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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

Effects of interaction and bioaccessibility of the cyanotoxins microcystin and cylindrospermopsin in aquatic and terrestrial species

Authors: Marisa Freitas^{1,2,3}, Joana Azevedo¹, António Paulo Carvalho^{1,2}, Sébastien Planchon⁴, Jenny Renaut⁴, Vera Mendes⁵, Bruno Manadas⁵, Edgar Pinto⁶, Aldo Barreiro¹, Joana Neves¹, Alexandre Campos¹, 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 Saúde do 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, *Cylindrospermopsis raciborskii*. 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 0.03 µg/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 withstand 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.

Conditions	Storage process			Cooking process		
	25° C	4° C	- 20° 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% to 48 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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