rodrigues[1], Isabel F. Loureiro[2]

1 *Research Centre on Environment and Health, Allied Health Sciences School of Polytechnic of Porto, Portugal*
2 *Centro ALGORITMI University of Minho, Guimarães, Portugal*

Presenting Author: Email: goncalo.asp@hotmail.com | Tel.: +351 916 622 121

INTRODUCTION:
Spine pathologies are not a recent problem. There is evidence that problems related to musculoskeletal disorders (MSDs) in children and adolescents are increasingly (Ebbehoj*et al.*, 2002), and the school environment and related tasks can have influence on it. The school environment is a factor of great influence in the development of postural changes and MSDs, for being the period of development of bone structure. At this period, improper habits in children and adolescents, such as incorrect posture, improper transport of backpacks or excess weight carried in backpacks can also be identified. Therefore, this is the best time to stimulate healthy habits to reduce the probability of occurrence of MSDs, irreversible in adult hood as in this period aspects related to overload and inappropriate postures become determinant to the development of the musculoskeletal system. The incorrect carrying of heavy backpacks can contribute greatly to this problem, which is the focus of this investigation study.

OBJECTIVES:
The aim of this study was to establish the problematic related to the use of backpacks by students. To achieve this issue a literature review was performed.

METHODS:
Research of the scientific publications was conducted on Science Direct and on the Library Knowledge Online (b-on). The keywords used for the papers research were: backpack; injuries; musculoskeletal; disorders; children. Several anatomy books available in municipal libraries were also consulted.

RESULTS AND DISCUSSION:
Usually the backpacks present different kinds of characteristics presenting one or two straps, with or without lumbar belt, with or without padded straps, among others. Therefore, one important step to prevent MSDs in infancy is to select an adequate backpack. To evaluate the most appropriate type of backpack, it is essential to take into account that the weight transported in a backpack transfers the centre of gravity of the body. To compensate this displacement, the body pulls the load towards the front and the centre of gravity moves relatively to the support the base. These changes lead to a rigidity of the spine and abdominal muscles, leading to back pain and injury. Taking this into consideration, it is considered that the most suitable backpack is the one that distributes the weight symmetrically on the shoulders and keeps the load close to the back. However, Oliveira (2013) showed that in most of cases, the backpack used by students is not adequate or it is not used correctly. One of the several identified problems is the overcharging due to excessive load of backpack. Overcharging is defined as a set of forces generated during maintenance of a posture or performing a given movement. When these forces are generated outside the body, are classified as external overload. The transport of excessive weight in backpacks, as a daily practice in the lives of children and adolescents, involves an overcharge on the musculoskeletal system, contributing to the emergence of pain in this population. In fact, there are numerous studies showing that there is an association between the use of backpacks and pains in the spine caused by excess weight (e.g. Bauer & Freivalds, 2009).Therefore, it is important to determine a safe limit related to the weight carried on the backpacks. This limit is defined as the weight from which the risk of MSDs begins to be detected, compromising the health of the individuals. There is no consensus in the literature regarding the acceptable weight in backpacks for children and

teenagers. Although this value varies between 10%-20% of the body weight, most authors suggest that the prevention of possible structural changes in the spine caused by overload should be considered for values below 10%(Bauer & Freivalds, 2009).

CONCLUSION:

This study showed that the use of backpack could be a problem for students. This fact could be related to the type of backpack used, the way that students do the transportation of the backpack and the weight of the backpack. Indeed, transporting overloaded backpacks contributes to increase the pressure on joints and ligaments, which may lead to postural changes. This can contribute to increase the development of musculoskeletal injuries. The literature review noticed that a maximum acceptable weight is always related to a certain group of children having a certain age. Nevertheless it is important to notice that children have different physical characteristics even though belonging to the same interval of age. Therefore, it is necessary to study and determine the ideal weight to carry in the backpacks, but restricting the sample to children with identical body mass index, so it is possible to indicate the maximum acceptable weight according to their personal characteristics and not according to their age.

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FOOD SAFETY AND FOOD SECURITY ABSTRACTS

Fungal contamination assessment in canteens from Portuguese faculties

Authors: Carla Viegas[1], Mateus dos Santos[1], Patrícia Neves[2], Ricardo Dias[1]

1 *Environmental Health RG - Lisbon School of Health Technology - Polytechnique Institute of Lisbon, Portugal*
2 *Lisbon School of Health Technology - Polytechnique Institute of Lisbon, Portugal*

Presenting Author: Email: carla.viegas@estesl.ipl.pt | Tel.: +351 218 980 430 | Fax: +351 218 980 469

INTRODUCTION:
Fungi are introduced into the indoor environment through natural sources, due to open windows and doors, mechanical ventilation systems, and also brought in by individual's shoes and clothing (Gots et al., 2003). Current evidence suggests that excessive moisture promotes mold growth (Fung et al., 2003) and some fungal species have been generally identified as the cause of irritative symptoms, respiratory infections, allergic diseases, alveolitis and organic dust toxic syndrome (ODTS) and other chronic pulmonary diseases (Fung et al., 2003).

OBJECTIVES:
This investigation was designed to assess the environmental fungal contamination in canteens from 8 faculties from Lisbon.

MATERIALS AND METHODS:
Air samples of 250L were collected from canteens in several places (kitchen, storeroom, dining room, scullery, microwave zone, self-service and student's lounge) through an impaction method with a flow rate of 140 L/min onto malt extract agar (MEA) supplemented with chloramphenicol (0.05%), using the Millipore air Tester (Millipore).An outdoor sample was also collected since this was the place regarded as reference.

Surfaces samples were collected by swabbing the surfaces of the same indoor sites, using a 10 by 10 cm square stencil disinfected with 70% alcohol solution between samples according to the International Standard ISO 18593 (2004). The obtained swabs were then plated onto MEA.

All the collected samples were incubated at 27ºC for 5 to 7 days. After laboratory processing and incubation of the collected samples, quantitative (colony-forming units - $CFU.m^{-3}$ and $CFU.m^{-2}$) and qualitative results were obtained with identification of the isolated fungal species. For species identification, microscopic mounts were performed using tease mount and lactophenol cotton blue mount procedures. Morphological Identification was achieved through macro and microscopic characteristics as noted by specific atlas for fungal identification.

RESULTS AND DISCUSSION:
It is suggested that fungal levels found indoors should be compared quantitatively and qualitatively with those found outdoors, because the former is dependent on the latter (Goyer et al., 2001). Five faculties presented higher fungal load than outdoor and in all analysed canteens some fungal species found inside were not identified outside, suggesting inside contamination (Kemp et al., 2002).

Penicillium sp. was the most frequent isolated in air (66.9%), followed by *Geotrichum sp.* (13.7%) and *Chrysonilia* sp. (9.7%). Regarding *Aspergillus* genus, species from 2 different complexes in indoor air were detected, namely species from *A. niger* (2.4%) and *A. Fumigates* (4.0%) complexes. *Alternaria* sp.(3.2%) was also detected in air samples (Figure 1).

In surfaces, the most frequent fungal species isolated were *Penicillium sp.* (61.9%) and *Chrysonilia sp.* (14.3%), followed by *Geotrichum* sp. (9.5%), *Rhizopus* sp.(9.5%) and *Alternaria* sp. (4.8%) (Figure 1).

We must be aware that species belong to *Aspergillus fumigates* complex and *Penicillium* genus are considered indicators of humidity problems and a potential health hazard when present as air contaminants (Goyer et al., 2001). Moreover, according to American Industrial Hygiene Association (AIHA), the confirmed presence from species belonging to *A. fumigates* complex requires the enforcement of corrective measures (AIHA, 1996).

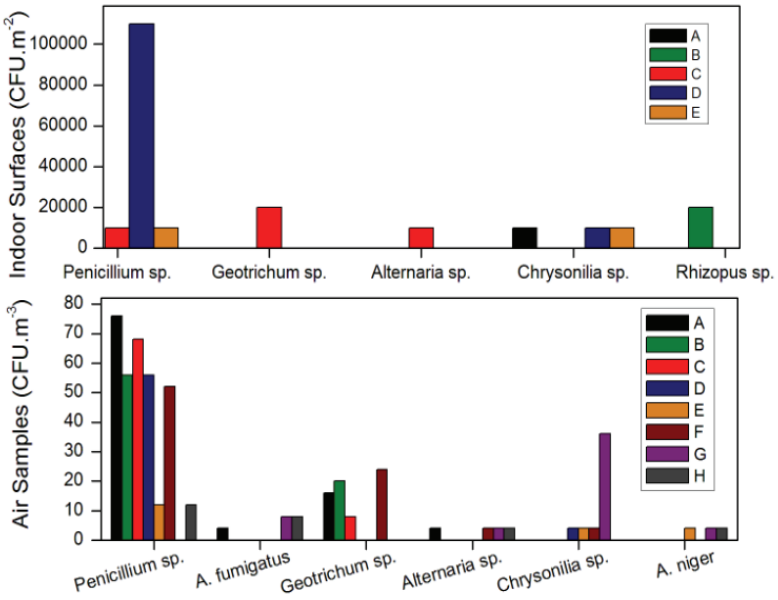


Figure 1 - Fungal distribution from the most prevalent species (*Strictu sensu*)in the eight faculties

CONCLUSION:

Considering the results, it was possible to study fungal contamination of the analyzed canteens and, as such, conclude that this contamination can pose a potential human health risk which could involve invoking corrective measures.

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The effect of skipping breakfast on fiber intake and sweetened beverages consumption in Polish adolescents

Authors: Joanna Kowalkowska, Lidia Wadolowska, Justyna Weronika Wuenstel, Małgorzata Anna Słowińska, Ewa Niedźwiedzka

Department of Human Nutrition, University of Warmia and Mazury in Olsztyn, Poland

Presenting Author: Email: joanna.kowalkowska@uwm.edu.pl | Tel.: +48 89 523 32 70

INTRODUCTION:

In accordance with the dietary recommendations the breakfast should be consumed every day as the one of the most important meal during a day. Breakfast consumption is associated with favourable nutrients intakes including higher intake of dietary fiber, total carbohydrate and lower total fat and cholesterol [1,2]. Consuming breakfast can also contribute to maintaining a body mass index within normal range and prevent obesity [3,4]. Breakfast consumption is also associated with more healthy lifestyle including physical activity. Between 10-30% of adolescents skip breakfast in the developed world [3-5]. More knowledge is needed to explain the relation between skipping breakfast and other dietary habits to predict overall impact of skipping breakfast on health.

OBJECTIVES:

To analyze the relation between skipping breakfast and chosen dietary habits such as fiber intake and sweetened beverages consumption in adolescents.

MATERIALS AND METHODS:

The research involved 1700 adolescents aged 13.0-18.9 years, 790 boys and 910 girls. Breakfast consumption was specified in 3 frequency categories: never (less than once a week), sometimes (1-6 times a week) and everyday. The validated Block's Questionnaire was used to assess fiber intake (Block Screening Questionnaire for Fruit/Vegetable/Fiber Intake, BSQFVF). First, the frequency of consumption of 9 foods rated into 5 categories: from less than once a week (0 points) to every day (4 points). On the basis of sum of points the girls were classified into one of two categories: unacceptable (0-19 points) or acceptable fiber intake (20-36 points) [6]. The sweetened beverages consumption was specified in 3 frequency categories: rarely (less than once a week), sometimes (1-6 times a week) and everyday. The reference groups in logistic regression analysis were boys and girls: (i) consuming breakfast every day, (ii) with acceptable fiber intake and (iii) consuming sweetened beverages rarely (OR=1.00). The odds ratio were adjusted by age and the significance of odds ratio was assessed by Wald's statistics.

RESULTS AND DISCUSSION:

More boys and girls skipping breakfast every day had unacceptable fiber intake and more often drank sweetened beverages every day (Table 1). The unacceptable fiber intake was in 67.4% of boys and 64.9% of girls who never consumed breakfast and 70.0% of boys and 57.0% of girls who consumed breakfast sometimes. Sweetened beverages were drunk every day by 31.6% of boys who never consumed breakfast and 20.5% of boys who consumed breakfast every day ($p<0.01$). Similarly, sweetened beverages were drunk every day by 18.5% of girls who never consumed breakfast and 11.7% of girls who consumed breakfast every day ($p<0.01$).

Table 1 - Fiber intake and sweetened beverages consumption by breakfast consuming in boys and girls aged 13.0-18.9 years (%) (n=1700)

	Breakfast consuming							
	Boys (n=790)			p-value	Girls (n=910)			p-value
	Everyday	Sometimes	Never		Everyday	Sometimes	Never	
Number of sample	435	260	95		443	316	151	
Fiber intake								
Unacceptable#	52.2	70.0	67.4	<0.0001	49.4	57.0	64.9	0.0026
Acceptable&	47.8	30.0	32.6		50.6	43.0	35.1	
Sweetened beverages consumption								
Rarely	14.5	8.5	10.5	0.0095	30.9	19.9	19.2	0.0010
Sometimes	65.1	72.7	57.9		57.3	67.4	62.3	
Everyday	20.5	18.8	31.6		11.7	12.7	18.5	

Fiber intake assessed by Block's Questionnaire: #unacceptable - 0-19 points; &acceptable - 20-36 points;
p-value - significance level of chi^2 test

The odds ratio (OR) for unacceptable fiber intake in girls who never consumed breakfast was 1.79 (95%CI: 1.21,2.64; p<0.01) and 1.91 (95%CI: 1.19, 3.05; p<0.01) in boys who never consumed breakfast (Figure 1). The odds ratio for everyday consuming of sweetened beverages in girls who never consumed breakfast was 2.70 (95%CI: 1.45, 5.04; p<0.01) and 2.13 (95%CI: 0.97, 4.71; p>0.05) in boys who never consumed breakfast.

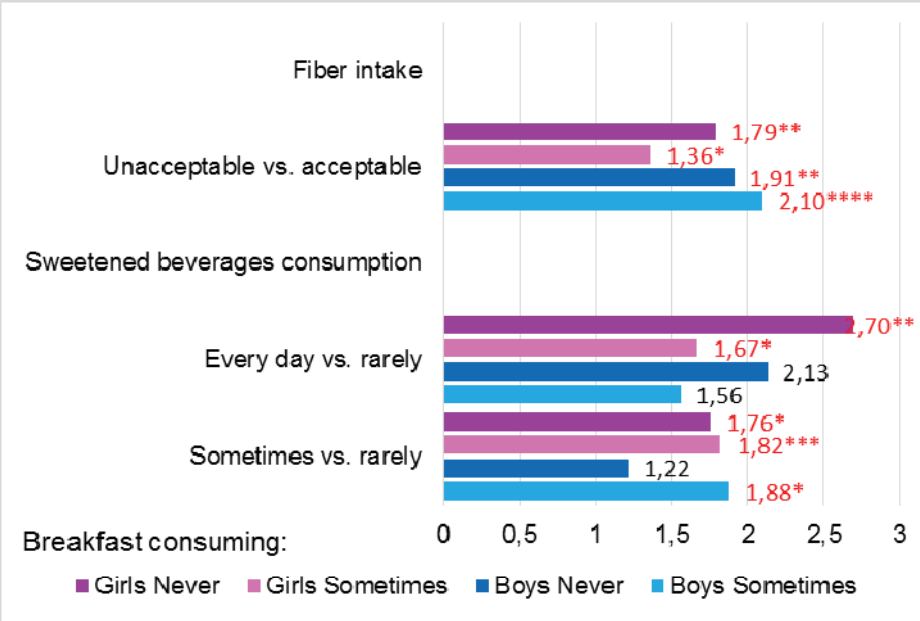


Figure 1 - Odds ratio (OR) of fiber intake and sweetened beverages consumption by breakfast consuming in girls and boys aged 13.0-18.9 years (n=1700)

Fiber intake assessed by Block's Questionnaire: unacceptable - 0-19 points; acceptable - 20-36 points;
* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001 (Wald's statistics)

CONCLUSION:

Skipping breakfast was related to other negative dietary habits. The low fiber intake and everyday consumption of sweetened beverages was found in skipping breakfast both girls and boys. In skipping breakfast adolescent the negative outcomes for health may be stronger because of common effect many of non-healthy dietary habits.

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Assessment of microbiological contamination in different catering units related to food handling in the district of Bragança

Authors: Salgado A[4], Cunha J[4], Abreu J[4], Barros P[5], Pires B[2], Nogueira A[1,3], Afonso A[1,2]

1 *Department of Therapeutic and Diagnostic Technologies, Polytechnic Institute of Bragança (IPB), Bragança, Portugal*
2 *Public Health Laboratory of Bragança, Bragança, Portugal*
3 *Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
4 *Students from the degree of Clinical Analysis and Public Health, School of Health Sciences of Bragança (IPB), Portugal*
5 *Clinical Pathology Laboratory, Hospital of Bragança*

Presenting Author: Email: ana.beatriz.salgado@live.com.pt | Tel.: +351 916 312 175
Email: joaninhaencarnada_2@hotmail.com | Tel.: +351 914 484 030
Email: jacinta-abreu@hotmail.com | Tel.: +351 918 653 323

INTRODUCTION:

The catering industry has developed over time, increasing the concern about the health and nutritional quality of the food. Therefore producers and food establishments are under pressure to improve the quality of their products and services in order to assure that food is safe and suitable for consumption.

The microbial contamination of food is a major public health problem since it affects millions of people worldwide. Poor hygiene practices within the food processing environment can cause the contamination of food with pathogenic particles, which is a risk for the consumers health and safety. In establishments where food is manufactured and served, several measures regarding the control of the microbiologic contamination are needed. The majors microorganisms associated to food contamination are the bacterias due to the fact that they have a high pathogenicity and diversity.

For an assessment of the hygienic conditions related to food handling is essential to know the sanitary conditions of the establishments since they can reduce the risks of food contamination. These food contaminations can cause adverse human health problems.

OBJECTIVES:

Assessment of microbial contamination of the materials and hands of food handlers in different restoration units in the district of Bragança.

MATERIALS AND METHODS:

In this project we analyzed 694 samples, 356hands and 338 utensils (crockery). It was conducted a microbiological assessment of the following microorganisms: *Staphylococcus aureus,* total and fecal coliforms and *Escherichia coli* in the hands of food handlers and analysis of total germs, total and fecal coliforms and *Escherichia coli* in handling utensils in different restoration units (day care centers, restaurants/snack bars, supermarkets/grocers; health center's/hospitals, schools/kindergartens; butchers, fishmongers; delicatessens; dairies; hotels; governmental establishments). All the samples belong to the areas of Alfândega da Fé, Bragança, Carrazeda de Ansiães, Freixo de Espada à Cinta, Macedo de Cavaleiros, Mirandela, Mogadouro, Torre de Moncorvo, Vila Nova de Foz Côa,Vila Flôr, Vimioso and Vinhais,and were analyzed in the Public Health Laboratory of Bragança, in the period between January and December 2013.
Excel - For the statistical analysis plan the program of Microsoft Office was used.

RESULTS AND DISCUSSION:

Regarding the hands of manipulators, 53.67% of the cases showed contamination. The hands samples data analysis showed 28.74% of total coliform, 14.96% of fecal coliforms, 3.23% of *Staphylococcus aureus* and

6.74% of *Escherichia coli*. The contamination of crockery, cutlery and other kitchen utensils, was confirmed in 28.80% of the samples with “unsatisfactory” results and 10.87% with "bad” results. These contaminations stand out 91.67% of the samples with the presence of total germs, 53.47% with total coliforms, 27.78% with fecal coliforms and 15:28% with *Escherichia coli*. It was also found that 60.33% of the samples had “satisfactory" results. The classification of the cleanliness of glassware and/or cutlery is classified as satisfactory if the number of colonies is less than or equal to 100 CFU (Colony Forming Units) per piece and the detection of coliforms is negative, it is classified as “unsatisfactory” if the number of colonies is greater than 100 CFU per piece and/or if the presence of coliforms is positive, it may still be classified as “bad” when the search of fecal coliforms and/or the research of *E. coli* is positive.

CONCLUSION:

We concluded that food handler contamination is the main cause of poor hygiene in the food manipulation process. The level of hygiene registered in most samples of the crockery, cutlery and other kitchen utensils used in food manipulation process is satisfactory. And when compared with the samples from the food handlers these samples have better results, even though we still recorded some levels of contamination. In general, it is necessary to implement appropriate food hygiene measures in order to reduce these contaminants in the catering units.

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Determination of antimicrobial residues in milk samples

Authors: Larissa Batista[1], Patrick Pais[1], Rúben Fernandes[1], Cristina Prudêncio[1], Mónica Vieira[1]

1 *Department of Chemical and Biomolecules Sciences, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

Presenting Author: Email: mav@eu.ipp.pt | Tel.: +351 222 061 000 | Fax: +351 222 061 001

INTRODUCTION:

In farming activities, antimicrobials are widely used as a therapeutic measure in the treatment of bacterial infections, disease prophilaxys and as a growth promoter (food additives) (Marshall & Levy, 2011). The presence of antimicrobial residues in milk may represent risks for human health, namely allergic reactions on hypersensitive individuals, or even cause problems indirectly by development of resistant strains of bacteria (Tillotson, Doern, & Blondeau, 2006).

OBJECTIVES:

This study aims the development of new clean-up and pre-concentration methods for milk samples, through a solid-phase extraction (SPE), in order to perform a better identification and quantification of antimicrobials by High-Performance Liquid Chromatography (HPLC).

MATERIALS AND METHODS:

The extraction method of the antibiotic residues involves the deproteinization of 27 spiked milk samples and their centrifugation, followed by a solid-phase extraction (SPE). Antimicrobial residues were, then, identified and quantificated by HPLC-DAD.

RESULTS AND DISCUSSION:

The recovery percentage for ciprofloxacin was 94.54 ± 6.90%. For sulfamethoxazole, the recovery percentage was 93,18% ± 5.60%. It was possible to identify the presence of sulfamethoxazole in one of the samples. The limits of detection (LD) were in the range of 2.43ng/mL and 162ng/mL and the limits of quantification (LQ) were ranging between 7,36 ng/mL and 492 ng/mL, which means that the developed new methods have LD and LQ below the maximum residue limits (MRLs) ruling in the European Union for five antimicrobials tested, in milk (Commission Regulation (EU) No 37/2010, OJ L15,20.1.2010, pl1).

Table 1 - Recovery percentage of antimicrobial residues, using the SPE-HPLC-DAD methods

Antimicrobial	Recovery (%)
Ciprofloxacin	94.54
Sulfamethoxazole	93.18

CONCLUSION:

The combination of the proposed methods of clean-up and pre-concentration by SPE and multi-residue HPLC-DAD allows, therefore, the detection and quantification of antimicrobial residues in milk, making this an important and useful alternative in quality control process for the food industry and other relevant areas.

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Seafood hazards: a review

Authors: Ana Cruz[1], Teresa Mateus[1,2], Filomena Ramalho[3], Humberto Rocha[1,2]

1 *Departamento de Medicina Veterinária, Escola Universitária Vasco da Gama, Coimbra, Portugal*
2 *CECAV, Centro de Ciência Animal e Veterinária, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*
3 *Gabinete Médico-Veterinário, Centro Oficial de Recolha de Animais de Companhia de Coimbra, Coimbra, Portugal*

Presenting Author: Email: mv.anacruz@gmail.com | Tel.: +351 961 226 443

INTRODUCTION:
Portugal was placed among the countries with the most elevated consumption of seafood *per capita*, comparatively to the European Union in 2007 and worldwide in 2010 (FAO/WHO, 2010).This fact emphasizes the importance of seafood safety in Portuguese alimentation. Furthermore, there are a number of risks associated to seafood consumption. In order to prevent or reduce the presence of contaminants in these food products, that may put at risk the consumer's health, there is a need to improve the awareness of the population, including seafood handlers and seafood industry operators.

OBJECTIVES:
Resorting to existent bibliography, we reviewed physical, chemical and biological hazards most frequently found in seafood meant for human consumption. The proposed poster intends to compile all the seafood hazards reviewed, in a visual and systematic way (for instance: using tables) for a quick consult and easy comprehension.

MATERIALS AND METHODS:
For the review, we used the available references, such as books and scientific articles related to food safety, technology and food microbiology directed to seafood. Food and Agriculture Organization (FAO) and World Health Organization (WHO) websites were used for acquiring statistical data and food safety guidelines. The online data base of the National Center for Biotechnology Information(NCBI) was the main search engine for most of the referred articles.

RESULTS AND DISCUSSION:
The possible hazards regarding seafood are numbered as following. Among chemical hazards: marine biotoxins (DSP, PSP, ASP and NSP syndrome associated toxins, ciguatera, tetrodotoxin, pirofeoforbid a and PTX), histamine, AZP, scombrotoxins, diotoxins, micotoxins, pesticides, PCB, HAP, toxins produced by bacteria, heavy metals, pharmacological residue and volatile nitrosamins (Veiga *et al.*, 2009; Gresham & Taylor, 2011).

Among the biological hazards, there are three big groups: bacteria, parasites and viruses. *Escherichia coli*, *Staphylococcus aureus, Vibrio cholerae, Clostridium botulinum, Listeria monocytogenes* are some of the described bacteria (WHO, 2008). The parasites here focused are *Anisakis simplex, Diphyllobothriumlatum, Toxoplasma gondii* and also others as *Chlonorchissinensis, Opistorchiswestermani* and *Paragonimus* spp.(Lv*et al.,* 2013). Here are also described physical hazards like radioactivity (Veiga*et al.*, 2009). According to the majority of references, shellfish, given their physiologic characteristics, have the highest prevalence of contamination by chemical and biological hazards. Certain population groups (pregnant, children, elderly and imuno-compromised) require some attention regarding this subject, assuming that they are especially susceptible to the development of foodborne diseases.

CONCLUSION:
In order to decrease the consumption risk associated with seafood, it is considered important to invest in disseminating information, mostly directed to the consumers and the food sector operators.

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Health risk assessment of veterinary drugs residues in food

Authors: Nina Zaitseva[1], Pavel Shur[1], Dmitry Kiryanov[1], Nina Atiskova[1], Olga Ustinova[1]

1 *Federal Budget Scientific Institution "Federal Scientific Center for Medical and Preventive Health Risk Management Technologies", Perm, Russia*

Presenting Author: Email: atiskova@fcrisk.ru | Tel.: +7(342)237-25-34

INTRODUCTION:

Health risk assessment of veterinary drugs residues in food in particular within the World Trade Organization, the Eurasian Economic Community and the Eurasian Economic Community customs union is one of the priority areas in field of consumer health safety. Health risk assessment is known as key element in food safety standards derivation [1].

OBJECTIVES:

Health risk assessment of tetracycline and ractopamine residues in food.

MATERIALS AND METHODS:

Health risk assessment of veterinary drugs residues in food comprised 4 stages: hazard identification, exposure assessment, dose-response assessment and risk characterization [1].

Maximum residue level (MRL) standards for ractopamine in meat products were chosen according to the Codex Alimentarius Commission (CAC) data and had values of 0.01 mg/kg for pork and beef, 0.04 mg/kg for liver, 0.09 mg/kg for kidney [2].

As tetracycline maximum residue levels standards, MRLs recommended by WHO (1990), FAO/WHO (1998) and MRLs accepted in USA and Russia were used (Table 1).

Table 1 - Tetracycline MRLs for different types of animal products (μg/kg)

Product	WHO 1990	FAO/WHO 1998	USA	Russia
Milk	100	100	300	10
Muscle	100	200	2000	10
Fat	10	-	10	10
Eggs	200	400	200	10
Liver	300	600	300	10
Kidney	600	1200	600	10

RESULTS AND DISCUSSION:

In case of health risk assessment of ractopamine residues in food on hazard identification stage it was revealed that ractopamine is used as feed additive and nowadays is banned in 80 countries including European Union but at the same time 22 countries use it in swine breeding. Main health effects due to ractopamine in food include cardiovascular and muscular skeletal systems diseases and metabolic disorders. On dose-response assessment stage for ractopamine residues in food carcinogenic and non - carcinogenic effects were evaluated. As a "dose-effect" model for carcinogenic risk estimation data on uterine leiomyoma incidences in long-termed study of ractopamine toxicity and carcinogenicity in mice were used [3]. Non-carcinogenic effects assessment was based on the evolutionary model of risk of cardiovascular functional disorders accumulation [4]. Exposure assessment in case of ractopamine was performed for two exposure scenarios taking into account average daily intake of animal products in Russia and ractopamine concentrations in animal products recommended as MRL by the Codex Alimentarius Commission (scenario 1) and detection limit of ractopamine residues in meat products (3-5 μg/kg) [5] (scenario 2). On the stage of risk characterization it was determined that using ractopamine MRL in animal food recommended by CAC,

carcinogenic risk level was 1.32×10^{-6} (acceptable risk). As a result of cardiovascular system disorders modelling values of normalized risk were obtained, for both scenarios risk level was unacceptable (0.47 and 0.141 for scenario 1 and scenario 2, respectively) [4] and could lead to life expectancy decrease due to additional cases of cardiovascular diseases (hypertensive diseases, ischaemic heart diseases).

In case of health risk assessment of tetracycline residues in animal food on hazard identification stage it was revealed that tetracyclines are bacteriostatic broad-spectrum antibiotics and can cause bacterial resistance increase among pathogenic and commensal bacteria of gut flora. As a critical health effect due to tetracycline impact on gut microflora was chosen, in particular gut flora alterations development. As a risk group children (1-11 years) with sensitive to tetracycline gut flora were considered. Dose-response assessment stage included mathematical modelling of facultative species growth against suppression of obligatory gut bacteria, particularly bifidus bacteria. On exposure assessment 4 scenarios were created taking into account average daily intake of animal products by Russian children and tetracycline MRLs recommended by WHO (1990), FAO/WHO (1998) and accepted in USA and Russia. On risk characterization based on results of gut flora alterations modelling it was founded that tetracycline residues concentration in food more than 10 μg/kg increases in children risk of digestive system diseases to 0.000461 (up to 4% of cases), risk of dermatitis to 0.000725 (up to 0.9% of cases), risk of alimentary allergy to 0.000149 (up to 0.1% of cases), risk of diseases of the blood to 0.001372 (up to 8% of cases).

CONCLUSION:

Consumption of food containing ractopamine on MRLs recommended by Codex Alimentarius Commission and even on ractopamine detection limit in meat products is prohibited because of ractopamine induced unacceptable health risk levels (cardiovascular functional disorders and cardiovascular diseases).

Health risk assessment of gut flora alterations and associated diseases in children showed that only 10 μg/kg tetracycline concentration in food (allowable residue level in Russia) do not lead to unacceptable risk of gut flora disorders.

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Gamma irradiation effects on microbial inactivation and antioxidant activity of *Melissa officinalis*

Authors: Márcia Meneses[1,2], Joana Madureira[1], Rita Melo[1], Amilcar L. Antonio[1,3], Sandra Cabo Verde[1]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Portugal*
2 *Environmental Health RG - Lisbon School of Health Technology - Polytechnique Institute of Lisbon, Portugal*
3 *Centro de Investigação de Montanha (CIMO), ESA, Instituto Politécnico de Bragança, Portugal.*

Presenting Author: Email: marcia17meneses@gmail.com | Tel.: +351 911 880 273 / 963 485 468

INTRODUCTION:
Many herbal products are traditionally being used as medicines and nutraceuticals in different regions of the world (Kumar et al., 2010). This increased consumption of natural products has become a public health problem. The concern in the safety of these products is due, in part, to the possible presence of pathogenic bacteria and fungi producing mycotoxins (Prado et al., 2009). Consequently the evaluations of the hygienic quality of medicinal plants, as well as the use of decontamination methods are significant steps towards the consumer safety and therapeutical efficiency (Soriani et al., 2005).

OBJECTIVES:
The aim of this study is to assess the effects of gamma irradiation on the microbial burden and antioxidant activity of medicinal plants, namely *Melissa officinalis*.

MATERIALS AND METHODS:
Samples of dried *Melissa officinalis* were irradiated in Co-60 experimental equipment (Precisa 22) located at the Campus Tecnológico e Nuclear, Instituto Superior Técnico, Sacavém, Portugal. The applied gamma radiation doses were 1; 2; and 4 kGy at a dose rate of 1.20 kGy/h. Non-irradiated samples followed all the experiments. Regarding the microbiological analyses, dried plant samples were blended on 100 mL of physiological solution with 0.1% of Tween 80. The samples were homogenized in a stomacher equipment during 15 minutes and filtrated using nitrocellulose membranes with a pore size of 45 m. Bacterial and fungal counts were carried out, in triplicate, on Tryptone Soya Agar (TSA) and Malt Extract Agar (MEA) at 30 ºC during 7 days. Microbiological counts were expressed as log colony forming units per gram. Morphological identification of fungi was achieved through macro and microscopic characteristics as proposed by specific atlas for fungal identification. Concerning the mesophilic bacterial isolates, all colonies were macroscopically, microscopically and biochemically typed by gram staining, catalase activity and oxidase test. The frequency of each phenotype was calculated based on the number of isolates and their characterization. Regarding to antioxidant activity, FRAP and DPPH assays were determined. For those experiments, water extract of *M. officinalis* (WEM) and ethanol extract of *M. officinalis* (EEM) were prepared. For preparation of WEM, 80 mg of each irradiated sample was added to 800 L bi-distilled water and the mixture was boiled for 2 min and stirred for 30 seconds. Concerning EEM, 80 mg of each irradiated sample was added to 800 L ethanol and this mixture was stirred for 30 seconds. The FRAP assay was carried out according to the method described by Benzie and Strain (1996). The free radical scavenging activity of the antioxidants of WEM and EEM based on the scavenging activity of stable DPPH free radical was determined according to the method of Brand-Williams et al, 1995. DPPH assay of *M.officinalis* extracts is ongoing.

RESULTS AND DISCUSSION:
The characterization of dried *M. officinalis* microbiota showed an average bioburden value of 10^2 colony-forming units (CFU)/g and a diverse microbial population predominantly composed by 1 morphological type: gram-positive rods (74%). The inactivation studies of the *M. officinalis* mesophilic population indicated linear inactivation kinetics (Figure 1), with a one log reduction of microbial burden (90% inactivation efficiency) for 4 kGy. The survivor microbiota was mainly constituted by gram-negative rods (75%).

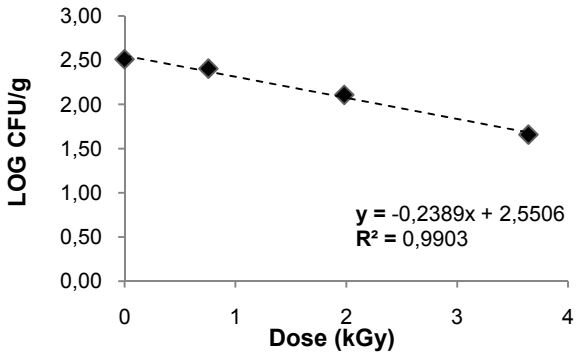


Figure 1 - Gamma radiation inactivation curve for the mesophilic microbial population of dried *M. officinalis.*

Concerning antioxidant activity evaluation, two different methods were applied. FRAP assay express the capability of antioxidants to reduce ferric (Fe^{3+}) ion to ferrous (Fe^{2+}) ion form in ferrous sulphate acidic solution. A higher absorbance at 593 nm indicates a higher ferric reducing power (Koksal et al., 2011). The obtained results are presented in the Figures 2 and 3

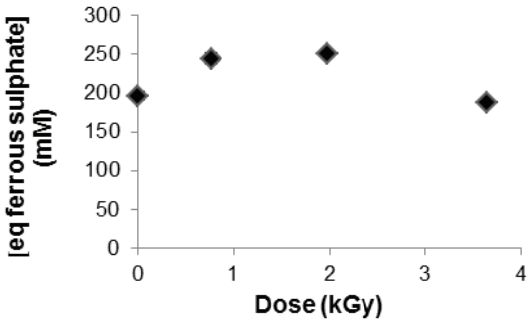


Figure 2 - Reducing power of water extracts of *M. officinalis* by FRAP assays.

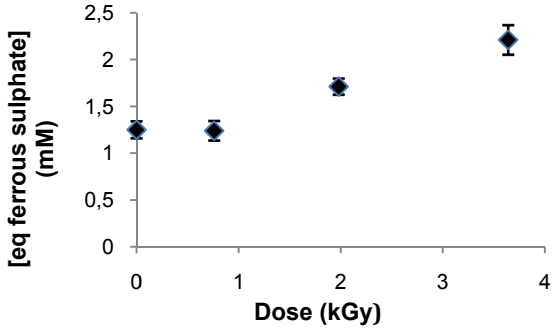


Figure 3 - Reducing power of ethanol extracts of *M. officinalis* by FRAP assays.

As shown in Figure 2 for water *M. officinalis* extract, the antioxidant activity reveals an increase of about 28% at 2 kGy. Regarding ethanol *M. officinalis* extracts, Figure 3 shows an increase of approximately 78% at 4 kGy. However, according to results of the present study, ferric reducing power of WEM was higher than EEM which could be related with antioxidants present in both extracts. This topic as well as DPPH assay is under study.

CONCLUSION:

The obtained results suggested that the gamma irradiation treatment could be advantageous in improving microbial safety of *M. officinalis* with the potential added-benefit of increasing its antioxidant content. The effect of higher irradiation doses on *M. officinalis* will be further investigated, in an attempt to augment the reduction of the microbial population.

ACKNOWLEDGMENTS:

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Good hygiene practices
A pillar for the production of safe food

Authors: Ana Oliveira[1], Carina Silva[2], Joana Barbosa[2], Paula Albuquerque[1]

1 *Professors of the degree course Environmental Health, Higher School of Health Technology of Lisbon, Lisbon Polytechnic Institute, Portugal*

2 *Students of the degree course Environmental Health, Higher School of Health Technology of Lisbon, Lisbon Polytechnic Institute, Portugal*

Presenting Author: Email: carinav_silva@hotmail.com | Tel.: +351 918 858 559

INTRODUCTION:

One of the main concerns of Public Health is foodborne diseases (Pinto, 2008).It is estimated that, each year, one in three people are affected by these diseases and food services account for over 50 % of the occurrence of these outbreaks (Barros, 2008).

The preparation of safe food is particularly important in school canteens, once any incident that occurs can affect a large number of consumers, most of them children and young people, due to the immaturity of their immune systems (Pereira, 2009).

In this framework, the approach to personal hygiene and hygiene of premises and equipment becomes unavoidable. Such operations are essential to minimize the risk of contamination. Although, despite its importance, they are sometimes neglected by professionals (Pinto, 2008).

OBJECTIVES:

The present work aims to analyze the food hygiene practices and attitudes of food handlers at the level of good personal hygiene practices and procedures of cleaning and disinfecting in school canteens from pre-school to secondary, located in rural areas.

MATERIALS AND METHODS:

An exploratory descriptive study was developed and used a quantitative method.

The target population of this study is food handlers by space meals of public schools from pre-school to secondary, with production on site and catering service. Of the target population, a sample of 61 food handlers from 11 food school facilities belonging to rural areas of the district of Lisbon was used.

Data collection was performed through a personal interview based on a pre- designed questionnaire. The collection took place between April and May 2012.

RESULTS AND DISCUSSION:

Analyzing the results obtained in the present study, it's verified that 59% of food handlers are aged between 35 and 54 years. Contrary to what has been observed in developed countries, the education average level of food handlers is relatively low, 74 % of professionals have only between 4 to 9 years of schooling. This value is relevant given that, currently, the minimum school level in Portugal is 12 years (Lei n.º 85/2009, de 27 de Agosto). Such evidence may be caused by the fact that the education of professionals not represent, in most cases, a criterion in the selection of workers for working in food establishments, the privilege is given to work experience in the sector (Pereira, 2009).

Related to the years of working in the food area, almost half of the interviewed (48 %) has worked in this area for more than five years, which could show up positive to guarantee food safety. However, in most cases

it corresponds to the existence of deeply rooted habits of work, which offers much reluctance to changing and updating knowledge (Egan *et al.*, 2007).

Regarding the specific training in Hygiene and Safety Food, 67 % of the handlers have it, and of these, 74 % did it about a year or less. Although the percentage of untrained professionals is considerably smaller (33%), even so, have a high value, taking into account the negative consequences that may result from the ignorance of good hygiene practices. The instability of contractual relationship that exists today can be one of the justifications for these values, a fact that is reinforced by the results obtained in this study, once 18 % of respondents has worked in the food area for less than a year.

Given the role that food handlers take in ensuring food safety, we denote the importance of the legal requirement in respect to education and/or proper training in Hygiene and Safety Food (Regulamento (CE) n.º 852/2004, de 29 de Abril). This training should be subject to periodic update to be effective and it is essential to check behavioral changes, because knowledge alone is insufficient (Egan *et al.*, 2007).

Bas *et al*, (2006) found that although 95% of participants in their study had received training, 63% admitted not carrying out appropriate behaviors from the point of view of food safety and hygiene.

According to Barros (2008), the content taught in training should have a practical component, complementing the theoretical part, which took into account the real work settings, so that workers could apply effectively their contents.

Regarding the use of protective clothing, it can be concluded that a considerable proportion of professional respondents uses uniform protection (97%) and caps (95%) whenever they manipulate food. To emphasize that all (100%) use this clothing exclusively in the kitchen, however, only 25% of professionals proceed to their daily hygiene.

According to the present study 49% of respondents always use disposable gloves, however, it is not proved that the use of the same is the safest method of handling food, compared to situations where they are not used and used an effective technique for washing hands (Pinto, 2008).

The use of gloves can promote poor hand hygiene practices. Green et al. (2006) suggest that some workers believe that the use of gloves eliminates the need to perform the washing of hands and their use is often responsible for a "false sense of security".

Indeed, to the question "What are the options that you consider essential to wash your hands?" (Graphic 1), the answers "before donning gloves" and "after removing gloves" were the least mentioned with 6 and 12 replies, respectively.

The answers "when I change task" (50 answers), "after using the bathroom" (44 answers), "before handling food" (38 answers) and "when hands are dirty" (34 replies) were the most commonly reported (Graphic 1). However, it is worrying that, professionals of the area, when confronted with this issue do not mention hand washing after using toilet, among other options, with greater frequency, as would be expected.

Graphic 1 - When do you consider essential to wash your hands?



With regard to cleaning and disinfection, it is verified that 67% of food handlers assumes that the cleaning of a work bench consists of a simplistic action, considering only the use of water and detergent to carry out this operation or using only the use of disinfectant, without the prior completion of a cleaning action. This is worrying, since improper cleaning of surfaces can be a source of food contamination, endangering the health of consumers.

The amount of chemical to use is itself a crucial factor for the effectiveness of cleaning operations. According to the study, it is noted that only 44% of professional determines it correctly, using a scoop (36%), consulting the hygiene plan (3%), or reading the product label (5 %). However, 56% do it "by eye", without any criteria.

For proper cleaning it is necessary to follow the instructions and concentrations recommended by the manufacturer, noted that there may be a decrease in level of hygiene if the concentration limit recommended is exceeded. Egan *et al.*, (2007) found that 12.1% of food handlers considers unimportant to check the concentration of cleaning solutions, showing that, in this aspect, food handlers denote lack of knowledge.

It should be noted that 30% of food handlers do not know if the establishment where they work has hygiene plan, and although 54% have been aware that it exists, 24% of them never consulted, which may be the cause of the number of incorrect responses associated with previously addressed issues concerning the procedures of cleaning and disinfecting. These data show that often the professionals do not give proper importance to documentation relating to hygiene which is available for consultation at the establishments.

Compared to what has been said, it is attested the need to raise awareness among food handlers for the consequences that might result from improper hygiene practices, appealing to a high sense of responsibility and professionalism thereof.

CONCLUSION:

It can be seen, that although a significant proportion (67 %) of food handlers have specific training in Food Safety and Hygiene, this is not always effective. Work habits ingrained by professionals working in the area for some years, and the unsuitability of the contents administered in formations are some of the possible causes that can induce professionals to adopt practices that increase the occurrence of contamination.

Awareness, sensitization and accountability of food handlers are vital to the successful implementation of good food hygiene practices. In this context, training in hygiene and food safety assumes a fundamental role. However, it is necessary that they are adequate in terms of content and effective in changing behaviors (Egan *et al.*, 2007).

Therefore it is relevant to include the evaluation of training effectiveness in future studies, in order to assess their suitability in terms of content as well as its effectiveness in changing behavior adopted by the handlers.

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The influence of serial repitching of *Saccharomyces* brewing biomass to produce flavor enhancer nucleotides

Authors: Elsa Vieira[1], Isabel M.P.L.V.O. Ferreira[1]

1 *Department of Bromatology and Hydrology, Faculty of Pharmacy of Porto University, Portugal*

Presenting Author: Email: elsavieiraf@gmail.com | Tel.: +351 220 428 642 | Fax: +351 226 093 390

INTRODUCTION:

Ribonucleotide derivatives and nucleosides are known to have various physiological effects and 5`nucleotides are widely used in food industry as flavor enhancers (Ferreira, Mendes, Gomes, Faria & Ferreira, 2001). Among all 5`nucleotides, guanosine 5`monophosphate (5`GMP) is an active flavor enhancer and adenosine 5`monophosphate (5`AMP) is a precursor of the well-known flavor enhancer inosine 5`monophosphate (5`IMP) (Ranogajec, Beluhan & Smit, 2010). Yeasts, namely, *Saccharomyces*, are the preferred source of nucleic acids for production of 5`nucleotides due to their high nucleic acid content, ˜8-11% RNA by dry weight (Charpentier, Aussenac, Charpentier, Prome, Duteurtre & Feuillat, 2005). Moreover, it is a GRAS microorganism and has good nutritional characteristics. Yeast extracts containing 5`GMP can be prepared by hydrolysis of RNA from baker's yeast cells by heating and using 5′-phosphodiesterases. Alternatively, brewer's spent yeast, the second major by-product from brewing industry, is an interesting raw material for the production of flavor-enhancing 5`nucleotides, due its low cost. In the brewing process, serial repitching of *Saccharomyces* biomass is usual, thus yeast is reused 4-6 times before its disposal (Ferreira, Pinho, Vieira & Tavarela, 2010). Thus, it can be of interest to understand the influence of yeast repitching for flavor enhancer's production.

OBJECTIVES:

The present work was undertaken to obtain 5′nucleotiderichyeast extracts from brewer's spent yeast without addition ofexogenous enzymes and evaluate the influence of serial yeast repitching on extract composition concerning monophosphatenucleotides and nucleosides. For the best of our knowledge, studies related with the influence of yeast generation on monophosphate nucleotides and nucleosides composition of brewer′s spent yeast have not been reported.

MATERIALS AND METHODS:

Yeast was harvested from the fermentation vessels (3000 hL) after 11 days of beer fermentation and successively inoculated (repitched) into fresh wort. Thirty-seven samples of 0.5 kg of brewer's spent yeast (*Saccharomyces pastorianus*) were collected; these included samples of yeast biomass with 2, 4, 5 and 6 serial repitchings in the brewing process, coded, respectively as R2, R4, R5 and R6. All samples were provided as slurry by the brewing industry Unicer, Portugal. Two procedures for disrupting cell walls that can be used at industrial scale were tested, and the optimum conditions for low-cost and efficient RNA hydrolysis were selected. A HILIC methodology coupled to diode array detection was validated for quantification ofadenosine, uridine, xanthosine, cytidine, guanosine, 5′GMP,5′IMP, 5′AMP, 5′CMP, 5′UMP, 5′XMP, 2′AMP, 3′AMP, 2′GMP and 3′GMP in yeast extracts. Statistical analyses were performed using the software SPSS for Windows, version 20.0 (SPSS, Chicago, IL,USA).

RESULTS AND DISCUSSION:

Extracts obtained by autolysis at 50°Cduring 24 h contained <18% of protein referred to w/w dry yeast cell, whereas those obtained by glass bead disruption contained between 43.5 and 47% of protein referred to w/w dry yeast cell. The RNA content of the yeast extracts varied between 4 and 8% (dry weight). Biomass groups R2 and R4 showed highest RNA mean content (8.97 and7.25%, respectively), followed by R5 and R6 (5.84 and 4.43%). Please see Figure 1. Under the RNA hydrolysis conditions selected previously,5 nucleosides and 9 nucleotides were identified in yeas textracts: adenosine, uridine, xanthosine, cytidine, guanosine,2`AMP,

3`AMP, 5`AMP, 5`UMP, 5`IMP, 2`GMP, 5`CMP,3`GMP, and 5`GMP.As expected, yeast extracts contained an array ofribonucleases, nucleotidases, and nucleosidases that contributed to the hydrolysis of RNA. Nucleotides accounted for 71.1-88.2% of the RNA products; 2`AMP was the most abundant (ranging between 0.08 and 2.89 g/100 g dry yeast) and 5`GMP content ranged between 0.082 and 0.907g/100g dry yeast. The sum of 5`GMP, 5`IMP, and 5`AMP represented between 25 and 32% of total nucleotides.

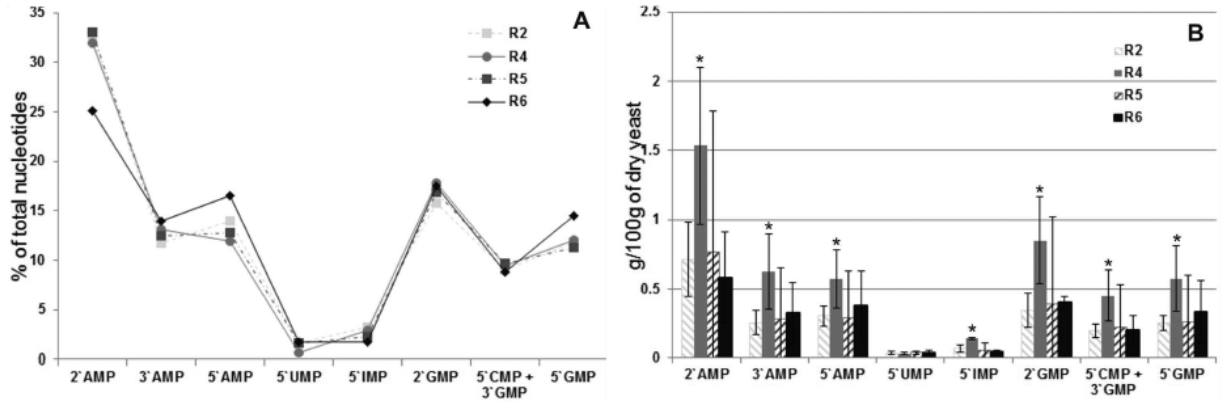


Figure 1 - Nucleotide composition of brewer's spent yeast extracts
∗ indicates significant differences (ANOVA and Duncan's post hoc, $p< 0.05$)

CONCLUSION:

This works highlights for the first time that although serial repitching influences the content of monophosphatenucleotides and nucleosides, the profiles of these RNA hydrolysis products are not affected. The proposed procedure can be scaled up to an industrial process easily and with low investment to produce flavour enhancers. However, the predominance of 3`ribonucleotides in the degradation products has practical implications because only 5`GMP and 5`IMP have flavor-enhancing properties and5`AMP is precursor of the well-known flavor enhancer 5`IMP.The use of inhibitors for 3`nucleotide forming RNases to increase 5`nucleotides yield is a field to explore.

ACKNOWLEDGMENTS:

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Microbiological quality of pre-cooked food: *Escherichia coli* and coagulase positive *Staphylococcus* prevalence

Authors: Bruna David[1], Manuela Amorim[1,2], Stephanie Ferreira[1,2], Jorge Condeço[1,2,3], Sandra Mota[1,2], Anabela Moreira[1], António Augusto[1,2,4]

1 *Department of Biomedical and Public Health Laboratory Sciences, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

2 *Research Centre of Health and Environment, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

3 *Portuguese Blood Bank and Transplantation Institute, Portugal*

4 *Biogerm, SA, Maia, Portugal*

Presenting Author: Email: mas@estsp.ipp.pt | Tel.: +351 222 092 128

INTRODUCTION:

Recent changes in society's lifestyle lead food industries to invest in the production of pre - cooked foods for an increasingly demanding consumer, who values its easiness and convenience.(Oliveira, 2009).These new eating habits increased foodborne diseases incidence which represents a public health problem (Newell et al., 2010).

The main genera of bacteria responsible for foodborne diseases are: *Salmonella, Staphylococcus, Escherichia, Vibrio, Bacillus, Clostridium, Listeria, Shigella* and *Campylobacter*(Newell et al., 2010).

To ensure the microbiological food quality, companies must implement a hazards and critical control points (HACCP) system of identification, analysis and control, ensuring compliance with national and international guidelines(Bolton & Maunsell, 2004).

OBJECTIVES:

To assess the prevalence of *Escherichia coli* and coagulase positive *Staphylococcus* in pre-cooked food; and to compare microbial load in the different categories of pre-cooked food.

MATERIALS AND METHODS:

We conducted a cross-sectional study on the results of microbiological laboratory control of pre-cooked food, performed in a national public health laboratory throughout the year 2013.Each result is a sample of a batch. Results included *E. coli* and coagulase-positive staphylococcicounts. These parameters were analyzed according to pre-cooked food category (meals, savory meat appetizers, savory fish appetizers, savory vegetables appetizers, and sweets and desserts) Samples were classified as "Satisfactory" (*E. coli* count <10UFC/g, coagulase-positive staphylococci count $<10^2$ UFC/g), and "Non-Satisfactory" (*E. coli* count ≥10UFC/g, coagulase-positive staphylococci count $\geq 10^2$ UFC/g), according to laboratory guidelines (INSA, 2005).

RESULTS AND DISCUSSION:

We studied 543 records. Results are summarized in table 1. Overall, we registered 15 non-satisfactory samples: 2 samples were considered non-satisfactory *for E. coli* (0,2%) and 13 (2,4%) for coagulase positive staphylococci, independently. Pre-cooked food category presenting higher frequency of non-satisfactory results was savoury meat and fish appetizers, of which5.08%fried meat balls and 8.33% fried codfish balls showed microbiological contamination.

Table 1 - Absolute and relative frequency of Satisfactory" and "Non-Satisfactory" samples regarding E. coli and coagulase-positive staphylococci counts, according to pre-cooked food category.

Pre-cooked food category	Coagulase-positive staphylococci counting (n, %)		E. coli (n, %)	
	Satisfactory	Non - Satisfactory	Satisfactory	Non - Satisfactory
savoury meat appetizers (n=260)	252 (96,9)	8 (3,1)	259 (99,6)	1 (0,4)
Meals(n=15)	15 (100,0)	0 (0,0)	15 (100,0)	0 (0,0)
savoury fish appetizers(n=250)	245 (98,0)	5 (2,0)	249 (99,6)	1 (0,4)
savory vegetables appetizers (n=13	13 (100,0)	0 (0,0)	13 (100,0)	0 (0,0)
sweets and desserts (n=5)	5 (100,0)	0 (0,0)	5 (100,0)	0 (0,0)
TOTAL (n=543)	530 (97,6%)	13 (2,4%)	541 (99,6%)	2 (0,4%)

CONCLUSION:

The relative frequency of contamination by E. coli is much lower than for coagulase-positive staphylococci. This could relate to the contamination from food handlers and is even more likely if we consider that the most manipulated foods are those with a higher frequency of contamination. Despite the low relative frequencies, the high number of precooked meals enhances the danger of these events, which have important consequences in involved consumers. Moreover if we consider that each output represents a sample of a batch type of food. These results are important not only in the evaluation of health hazards but also from the economic point of view, since it can result in significant losses. Companies must implement a hazards and critical points (HACCP) system in order to ensure compliance with national and international guidelines.

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Food additives, a negative health externality

Authors: Dan Sava[1]

1 *Private consultant, Romania*

Presenting Author: Email: dsava22@gmail.com | Tel.: +40 31 410 9565

INTRODUCTION:

Food industry uses various additives for commercial purposes. Some of these additives were proven to be dangerous to human health, and excluded for use in EU. However other food additives are still in use and their potential to harm human health is not assessed yet. From the health economics perspective harmful additives are negative externalities produced by food industry.

OBJECTIVES:

The objective of the paper is to shed a new light on food additives from the health economics perspective. This view is important because the impact of those additives on human health is increasingly questionable.

MATERIALS AND METHODS:

The paper tries to review the literature on the harmful effect of food additives on health and will use health economics tools to asses them as bad externalities. Until now, food additives have been regarded as chemicals, but the paper tries to provide a new perspective on them.

RESULTS AND DISCUSSION:

The food industry uses various additives for economic reasons (marketing, logistics, etc.). The scope of food additives comprises colors adding, preservatives, antioxidants, emulsifiers, stabilizers, thickeners, break-up agents, sweeteners, flavor enhancers and others. Some of these additives make certain foods look better, others lengthen their shelf life, some interfere with hormones regulating appetite and satiety. From the economic point of view, the use of these additives has as the ultimate purpose, the increase of sales. However some of them seem to be harmful for human health. The literature is not very clear, and it is certainly not enough to tell beyond reasonable doubt how harmful are for example sorbitol (E420), or sodium benzoate (E 211), or sodium nitrate (E 250), or carrageen an (E 407). However, the history of food additives is just a one way stream. Food additives like tartrazine (E 102), aspartame (E 951), sodium glutamate (E 621), were widely used once, until they were proven to be harmful. This suggests there is a need for new and more comprehensive studies regarding the potential harmful effects of food additives. Because of this, food additives can be regarded as bad externalities, like pollution for example. From this perspective, not only public health policies can be developed for tackling this problem, but pure economic measures can be envisaged to redress the situation.

CONCLUSION:

There is a need for more systematic and thorough studies regarding the effects of food additives on human health. If food additives are regarded as bad externalities, governments will have the scientific support to take economic measures for mitigating the impact of harmful food additives. The result of these new policies and actions will certainly improve the health of citizens but might require changes in the way food industry is doing business.

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Assessment of hygienic quality of surfaces and food handlers in Portuguese school canteens

Authors: Joana Ferreira[1], Andreia Rebelo[2], Joana Santos[2], Manuela Vieira da Silva[2]

1 *Research Centre on Environment and Health, School of Allied Health Sciences of Polytechnic of Porto, Portugal, Graduate Project on Environmental Health*

2 *Research Centre on Environment and Health, School of Allied Health Sciences of Polytechnic of Porto, Portugal*

Presenting Author: Email: joana_fpf@hotmail.com | Tel.: +351 912 781 655

INTRODUCTION:

The incidence of food borne outbreaks in school canteens represents a major problem for society, since they serve a youthful population who are particularly susceptible to health risks. According to the World Health Organization (2003), 31% of the reported outbreaks between 1999 and 2000 in Portugal, were originated in schools and kindergartens, which demonstrate the importance of such establishments in the transmission of food borne diseases. Food handlers have a major role in the prevention of these diseases, mainly during the production and distribution of food since they may cross-contaminate raw and processed foodstuffs.

OBJECTIVES:

The aim of this study was to evaluate the hygienic quality of surfaces and food handlers from high school canteens and also to assess the knowledge of these operators, regarding to hygiene and food safety.

MATERIALS AND METHODS:

This study was conducted in three school canteens located in Maia, Portugal. To assess the hygiene and food safety knowledge of food handlers, it was designed a short questionnaire with open and closed questions, about cross-contamination, cleaning and temperature control. These questionnaire was applied as face-to-face interviews. In addition, samples were taken from surfaces in contact with foods (including work surfaces and cutting equipments) and hands of food handlers, after normal cleaning procedures. Depending the sampling sites, samples were collected using two traditional microbiological methods (swabbing and contact plates) and an indirect method of ATP bioluminescence(using the ATP analyzer Hy-lite).The microbiological parameters investigated and relative identification techniques are described in Table 1.

Table 1 – Microbiological parameters and identification techniques studied

Microbiological parameters	Culture Medium	Incubation conditions	Growth and types of colonies
Total Viable Count (TVC)	Nutrient Agar (Liofilchem)	25 ± 2°C for 24h	Enumeration of cell forming units
Enterobactereaceae	Violet Red Bile Glucose Agar (Liofilchem)	37± 2°C for 48h	Enumeration of cell forming units (typical colonies)
Staphylococcus aureus	Baird Parcker Agar Base (Liofilchem)	37± 2°C for 48h	Enumeration of cell forming units (typical colonies)

RESULTS AND DISCUSSION:

A total of 7 food handlers were interviewed. The majority of the participants (85.7%) answered incorrectly at least one of the questions, particularly with regard to temperature control. In general, food handlers are not aware of the importance of temperature control requirements for the control of microbial hazards, assuming that this parameter is only important for organoleptic reasons. The participant who had more incorrect answers, had a lower educational level compared to the others. According to Milan et al. (2004) a higher level of education is responsible for a higher level of hygiene and food safety knowledge. Only one participant demonstrated a high level of knowledge, answering correctly to all the questions. Although cross-contamination seems to be a known issue for food handlers, is not always perceived by them. As regards the hygiene level of surfaces and food handlers (n=33) the results indicates that, in general, was adequately high (Table 2). Only 33.3% of cutting equipments did not conform with the advisory standards for

the TVC(≥1.3 $Log_{10}cfu/cm^2$) and *Enterobactereaceae* (≥1 $Log_{10}cfu/cm^2$), mainly in knifes and in a vegetable cutting blade. Worktops and Teflon chopping boards were in general in compliant with the microbiological advisory standards. Regarding hands hygiene, *S.aureus* were found in 62.5%of food handlers. However, only 12.5 % had an unsatisfactory result (≥1 $Log_{10}cfu/cm^2$). Although people that carry out *S.aureus* in their hands are allowed to work in food production, it is crucial that they recognise the importance of careful washing and disinfection. An unsatisfactory contamination with TVC and *Enterobactereaceae* were also observed in 25% and 12.5% of food handlers, respectively. These results show that the washing procedures applied by some food operators are not being effective.

Table 2 - Conformity of surfaces in contact with food and washed hands to microbiological advisory standards [a]

Surfaces	TVC at 32°C		*Enterobactereaceae*		*S.aureus*	
	Satisfactory (<1.3 $Log_{10}cfu/cm^2$) (%)	Unsatisfactory (≥1.3 $Log_{10}cfu/cm^2$) (%)	Satisfactory (<1 $Log_{10}cfu/cm^2$) (%)	Unsatisfactory (≥ 1 $Log_{10}cfu/cm^2$) (%)	Satisfactory (<1 $Log_{10}cfu/cm^2$) (%)	Unsatisfactory (≥1 $Log_{10}cfu/cm^2$) (%)
Work surfaces (worktops, Teflon chopping boards) n= 19	100	0	100	0	100	0
Cutting equipments (Knives, vegetable cutting blade) n= 6	66.6	33.3	66.6	33.3	100	0
Food handlers' hands n=8	75.0	25.0	87.5	12.5	87.5	12.5
All surfaces n = 33	87.9	12.1	90.9	9.1	97.0	3.0

[a]Henroid et al., 2004; Sneed et al., 2004

As regards the detection of ATP, it was only performed in 7 work surfaces (2 worktops and 7 Teflon chopping boards). The results ranged between 19 and 440 RLU's/100cm^2 being in conformity with the guidelines proposed by Grbalová et al. (2003), which indicates the cleanliness of these surfaces. This method proves to be more advantageous over traditional methods since takes into account the presence of foreign matter which may represent a nourishment source for microorganisms.

CONCLUSION:

Out of the total number of samples taken for testing, 24% were not in conformity with the advisory standards. Excessive amounts of TVC and *Enterobactereaceae* were mainly recorded in cutting equipments. Although the hygiene levels of surfaces and food handlers reveals to be overall satisfactory, showing that the current cleaning procedures applied are effective, there are still some limitations regarding the hygiene and food safety knowledge of food handlers. Temperature control is particularly an improvement area. This study provides more information about the reality of Portuguese school canteens as regards the hygiene and food safety.

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Comparative risk assessment of listeriosis under different L. monocytogenes contamination levels in food according to EU, Codex Alimentarius and Russian Federation standards

Authors: N.V. Zaitseva[1], P.Z. Shur[1], C.V. Romanenko[1], N.G. Atiskova[1], D.A. Kiryanov[1], M.R. Kamaltdinov[1]

1 *Federal Budget Scientific Institution "Federal Scientific Center for Medical and Preventive Health Risk Management Technologies"*

Presenting Author: Email: romanenko@fcrisk.ru | Tel.: +79 222 448 812

INTRODUCTION:

There is sufficient evidence in the United States, the European Union, and the Russian Federation of L. monocytogenes contaminated food consumption [1,2].

As far as listeriosis is a severe disease (mortality rate is about 21%) [3], and all established standards in the EU, Codex Alimentarius and the Russian Federation are different and based on health risk assessment procedure, the comparative health risk assessment to determine the standard providing consumers health security is reasonable.

OBJECTIVES:

To carry out a comparative risk assessment of listeriosis under different levels of food contamination by L. monocytogenes in order to protect consumers health.

MATERIALS AND METHODS:

Microbiological risk assessment was based on general principles of Codex Alimentarius stated in CAC/GL-30 - 1999 " Principles and Guidelines for the installation of microbiological risk assessment" [4]. Standards of L. monocytogenes in food were selected in accordance with relevant regulations of Codex Alimentarius, the European Union and the Russian Federation.

To evaluate "exposure- effect" relation an exponential model was used [5]:

$$P_i = 1 - \exp[-r_i * N_i],$$

where P_i - the probability of illness after consumption of the i-th product, N_i - dose of microorganisms, CFU L. monocytogenes/day, r_i - the probability of illness from ingestion of one microorganism[5].

To calculate the probability of the illness for the general population specific coefficients for three product types (smoked fish, chocolate milk and maize-tuna salad) were used (for sensitive groups a different coefficient was applied) according to FAO/WHO [5].

RESULTS AND DISCUSSION:

Risk assessment procedure comprised hazard identification, hazard characterization, exposure assessment and risk characterization [5].

Hazard identification revealed that L. monocytogenes is a Gram-positive, facultatively anaerobic, non-sporeforming rod and has been often isolated from food processing environment characterized by low temperatures and high humidity. L. monocytogenes frequently presents in foods such as milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw meat and cooked sausages, raw and cooked poultry, raw meats, and raw and smoked seafood [4].

Hazard characterization determined that L. monocytogenes is widely distributed in the environment and foods. Despite the fact that a wide variety of foods may be contaminated with L. monocytogenes, outbreaks and sporadic cases of listeriosis are predominately associated with ready-to- eat (RTE) products. As a negative health effect in terms of health risk assessment development of listeriosis was chosen.

On the exposure assessment stage twelve exposure scenarios were worked out taking into account the average daily food consumption (actual and recommended) for general population and the most sensitive groups and the acceptable L. monocytogenes contamination levels on the end of product manufacture (0.04 CFU L. monocytogenes/g) and the maximum L. monocytogenes contamination level at the point of sale (100 CFU L. monocytogenes/g) as the most frequently discussed standards for L. monocytogenes in foods. Vegetables, dairy products, fish (seafood) were taken as high risk food groups. The exposure assessment revealed that maximum daily intake of L. monocytogenes with food varied from 44 (L. monocytogenes absence in 25g of product standard) to 185 660 CFU (100 CFU L. monocytogenes/g standard).

Probability values of listeriosis development for 12 exposure scenarios were obtained as a result of "dose-effect" modelling (Table 1).

Table 1 – The risk of listeriosis for different exposure scenarios

Scenarios	Standard	Population	Average daily food consumption	The total risk
Scenario 1	Absence in 25 g	General population	Recommended average daily food consumption for general population	$2,77^{-07}$
Scenario 2	100 CFU			$6,93^{-04}$
Scenario 3	Absence in 25 g	General population	Actual average daily food consumption for general population	$1,42^{-07}$
Scenario 4	100 CFU			$3,54^{-04}$
Scenario 5	Absence in 25 g	Pregnant women	Recommended average daily food consumption for pregnant women (vegetables, fish (seafood); Recommended average daily food consumption for general population (milk)	$7,71^{-06}$
Scenario 6	100 CFU			$2,55^{-03}$
Scenario 7	Absence in 25 g	Pregnant women	Recommended average daily food consumption for pregnant women (vegetables, fish (seafood); Actual average daily food consumption for general population (milk)	$7,57^{-06}$
Scenario 8	100 CFU			$2,21^{-03}$
Scenario 9	Absence in 25 g	Lactating women	Recommended average daily food consumption for lactating women (vegetables, fish (seafood); Recommended average daily food consumption for general population (milk)	$8,97^{-06}$
Scenario 10	100 CFU			$2,86^{-03}$
Scenario 11	Absence in 25 g	Lactating women	Recommended average daily food consumption for lactating women (vegetables, fish (seafood); Actual average daily food consumption for general population (milk)	$8,83^{-06}$
Scenario 12	100 CFU			$2,52^{-03}$

Risk characterization showed that application of 100 CFU L. monocytogenes/g standard could lead to unacceptable risk levels of listeriosis among both general population and the most sensitive groups. Acceptable risk level for serious diseases ($<1 \times 10^{-4}$) was not exceeded for all studied population under absence of L. monocytogenes in 25 g standard (0.04 CFU L. monocytogenes/g), that ensures public health safety.

CONCLUSION:

It was determined that risk of foodborne listeriosis under absence of L. monocytogenes in 25 g standard does not exceed the maximum acceptable risk level. Usage of 100 CFU L. monocytogenes/g standard could result in unacceptable risk levels of listeriosis for general population and the most sensitive groups. At the same time the risk of listeriosis in general population is associated with the consumption of vegetables, while the main group of products forming health risk for pregnant and lactating women is dairy products.

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Gastroenteritis outbreak following funeral meal in Tjakastad, Chief Albert Luthuli Sub-district, South Africa, August 2010

Authors: Themba Sigudu[1,2], Kaizer Ndlovu[2], Thembi Zulu[3]

1 *Field Epidemiology and Laboratory Training Programme, National Institute for Communicable Diseases*
2 *Environmental Health, Department of Health, Mpumalanga Provincial Government*
3 *Communicable Disease Control, Department of Health, Mpumalanga Provincial Government*

Presenting Author: Email: thembas@uj.ac.za | Tel.: +27 72 130 7992 | Fax: +27 86 207 4037

INTRODUCTION:

Gastroenteritis, also known as food poisoning, occurs when a person gets sick from eating food that has been contaminated by pathogenic microorganisms and it usually arises from improper handling, preparation, transportation or storage of food (Gerald L et al, 2005). On the 17th of August 2010 an NHLS Laboratory technician reported to the Environmental Health unit about the suspected gastroenteritis outbreak following funeral meal in Tjakastad. Upon receiving such report, the Environmental Health unit initiated an outbreak investigation.

OBJECTIVES:

An investigation was carried out to characterise the outbreak in terms of time, place and person, to determine the source of the outbreak, to identify the causative organism, and to recommend and to implement appropriate prevention and control measures.

MATERIALS AND METHODS:

A retrospective cohort study was conducted among 95 individuals who attended the funeral. A case was defined as any person who ate the served meal and developed diarrhoea, abdominal cramps and vomiting within 6 hours after eating the meal (NDoH, 2006). Diarrhoea was defined as three or more stools in a 24-hour period (CDC, 1997). A line list was used for screening and obtaining information on demographic characteristics, clinical features of cases, exposure to food items and outcome data which was analysed by making use of Epi Info. Rectal swabs were collected from 9 of the cases for bacterial culture. Left-over food samples were taken for laboratory testing.

RESULTS AND DISCUSSION:

The results of the investigation confirm that people who attended the funeral became ill with Non-typhoid *Salmonella* after consuming the meal served on the 13th -14th of August 2010.Out of the 95 people interviewed, (n=88, 93%) people reported eating the served funeral meal. Among those who ate the meal, (n=56, 64%) met the outbreak case definition. On average the male to female case ratio was 1:1.5. Median age was 50 years (range: 1-90 years). Onset of symptoms ranged from 3 hours to 11 hours after the lunch, with a median incubation period of 6 hours (range: 3-11 hours). Please see Figure 1. The majority of the cases i.e. 14 (25%) experienced illness within 8 hours of food consumption, as seen by the highest peak. The sharp increase and slow decline in the number of cases over a short period of time demonstrate a common-source outbreak. Duration of illness ranged from 1-3 days. Symptoms commonly reported were: watery diarrhoea (n=53, 95%), abdominal discomfort (n=50, 89%) and vomiting (n=50, 50%). Of the food items served, porridge was statistically implicated as a vehicle for disease transmission. People who consumed the porridge were more likely to become ill compared to those who did not eat porridge
(RR = 1.01; 95%CI = 0.57-1.81). Please see table 1

Table 1 - Risk of illness by food item served during the funeral meal, Tjakastad, Albert Luthuli Sub-district, August 2010.

Food served	Ate food			Did not eat food				
	Ill	Not ill	AR%	Ill	Not ill	AR%	RR	95%CI
Porridge	37	21	64	19	11	63	1.01	0.57-1.81
Pumpkin	16	17	48	40	15	73	0.53	0.31-0.91
Cabbage	19	16	54	37	16	70	0.66	0.38-1.14
Chicken stew	38	29	57	18	03	86	0.33	0.11-0.97
Cakes	34	22	61	22	10	69	0.80	0.43-1.46
Potatoes	37	27	58	19	05	79	0.49	0.22-1.13

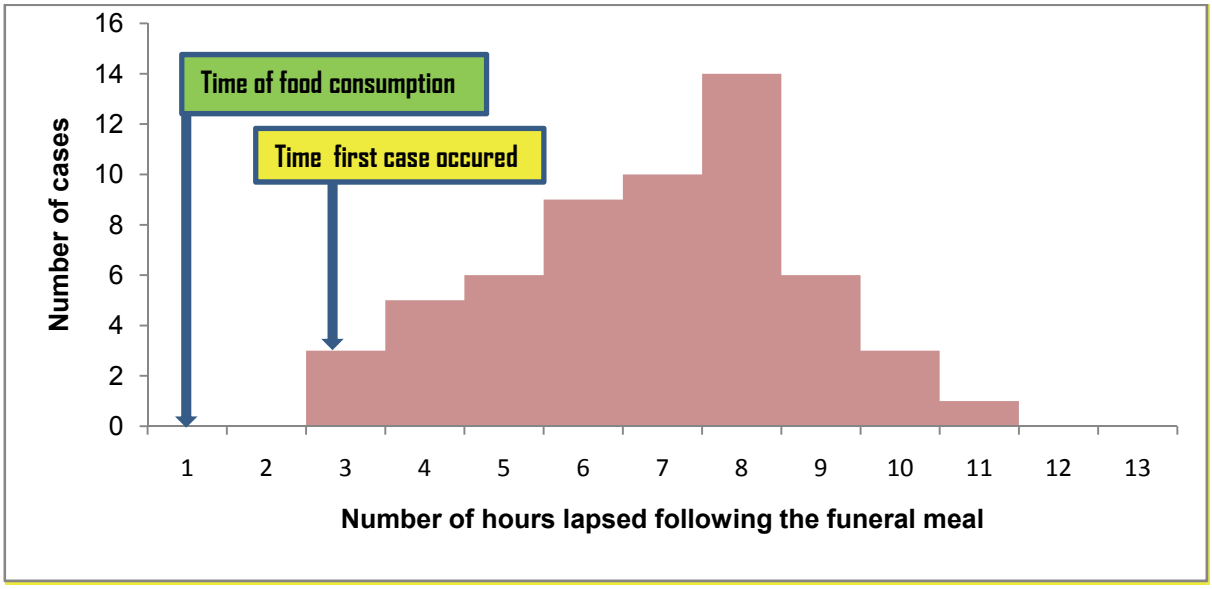


Figure 1 - Epi curve showing time of onset of the gastroenteritis outbreak following a funeral meal, Tjakastad, Chief Albert Luthuli Sub-district, August 2010.

CONCLUSION:

Food poisoning and infections have been subjected to under reporting in Mpumalanga Province including Albert Luthuli Sub-district. Although porridge appears to have been the most likely vehicle, lack of medical examination of food handlers posed a limitation in identifying the organism. The means of contamination could not be identified. Shortage of human resources with the necessary skills to conduct adequate and credible disease outbreak investigations as well as the lack of cooperation on the part of the nursing and medical staff forms the major obstacle to controlling food-borne disease outbreaks.

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ORGANISING COMMITEE OF ICEH2014:

ESTSP | POLITÉCNICO DO PORTO
Escola Superior de Tecnologia da Saúde

Rua de Valente Perfeito, 322
4400-330 Vila Nova de Gaia
Porto - PORTUGAL

t. +351 222 061 000 **e.** geral@estsp.ipp.pt
f. +351 222 061 001 **w.** www.estsp.ipp.pt