Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals

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ABSTRACT

The impact of effluent wastewaters from four different hospitals: a university (1456 beds), a general (350 beds), a pediatric (110 beds) and a maternity hospital (96 beds), which are conveyed to the same wastewater treatment plant (WWTP), was evaluated in the receiving urban wastewaters. The occurrence of 78 pharma-ceuticals belonging to several therapeutic classes was assessed in hospital effluents and WWTP wastewaters (influent and effluent) as well as the contribution of each hospital in WWTP influent in terms of pharmaceu- tical load. Results indicate that pharmaceuticals are widespread pollutants in both hospital and urban waste- waters. The contribution of hospitals to the input of pharmaceuticals in urban wastewaters widely varies, according to their dimension. The estimated total mass loadings were 306 g d^{-1} for the university hospital, 155 g d^{-1} for the general one, 14 g d^{-1} for the pediatric hospital and 1.5 g d^{-1} for the maternity hospital, showing that the biggest hospitals have a greater contribution to the total mass load of pharmaceuticals. Fur- thermore, analysis of individual contributions of each therapeutic group showed that NSAIDs, analgesics and antibiotics are among the groups with the highest inputs.

Removal efficiency can go from over 90% for pharmaceuticals like acetaminophen and ibuprofen to not removal for β-blockers and salbutamol. Total mass load of pharmaceuticals into receiving surface waters was estimated between 5 and 14 g/d/1000 inhabitants.

Finally, the environmental risk posed by pharmaceuticals detected in hospital and WWTP effluents was assessed by means of hazard quotients toward different trophic levels (algae, daphnids and fish). Several pharmaceuticals present in the different matrices were identified as potentially hazardous to aquatic organ- isms, showing that especial attention should be paid to antibiotics such as ciprofloxacin, ofloxacin, sulfameth- oxazole, azithromycin and clarithromycin, since their hazard quotients in WWTP effluent revealed that they could pose an ecotoxicological risk to algae.

Keywords: Pharmaceuticals, Hospital effluent, Wastewaters, Removal efficiency, Environmental risk assessment

1. Introduction

Over the last decades, the worldwide consumption of pharmaceuticals has increased as well as their detection in wastewaters and surface waters, which represents a major concern in terms of their potential impact on the environment and human health. Wastewaters have been pointed out as the main route of entry of pharmaceuticals into the environment (Daughton and Ruhoy, 2009), since they gather the residues excreted after ingestion, which are excreted in urine and feces, either as unchanged compounds or metabolites. Several studies pointed out that Wastewater Treatment Plants (WWTPs) are not able to completely remove pharmaceuticals (Behera et al., 2011; Gracia-Lor et al., 2012; Jelic et al., 2011; Kosma et al., 2010; Zorita et al., 2009). Besides urban wastewaters, hospital wastewaters have also stood up as an important environmental exposure pathway of pharmaceuticals (Verlicchi et al., 2010b).

Due to their specific nature, it is expected that hospital effluents present a mixture of compounds, including not only pharmaceuticals and their metabolites, but also diagnostic agents, disinfectants, among others, resulting from diagnostic, laboratory and research activities and principally from medicine excretion from patients (Verlicchi et al., 2010b). Consumption, use and application of pharmaceuticals in a hospital may vary over the year and from country to country (Schuster et al., 2008), due to the predominance of diseases and to the hospital activity, as well as to the local list of pharmaceuticals suggested for the treatment of different diseases. These changes will have impact on pharmaceuticals detected in hospital effluents, since they are closely related with the substances that are being administered in a certain hospital as well as their quantities. Several authors have shown the presence of pharmaceuticals in hospital wastewaters (Gómez et al., 2006; Lin and Tsai, 2009; Sim et al., 2011; Verlicchi et al., 2012a; Weissbrodt et al., 2009). Furthermore, hospital effluents also play an important role in the introduction of pathogens into public wastewaters, especially concerning multi-resistant bacteria, contributing to the spread of antibiotic resistance into the environment (Kümmerer, 2009).

Hospitals generate different quantities of wastewaters depending on factors like number of beds, hospital age, general services present inside the structure (kitchen, laundry, etc.), number and types of wards and units, institution management policies, cultural and geographical factors, among others (Verlicchi et al., 2010b). Usually hospital effluents are directly discharged into public sewer network, being co-treated with domestic wastewaters in municipal WWTPs. This practice has been questioned by some authors (Pauwels and Verstraete, 2006; Verlicchi et al., 2012a), who suggested the adoption of a more dedicated treatment for hospital effluents before being discharged into public wastewaters and then both urban and hospital wastewaters would be subsequently treated in WWTPs (Pauwels and Verstraete, 2006; Verlicchi et al., 2010a). This approach has benefits like avoiding the dilution of hospital wastewaters with urban wastewaters, which may result in the inhibition of biomass and reduction of removal efficiency in WWTPs, as well as to avoid losses into the environment due to sewer leakage and combined sewer overflows (Kovalova et al., 2012). At the same time, it is possible to avoid the spread of multi-antibiotic resistant bacteria (Kümmerer, 2009) and the input of chemical substances (pharmaceuticals, diagnostic agents, etc.) that in some cases are genotoxic (Gupta et al., 2009).

Several monitoring studies have reported the presence of pharmaceuticals in urban wastewaters (Al-Rifai et al., 2007; Brown et al., 2006; Bueno et al., 2012; Gracia-Lor et al., 2011; Gros et al., 2006; Pedrouzo et al., 2011) and surface waters (Daneshvar et al., 2010; González Alonso et al., 2010; Kolpin et al., 2002; Martín et al., 2011; Spongberg et al., 2011; Vystavna et al., 2012). Nevertheless, few data is available on the contribution of hospital effluents towards the load of pharmaceuticals in WWTPs (Beier et al., 2011; Langford and Thomas, 2009; Ort et al., 2010; Thomas et al., 2007; Verlicchi et al., 2012a). At the same time, available data regarding the environmental risk posed by hospital effluents to aquatic organisms is still sparse and often limited to predicted (Escher et al., 2011; Souza et al., 2009) rather than measured concentrations (Verlicchi et al., 2012a).

Due to their bioactive intrinsic properties, pharmaceuticals are recognized as being able to cause potential effects in aquatic organisms; therefore environmental risk assessment (ERA) studies are recommended, in order to consider the potential effect of pharmaceuticals at their exposure levels (von der Ohe et al., 2011). According to the guidelines set out by the European Medicines Agency (EMA), new pharmaceuticals require an ERA, which is assessed in a step-wise approach, divided in two phases. In Phase I, environmental exposure of the pharmaceuticals is estimated and if their predicted environmental concentration (PEC) exceeds a threshold safety value of 10 ng L^{-1}, Phase II studies are required, in order to assess their ecotoxicological potential (EMEA, 2006).

In this context, the aim of the present work was to monitor the occurrence of 78 pharmaceuticals of major human consumption in four hospitals located in Coimbra (Portugal) with different capacities, wards and units, namely a university hospital (1456 beds), a general hospital (350 beds), a pediatric hospital (110 beds) and a maternity hospital (96 beds), as well as in the influent and effluent wastewaters of the WWTP that receives and co-treats their wastewaters. The impact and individual contribution of each hospital to the load of pharmaceuticals into the receiving urban wastewaters was evaluated, being one of the few studies that embraced a high number of compounds belonging to several therapeutic classes. In addition, removal efficiency for all target compounds was also evaluated in WWTP. Finally, the potential ecotoxicological risk posed by pharmaceuticals to aquatic organisms when exposed to the studied hospital and WWTP effluents was assessed and prioritization lists of potentially hazardous pharmaceuticals that should be included in monitoring programs and that might be considered for inclusion in future regulations were established.

2. Materials and methods

2.1. Sampling site, sample collection and sample pre-treatment

Effluents from four hospitals with different dimensions, units and wards located in Coimbra (Portugal) were sampled in this study, together with the influent and effluent of the receiving WWTP. Studied hospitals included:

- University hospital: large hospital with 1456 beds and with a broad range of clinical and services and medical specialities as well as a center of research. It serves a population of approximately 430,000 inhabitants and it is also a reference hospital for the center region of Portugal;
- General hospital: medium-sized hospital with 350 beds and thirteen main wards. It serves a population of approximately 369,000 inhabitants;
- Pediatric hospital: small hospital with 110 beds and nine main wards. It serves a population of approximately 90,000 inhabitants and it is a reference hospital that supports pediatric units of hospitals located in the center region of Portugal;
- Maternity: small hospital with 96 beds, not including the baby unit, and three main wards, namely gynecology, obstetrics and neonatology. It serves a population of approximately 507,000 inhabitants (women).

The WWTP is designed for 213,000 population equivalent and it has a primary and secondary treatment operating with trickling filters. The WWTP receives urban wastewaters (including domestic wastewaters and hospital effluents – from the four mentioned hospitals) combined with rain waters. The biological treatment is performed by four trickling filters that work in parallel. They are 3 m high and 36 m in diameter, having a unitary volume of 3030 m^3.

Sampling campaigns were performed between February 2011 and May 2011, embracing a total of nine sampling periods for hospitals and seven for WWTP (influent and effluent). Samples from hospital effluents and WWTP wastewaters were collected in the same days, with the exception of two days where it was only possible to collect samples from hospital wastewaters (namely 28th March 2011 and 4th April 2011). Wastewater samples were collected in amber glass bottles previously rinsed with ultra-pure water as grab samples to hospital effluents, which were all collected at the same time frame (10–11 a.m.), and time proportional 24-h composite samples to WWTP influent and effluent. Samples were kept refrigerated (± 4 °C)

Table 1

Range of concentrations and mean concentration (± standard deviation), expressed in ng L^{-1}, of pharmaceuticals in hospital effluents and in WWTP influent and effluent.

Therapeutic group	Compound	University hospital		General hospital		Pediatric hospital		Maternity hospital		WWTP influent		WWTP effluent	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Analgesics and anti-inflammatories	Ketoprofen	bMQL-199	99.3 ± 66.3	143-3250	1107 ± 1269	83.6-180	124 ± 29	79.3-264	146 ± 66	289-589	458 ± 112	158-320	218 ± 52
	Naproxen	45.4-6042	1837 ± 2057	bMDL-4046	608 ± 1293	bMQL-5625	674 ± 1857	36.5-1638	504 ± 628	8.84-1617	741 ± 522	bMQL-774	303 ± 275
	Ibuprofen	232-5815	1965 ± 2082	237-11,333	3082 ± 4200	1263-38,148	7090 ± 11,995	1952-16,630	7728 ± 5286	bMDL-4926	1596 ± 1715	bMQL-369	119 ± 136
	Indomethacine	n.d.-bMQL	bMDL	n.d.-150	bMQL	n.d.	n.d.	n.d.-79.5	bMDL	bMDL-51.0	bMDL	bMDL-bMQL	bMDL
	Acetaminophen	13,029-58,857	27,700 ± 16,107	12,557-47,143	24,687 ± 12,201	2271-57,143	18,235 ± 15,503	211-13,986	9211 ± 4629	80.7-9286	2463 ± 3454	83.1-106	96.1 ± 8.1
	Salicylic acid	383-2817	1822 ± 825	bMDL-2272	1255 ± 806	6.89-4681	1256 ± 1546	72.2-4624	1343 ± 1486	bMDL-257	51.8 ± 92.6	n.d.-bMDL	bMDL
	Diclofenac	bMQL-189	80.8 ± 59.9	bMQL-63.5	bMQL	bMQL-169	46.6 ± 47.7	bMQL-103	47.0 ± 28.4	bMQL-269	69.7 ± 89.4	24.6-83.1	42.9 ± 19.5
	Phenazone	60.5-271	121 ± 73	51.7-146	84.4 ± 27.3	bMDL-10.4	bMQL	bMDL-64.4	14.1 ± 20.1	11.1-43.8	25.7 ± 12.5	12.6-52.9	29.5 ± 12.9
	Propyphenazone	bMDL-1.72	bMQL	bMDL-1.47	bMQL	bMDL-1.53	bMQL	n.d.-bMQL	bMDL	bMDL-bMQL	bMDL	bMDL-bMQL	bMQL
	Piroxicam	n.d.-51.2	9.25 ± 19.0	n.d.-bMDL	bMDL	n.d.-bMQL	bMDL	n.d.-bMQL	bMDL	bMDL-bMQL	bMQL	bMDL	bMDL
	Tenoxicam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.-bMDL	bMDL
	Meloxicam	n.d.	n.d.	n.d.-b MDL	bMDL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Oxycodone	n.d.-bMDL	bMDL	n.d.-bMDL	bMDL	n.d.-23.5	bMQL	bMDL-12.9	bMQL	bMDL	bMDL	bMDL	bMDL
	Codeine	8.08-2837	467 ± 914	9.58-1006	295 ± 337	2.68-429	67.1 ± 140	3.49-2760	404 ± 896	153-283	206 ± 48	16.9-261	138 ± 86
Lipid regulators and cholesterol lowering statin drugs	Bezafibrate	bMDL-1350	258 ± 452	bMDL-659	86.9 ± 216	n.d.-17.6	bMQL	n.d.-242	76.2 ± 87.3	382-623	490 ± 93	93.8-635	409 ± 214
	Gemfibrozil	n.d.-285	32.8 ± 94.5	n.d.	n.d.	n.d.-1126	125 ± 375	n.d.-224	38.6 ± 72.8	n.d.-22.5	bMQL	n.d.-bMQL	bMDL
	Pravastatin	bMQL-1200	305 ± 368	bMDL-332	75.5 ± 105	n.d.-2086	306 ± 673	n.d.-b MQL	bMDL	124-327	218 ± 72	118-395	239 ± 111
	Fluvastatin	n.d.-27.8	bMQL	n.d.-bMDL	bMDL	n.d.-bMDL	bMDL	n.d.-bMDL	bMDL	n.d.-bMDL	bMDL	n.d.	n.d.
	Atorvastatin	n.d.-60.0	9.86 ± 19.9	n.d.	n.d.	n.d.	n.d.	n.d.-65.1	13.1 ± 24.1	n.d.-bMQL	bMDL	n.d.	n.d.
Psychiatric drugs	Carbamazepine	428-1050	771 ± 213	128-1123	650 ± 371	19.3-2042	295 ± 658	bMQL-344	64.5 ± 112	437-673	565 ± 74	364-496	460 ± 45
	Acridone*	n.d.	n.d.	n.d.	n.d.	n.d.-2.86	bMQL	n.d.	n.d.	n.d.-bMDL	bMDL	n.d.-bMDL	bMDL
	Sertraline	bMDL-bMQL	bMDL	bMDL	bMDL	bMDL-bMQL	bMDL	bMDL-bMQL	bMDL	bMDL-bMQL	bMQL	n.d.-bMQL	bMQL
	Citalopram	31.0-232	110 ± 86	9.43-122	58.3 ± 45.2	11.4-888	196 ± 335	11.2-457	145 ± 162	12.7-34.3	23.3 ± 7.8	17.0-49.1	34.0 ± 11.5
	Venlafaxine	81.3-880	325 ± 310	53.3-662	227 ± 194	13.0-972	245 ± 319	38.5-1914	545 ± 619	68.0-268	181 ± 79	184-322	272 ± 54
	Olanzapine	1.62-824	236 ± 267	bMQL-102	29.3 ± 35.6	n.d.-303	38.3 ± 99.4	n.d.-9.97	1.71 ± 3.58	n.d.-15.3	4.52 ± 6.23	15.0-36.1	26.2 ± 8.4
	Trazodone	5.37-51.1	16.2 ± 13.8	bMQL-31.1	11.4 ± 9.6	bMQL-36.6	7.86 ± 11.1	bMDL-36.9	12.5 ± 13.8	3.06-11.1	6.72 ± 2.72	bMQL-6.37	4.03 ± 1.84
	Fluoxetine	34.8-105	70.1 ± 37.6	18.3-43.6	31.0 ± 9.3	n.d.-44.5	19.3 ± 15.6	n.d.-128	36.7 ± 46.3	bMDL-29.7	bMQL	n.d.-bMQL	bMDL
	Norfluoxetine*	bMQL-49.1	15.3 ± 16.7	bMQL-40.9	24.7 ± 13.3	bMQL-33.4	10.3 ± 8.9	bMQL-85.1	26.1 ± 31.9	45.1-226	112 ± 67	bMDL-99.6	36.1 ± 38.4
	Paroxetine	bMDL-bMQL	bMDL	bMDL	bMDL	bMDL	bMDL	bMDL	bMDL	bMDL	bMDL	bMDL-bMQL	bMQL
	Diazepam	12.2-31.1	18.5 ± 6.9	bMDL-29.6	10.5 ± 7.8	bMDL-31.9	bMQL	bMQL-49.1	17.7 ± 18.1	bMQL-7.63	6.46 ± 0.84	6.53-8.81	7.16 ± 0.82
	Lorazepam	107-1325	441 ± 374	151-520	308 ± 142	28.2-320	110 ± 94	43.9-551	289 ± 165	221-446	299 ± 81	175-347	294 ± 59
	Alprazolam	37.5-81.5	44.9 ± 13.9	42.2-168	106 ± 40	6.87-143	34.3 ± 42.5	4.58-96.7	46.5 ± 26.0	19.1-49.1	32.3 ± 11.1	11.3-33.5	27.5 ± 7.9
Histamine H_1 receptors antagonists	Loratadine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Desloratadine*	n.d.-10.2	2.66 ± 3.33	n.d.-0.713	bMQL	n.d.-1.07	bMQL	n.d.-1.31	0.224 ± 0.469	n.d.	n.d.	n.d.	n.d.
Histamine H_2 receptors antagonists	Ranitidine	31.3-12,240	2152 ± 4171	255-19,840	4164 ± 6366	16.2-856	115 ± 278	44.4-3240	477 ± 1046	41.6-359	211 ± 106	31.7-313	149 ± 98
	Famotidine	bMQL-14.5	4.11 ± 4.38	bMQL-212	26.1 ± 69.7	bMQL-1.32	bMQL	bMQL-2.58	bMQL	bMQL-3.96	2.01 ± 1.07	1.36-2.82	1.99 ± 0.48
	Cimetidine	bMDL-24.9	4.55 ± 8.20	2.49-479	58.4 ± 158	n.d.-1.80	bMDL	n.d.-2.47	bMQL	2.40-14.6	7.07 ± 4.20	2.00-11.9	7.40 ± 3.41

β-Blockers	Atenolol	76.3-2000	706 ± 575	172-1171	595 ± 361	8.55-8037	1069 ± 2628	45.5-5908	1063 ± 1852	361-751	522 ± 132	411-782	600 ± 152
	Sotalol	bMDL-345	89.1 ± 122	23.7-142	56.9 ± 36.9	n.d.-bMDL	bMDL	n.d.-172	20.5 ± 56.9	85.7-144	117 ± 24	83.1-186	154 ± 34
	Propranolol	bMQL-53.6	21.3 ± 16.9	4.19-81.0	18.0 ± 24.7	n.d.-812	98.9 ± 268	6.36-243	66.6 ± 83.7	2.61-23.9	8.98 ± 8.05	4.28-10.6	8.27 ± 2.07
	Metoprolol	n.d.-280	35.6 ± 92.0	bMQL-441	59.9 ± 144	n.d.-148	18.8 ± 48.5	n.d.-5.29	bMQL	bMQL-15.2	bMQL	5.50-18.4	11.9 ± 4.28
	Nadolol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.-2.14	bMQL	n.d.	n.d.
	Carazolol	6.05-7.37	6.63 ± 0.39	5.79-6.39	6.01 ± 0.19	5.66-6.89	6.19 ± 0.44	5.68-6.87	6.11 ± 0.44	2.82-3.11	2.91 ± 0.11	bMQL	bMQL
Diuretic	Hydrochlorothiazide	692-810	767 ± 41	590-863	764 ± 102	223-825	565 ± 238	239-997	518 ± 257	359-424	393 ± 22	223-233	229 ± 4
	Furosemide	4763-22,326	12,014 ± 6337	2363-21,488	11,121 ± 5671	535-32,558	5444 ± 10,241	434-9953	3574 ± 3652	1591-4577	2726 ± 1043	267-2214	1183 ± 609
	Torasemide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antidiabetic	Metformin	18.6-2844	972 ± 859	484-3836	1346 ± 1105	16.1-716	174 ± 242	bMQL-4040	1163 ± 1329	bMQL-1568	720 ± 551	3.87-299	164 ± 124
	Glibenclamide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antihypertensives	Amlodipine	bMQL-195	93.9 ± 56.9	bMQL-101	37.8 ± 27.6	bMQL-45.5	bMQL	bMDL-110	36.5 ± 31.8	14.9-85.7	48.5 ± 24.6	bMQL-78.7	41.4 ± 30.1
	Losartan	72.0-910	259 ± 264	59.0-433	178 ± 113	bMDL-333	141 ± 108	bMQL-257	92.8 ± 85.0	90.0-658	237 ± 205	bMQL-364	143 ± 106
	Irbesartan	90.0-2120	539 ± 620	18.4-1850	520 ± 562	bMDL-1830	491 ± 596	39.9-3860	670 ± 1206	278-1170	591 ± 317	116-790	410 ± 237
	Valsartan	902-19,822	8936 ± 7423	407-4489	1562 ± 1327	104-11,733	1873 ± 3774	280-7822	1846 ± 2369	2956-8400	5117 ± 2009	20.8-4860	2377 ± 2100
Antiplatelet agent	Clopidogrel	37.1-396	162 ± 121	33.3-175	98.1 ± 44.8	3.03-199	32.6 ± 62.7	2.39-184	31.4 ± 59.6	2.57-53.4	20.6 ± 20.0	4.21-16.8	11.1 ± 4.3
Prostatic hyperplasia	Tamsulosin	2.26-3.20	2.62 ± 0.31	1.69-2.37	1.99 ± 0.26	1.62-2.24	1.93 ± 0.22	1.47-2.02	1.62 ± 0.17	0.781-1.37	1.04 ± 0.23	bMQL-0.872	0.719 ± 0.143
β-Agonist	Salbutamol	n.d.-2595	383 ± 832	56.1-199	136 ± 51	11.9-279	77.6 ± 88.9	n.d.-43.4	6.78 ± 14.4	0.967-12.1	7.34 ± 3.62	4.43-26.8	16.1 ± 7.5
Anticoagulant	Warfarin	3.57-8.28	6.17 ± 1.80	2.21-8.02	4.50 ± 2.01	bMQL-2.85	1.42 ± 0.71	bMQL-2.47	1.54 ± 0.73	bMQL-7.10	3.55 ± 1.75	1.56-3.87	2.42 ± 0.76
X-ray contrast agent	Iopromide	66,286-550,857	19,5683 ± 168,147	50,229-611,429	26,0908 ± 216,851	880-24,743	7493 ± 7606	205-1243	461 ± 341	23,543-164,000	79,527 ± 46,533	33,885-85,000	49,286 ± 16,933
Antihelmintics	Albendazole	n.d.-bMQL	bMDL	n.d.-28.3	3.38 ± 9.37	n.d.-bMDL	bMDL	n.d.-bMQL	bMDL	n.d.-1.79	0.526 ± 0.739	n.d.	n.d.
	Thiabendazole	n.d.-9.77	bMQL	n.d.-494	58.2 ± 164	n.d.-1746	398 ± 572	n.d.-97.7	31.0 ± 39.9	n.d.-15.3	2.77 ± 5.75	0.493-12.1	4.95 ± 4.18
	Levamisole	n.d.-39.5	5.82 ± 12.9	n.d.	n.d.	n.d.-182	20.4 ± 60.7	n.d.-74.3	25.5 ± 30.2	6.96-24.0	11.7 ± 5.9	8.72-31.5	19.1 ± 7.9
Synthetic glucocorticoid	Dexamethasone	72.4-352	127 ± 87	bMQL-61.8	28.4 ± 19.5	n.d.-31.0	bMQL	bMDL-278	66.9 ± 98.9	n.d.-bMQL	bMQL	bMDL-bMQL	bMQL
Sedation and muscle relaxation	Xylazine	n.d.	n.d.	n.d.	n.d.	n.d.-13.6	bMQL	n.d.-24.4	bMQL	n.d.	n.d.	n.d.	n.d.
Tranquilizer	Azaperone	bMDL-2.70	bMQL	bMDL-3.87	bMQL	bMDL-bMQL	bMQL	n.d.-bMQL	bMDL	bMDL	bMDL	n.d.-bMDL	bMDL
	Azaperol*	n.d.-bMQL	bMDL	n.d.-bMDL	bMDL	n.d.-bMDL	bMDL	n.d.	n.d.	n.d.-bMDL	bMDL	n.d.-bMDL	bMDL
Antibiotics	Erythromycin	bMQL-1075	209 ± 355	n.d.-22.2	bMQL	n.d.-913	108 ± 302	47.8-7545	1407 ± 2350	9.64-220	92.7 ± 77.9	20.4-134	71.2 ± 40.6
	Azithromycin	1227-7351	3748 ± 2331	89.2-4492	1889 ± 1299	bMQL-376	85.8 ± 116	bMQL-2665	840 ± 917	79.7-295	186 ± 79	93.7-297	171 ± 68
	Clarithromycin	2.56-199	62.6 ± 71.6	n.d.-45.6	7.56 ± 15.6	n.d.-960	135 ± 312	n.d.-165	32.5 ± 59.0	n.d.-52.3	22.2 ± 17.8	12.0-40.0	22.4 ± 11.4
	Tetracycline	n.d.-bMDL	bMDL	n.d.-bMDL	bMDL	n.d.-bMDL	bMDL	n.d.-bMDL	bMDL	bMDL-32.3	12.1 ± 12.7	bMDL-22.8	bMQL
	Ofloxacin	3135-24,811	12,222 ± 6786	1986-12,865	7302 ± 3741	n.d.-662	104 ± 219	n.d.-bMQL	bMDL	51.9-4986	946 ± 1790	110-366	233 ± 79
	Ciprofloxacin	2259-38,689	11,624 ± 11,340	457-13,344	3673 ± 3786	120-1334	503 ± 443	101-2000	572 ± 574	107-330	221 ± 88	127-1396	369 ± 455
	Sulfamethoxazole	307-8714	3015 ± 3012	191-5524	1897 ± 1656	41.0-1288	401 ± 447	n.d.-695	89.6 ± 230	529-1662	912 ± 391	340-1679	950 ± 460
	Trimethoprim	837-3963	1849 ± 1272	30.5-1182	528 ± 431	12.5-1089	337 ± 340	n.d.-122	13.5 ± 40.5	n.d.-360	124 ± 131	66.6-299	167 ± 78
	Metronidazole	n.d.-12,315	1638 ± 4037	bMDL-1569	192 ± 517	bMQL-4315	586 ± 1410	bMDL-5008	751 ± 1633	bMDL-113	51.1 ± 49.8	19.4-83.5	51.1 ± 21.1
	Metronidazole-OH*	n.d.-11,344	1604 ± 3690	n.d.-2125	261 ± 700	n.d.-523	121 ± 191	n.d.-990	229 ± 350	n.d.-145	62.9 ± 69.0	64.7-158	102 ± 33
	Ronidazole	n.d.-bMQL	bMDL	n.d.-bMQL	bMDL	n.d.-bMQL	bMDL	n.d.-bMQL	bMDL	n.d.-bMDL	bMDL	n.d.-bMDL	bMDL
Calcium channel blockers	Diltiazem	416-1470	814 ± 348	161-886	414 ± 263	15.5-174	58.4 ± 50.5	bMQL-346	50.9 ± 111	74.3-489	283 ± 167	154-231	189 ± 33
	Verapamil	5.68-67.2	14.2 ± 19.9	4.14-12.0	6.80 ± 2.81	4.00-5.83	4.78 ± 0.56	4.17-6.55	5.21 ± 0.68	2.83-4.88	4.12 ± 0.76	1.22-3.04	2.20 ± 0.60
	Norverapamil*	bMDL-5.13	bMQL	n.d.-8.93	bMQL	n.d.-bMQL	bMDL	n.d.-4.00	bMDL	n.d.-0.908	bMQL	n.d.-bMDL	bMDL

* Metabolites; bMDL – below method detection limit; bMQL – below method quantification limit; n.d. – not detected.

during the transport to the laboratory. Upon reception, samples were vacuum filtered through 1.0 μm glass microfiber filters (GF/C, Whatman, UK), followed by 0.45 μm nylon membrane filters (Whatman, UK) and stored at −20 °C, until extraction. As the suspended solids are removed during sample preparation, the measured concentrations of pharmaceuticals correspond to their dissolved fraction.

2.2. Investigated pharmaceutical compounds

In this study, a total of 78 pharmaceuticals belonging to 20 different therapeutic classes were studied. The list of selected therapeutic classes was as follows: analgesics and anti-inflammatories (14 compounds); lipid regulators and cholesterol lowering statin drugs (5 compounds); psychiatric drugs (13 compounds); histamine H_1 receptors antagonists (2 compounds); histamine H_2 receptors antagonists (3 compounds); β-blockers (6 compounds); diuretics (3 compounds); oral antidiabetics (2 compounds); antihypertensives (4 compounds); antiplatelet agent (1 compound); prostatic hyperplasia (1 compound); β-agonist (1 compound); anticoagulant (1 compound); X-ray contrast agent (1 compound); antihelmintics (3 compounds); synthetic glucocorticoid (1 compound); sedation and muscle relaxation (1 compound); tranquilizer (2 compounds); antibiotics (11 compounds); and calcium channel blockers (3 compounds). For more detailed information, see Table S1, Supporting information.

2.3. Chemicals and reagents

For more detailed information, see Supporting information.

2.4. Analytical method

Preparation and analysis of the samples was adapted from the protocols described in Gros et al. (2009, 2012). Briefly, after filtration, an appropriate volume of aqueous solution of 5% Na_2EDTA was added to 200 mL of effluent and 100 mL of influent wastewaters, and 50 mL of hospital effluent, in order to achieve a final Na_2EDTA concentration of 0.1%. Afterwards, samples were pre-concentrated onto Oasis HLB cartridges (60 mg, 3 mL), previously conditioned with 5 mL of methanol and 5 mL of HPLC grade water, using a vacuum manifold system (Phenomenex, USA) at a flow rate of approximately 5 mL min^{-1}. After that, cartridges were rinsed with 5 mL of HPLC grade water and dried under vacuum for 15–20 min, to remove excess of water. Finally, analytes were eluted with 6 mL of pure methanol at a flow rate of 1 mL min^{-1}. Extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted with 1 mL of methanol/water (10:90, *v/v*). Lastly, 10 μL of a 1 ng μL^{-1} standard mixture containing all isotopically labeled standards were added in the extract as internal standards.

Instrumental analysis was performed in a Waters Acquity Ultra-Performance™ liquid chromatography system, equipped with two binary pumps systems (Milford, MA, USA), and coupled to a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer with a turbo Ion Spray source (Applied Biosystems, Foster City, CA, USA). Chromatographic separation was achieved using an Acquity HSS T_3 column (50 × 2.1 mm i.d., 1.7 μm particle size) for the compounds analyzed under positive electrospray ionization (PI) and an Acquity BEH C_{18} column (50 × 2.1 mm i.d., 1.7 μm particle size) for the ones analyzed under negative electrospray ionization (NI), both purchased from Waters Corporation. For the analysis in PI mode, methanol was used as eluent A and 10 mM formic acid/ammonium formate (pH 3.2) as eluent B at a flow rate of 0.5 mL min^{-1}, whereas the analysis in NI mode was carried out using acetonitrile as eluent A and 5 mM ammonium acetate/ammonia (pH 8) as eluent B at a flow rate of 0.6 mL min^{-1}. For both modes, the injection volume was 5 μL.

Quantification of analytes was performed by SRM, monitoring two transitions between the precursor ion and the most abundant fragment ions for each compound, as described in detail elsewhere (Gros et al., 2012). Detailed information on the optimized mass spectrometer parameters (two SRMs, collision energies, and ion ratio) for each investigated compound in negative and positive ionization modes as well as on the internal standards used for quantification is given in Supporting information (Tables S1 and S4).

2.5. Mass loading estimations

Mass loadings of pharmaceuticals were calculated for each sampling period by multiplying individual concentrations of each pharmaceutical found by the mean daily flow rate of wastewater provided by the WWTP (Table S2, Supporting information). In the case of hospitals, their individual mass loadings were evaluated using the estimated daily water consumption data provided by the hospitals (Table S3, Supporting information). For the WWTP, loads were normalized by the population equivalent.

Individual contribution of each hospital effluent into the load of pharmaceuticals in the receiving WWTP was obtained by dividing the total mass load of the considered therapeutic group in the hospital effluent by the total mass load of the same therapeutic group in the WWTP influent multiplied by 100. The total mass loads of each therapeutic group used to calculate the contribution of hospitals refers to the mean value of the seven sampling campaigns performed.

Removal efficiency of pharmaceuticals was evaluated by means of Eq. (1):

$$\text{Removal efficiency (\%)} = \frac{(m_{inf} - m_{eff})}{m_{inf}} \times 100 \quad (1)$$

where m_{inf} is the load of pharmaceutical in WWTP influent and m_{eff} is the load of pharmaceutical in WWTP effluent.

2.6. Environmental risk assessment

Prioritization of pharmaceuticals based on environmental risk assessment was defined regarding their hazard quotient (HQ), using three different trophic levels representatives of the aquatic ecosystem (algae, daphnids and fish). HQs were calculated according to EU guidelines (European Comission, 2003) as the quotient between measured environmental concentration (MEC) and predicted no-effect concentration (PNEC), where the maximum individual concentrations of pharmaceuticals found in the different wastewaters were used as MEC. When the reported concentration was below the method quantification limit (bMQL), half of the MQL value was considered (von der Ohe et al., 2011). PNEC values were estimated using the lowest acute ecotoxicological data reported in the literature (EC_{50} or LC_{50}) for short term standard toxicity studies using three different species from several trophic levels (fish, *Daphnia* and algae) and applying an assessment factor (usually 1000) (European Comission, 2003), in order to take into account the extrapolation from inter- and intra-species variability in sensitivity (Sanderson et al., 2003). When no experimental values were available, EC_{50} values estimated with ECOSAR (Sanderson et al., 2003) were used (Table S7, Supporting information). If HQ is equal or above 1 there is a potential environmental risk situation, whereas when values are lower than 1, no risk is expected (Straub, 2002).

3. Results and discussion

3.1. Occurrence of pharmaceuticals in hospital effluents

Table 1 presents the occurrence data of the selected pharmaceuticals in the effluents of the four hospitals studied in this work, namely a university, a general, a pediatric and a maternity hospital. A similar number of pharmaceuticals was detected in all hospitals, specifically 67 compounds in the university and maternity hospitals, 63 in the pediatric one and 62 in the general one (Table 1). Only 7 out of the 78 pharmaceuticals studied (tenoxicam, meloxicam, loratadine, nadolol, torasemide, glibenclamide and tetracycline) had never been detected in any of the hospitals. However, differences in pharmaceutical concentrations between the effluents of the four hospitals were observed, since those reflect the variation in pharmaceuticals consumption of each healthcare facility, which is strictly connected with their number of beds as well as the number and type of wards and units, and to the consumption patterns defined by the National Guidelines for the Proper Use of Pharmaceuticals for the Different Diseases. Therefore, the highest concentrations were found in the effluents of the university and general hospitals rather than the other ones and different consumption patterns for the four hospitals could be established. Taking into account the relative concentration of pharmaceuticals for each hospital, it can be observed that in the pediatric and the maternity hospitals more than 50% of total pharmaceuticals belong to analgesic and anti-inflammatory drugs, which are by far much higher levels than those found in the university and the general hospitals (less than 15%). Diuretics also have an important pattern in pediatric and maternity hospitals with a relative percentage of the total concentration of approximately 12%. On the other hand, iopromide accounts for 65 and 75% of the total concentration of pharmaceuticals detected in university and general hospitals, respectively, in opposition to the low contribution in the other hospitals. University hospital also has a more pronounced consumption of antibiotics (about 7%) than the other hospitals.

Analgesics/anti-inflammatories, antibiotics and X-ray contrast agent are amongst the therapeutic groups most widely detected in hospital effluents, as was previously reported by Verlicchi et al. (2010b).

From all studied pharmaceuticals, the highest concentration was detected for the X-ray contrast agent iopromide in the general hospital (611,429 ng L^{-1}), followed by the university one (550,857 ng L^{-1}). Nevertheless, concentrations of one order of magnitude higher were reported in effluents from a hospital in Switzerland (Weissbrodt et al., 2009).

Acetaminophen and ibuprofen are among the analgesics/ anti-inflammatories pharmaceuticals with highest concentrations detected in all hospital effluents (up to 58,857 ng L^{-1} and 38,148 ng L^{-1}, respectively). Comparatively to previous findings, Thomas et al. (2007) reported higher levels of acetaminophen (329,852 ng L^{-1}) in the effluents of hospitals from Oslo (Norway), while ibuprofen did not exceed 8957 ng L^{-1}.On the other hand, lower concentrations of these pharmaceuticals were detected in the effluents of two Italian hospitals, where acetaminophen levels went from 1400 to 5900 ng L^{-1} and ibuprofen from 380 to 3200 ng L^{-1} (Verlicchi et al., 2012a). Opposite to this trend, Sim et al. (2011) never detected acetaminophen or ibuprofen in effluents from four general hospitals in Korea. These findings may be correlated with differences in pharmaceuticals consumption among countries. Another anti-inflammatory commonly detected in hospital effluents is salicylic acid. In the present study it was found at levels ranging from b2.7 to 4681 ng L^{-1} (Table 2), while in Greece its concentration reached 14,600 ng L^{-1} (Kosma et al., 2010) and in Italy did not exceed 2400 ng L^{-1} (Verlicchi et al., 2012a).

Among the analgesics opiates, codeine is one of the most often used in hospitals; therefore it was detected in the effluents of the four hospitals at concentrations up to 2837 ng L^{-1}. These concentrations are in agreement with codeine levels previously reported in Italy (Verlicchi et al., 2012a), while in Taiwan its maximum concentration was 378 ng L^{-1} (Lin et al., 2010).

The highest concentrations of antibiotics were found in the university and general hospitals, being the most prevalent compounds the fluoroquinolone antibiotics ofloxacin and ciprofloxacin (24,811 and 38,689 ng L^{-1}, respectively), followed by sulfamethoxazole (8714 ng L^{-1}) and azithromycin (7351 ng L^{-1}), in contrast to the maternity hospital where erythromycin was the most abundant antibiotic (7545 ng L^{-1}). Several studies have reported the presence of antibiotics in hospital effluents, being the fluoroquinolones among the most detected. For instance, the measured concentrations of ciprofloxacin are in agreement with previous findings reported in literature to hospital effluents in Norway (up to 23,336 ng L^{-1}) (Thomas et al., 2007), Switzerland (31,980 ng L^{-1}) (Kovalova et al., 2012) and

Table 2
Loads detected in both WWTP influent and effluent (mg/d/1000 inhabitants) for the different therapeutic groups and removal efficiency, including all the seven sampling campaigns.

Therapeutic group	Load in WWTP influent (mg/d/1000 inhabitants)		Load in WWTP effluent (mg/d/1000 inhabitants)		Removal efficiency (%)	
	Range	Mean	Range	Mean	Range	Mean
NSAIDs	80-988	407	46-136	87	42-93	79
Analgesics	74-1149	359	25-121	57	13-95	84
Lipid regulators and cholesterol lowering statin agents	64-142	106	46-106	78	NE-61	26
Psychiatric drugs	141-279	186	140-213	161	NE-24	13
Histamine H_2 receptor antagonists	6.9-44	33	7.1-38	20	NE-83	40
β-blockers	63-159	98	74-132	106	NE-17	NE
Diuretics	241-668	455	125-279	181	12-81	60
Oral antidiabetic (metformin)	0.04-19	10	0.05-4.0	2.2	NE-99	77
Antihypertensives	409-1340	892	66-694	352	NE-94	61
Calcium channel blockers	10-91	49	19-42	27	NE-65	45
Antibiotics	174-1612	512	229-362	294	NE-85	43
- Fluoroquinolone antibiotics	19-1337	281	45-217	83	NE-95	70
- Macrolide antibiotics	24-53	43	21-52	35	NE-60	19
- Sulfametoxazole	75-199	135	57-201	120	NE-41	11
- Trimethoprim	n.d.-43	20	15-36	22	NE-20	NE
- Others antibiotics	2.0-67	24	13-32	22	NE-58	7
Antiplatelet agent (clopidogrel)	0.3-6.1	3.1	0.5-2.9	1.6	NE-69	48
Prostatic hyperplasia (tamsulosin)	0.09-0.3	0.2	0.04-0.2	0.09	NE-71	46
β-agonist (salbutamol)	0.1-1.7	1.1	1.1-3.2	1.9	NE-15	NE
Anticoagulant (warfarin)	0.2-1.0	0.5	0.3-0.4	0.3	NE-59	28
X-ray contrast agent (iopromide)	6966-22,965	12,202	4394-11,902	7241	NE-61	41
Antihelmintics	1.1-3.1	2.1	2.4-3.9	3.1	NE-7	NE
Total load	9948-25,295	15,318	5339-13,719	8613		

n.d. – Not detected; NE – Not eliminated (compounds for which the concentrations found in WWTP effluent were higher than the concentrations found in WWTP influent).

Italy (1400-26,000 ng L^{-1}) (Verlicchi et al., 2012a), while Duong et al. (2008) found lower concentrations of ciprofloxacin (1100-10,900 ng L^{-1}) in Taiwan. On the other hand, higher levels were detected in a university hospital in Germany (up to 51,000 ng L^{-1}) (Ohlsen et al., 2003) and in Sweden (3600-101,000 ng L^{-1}) (Lindberg et al., 2004). However, for ofloxacin higher levels were detected in Italy (3300-37,000 ng L^{-1}) (Verlicchi et al., 2012a), USA (up to 35,500 ng L^{-1}) (Brown et al., 2006) and Germany (up to 31,000 ng L^{-1}) (Ohlsen et al., 2003), but not in Sweden (200-7600 ng L^{-1}) (Lindberg et al., 2004). Relatively to sulfamethoxazole, the concentrations found reaching up to 8714 ng L^{-1}, which were, in general, higher than data reported in literature (Brown et al., 2006; Kovalova et al., 2012; Ohlsen et al., 2003; Thomas et al., 2007; Verlicchi et al., 2012a). Nevertheless, levels up to 12,800 ng L^{-1} were detected in hospital effluents in Sweden (Lindberg et al., 2004). Sim et al. (2011) studied the presence of different antibiotics in the effluents of four general hospitals, in Korea, showing that only trimethoprim had higher concentrations (95,100 ng L^{-1}) than those reported in the present study (3963 ng L^{-1}), while ciprofloxacin, sulfamethoxazole and erythromycin showed lower concentrations (up to 3080, 3840 and 470 ng L^{-1}, respectively). Moreover, Ohlsen et al. (2003) also determined the presence of several antibiotics in the effluent of a university hospital in Würzburg (Germany), reporting concentrations of erythromycin (up to 6000 ng L^{-1}), which are in agreement with the present findings (up to 7545 ng L^{-1} in the maternity hospital).

Furosemide was the most prevalent diuretic at the four hospitals, being detected at concentrations from 434 ng L^{-1} in maternity hospital to 32,558 ng L^{-1} in pediatric hospital. In general, the studied hospitals presented higher concentrations of furosemide than those reported in Switzerland (2037 ng L^{-1}) (Kovalova et al., 2012) and in Italy (5300-18,000 ng L^{-1}) (Verlicchi et al., 2012a). In the case of antihypertensives, valsartan was the most predominant pharmaceutical, with levels up to 19,822 ng L^{-1} in university hospital. Similar findings were found in USA (14,572 ng L^{-1}) (Nagarnaik et al., 2010), while in Switzerland lower concentrations were detected (3032 ng L^{-1}) (Kovalova et al., 2012).

Glibenclamide has been described as the oral antidiabetic most often used in hospitals (Verlicchi et al., 2010b) and its presence has been reported in their effluents at concentrations from 50 to 110 ng L^{-1} (Verlicchi et al., 2012a). Nevertheless, in this study only metformin was detected in hospital effluents, rising levels up to 4040 ng L^{-1} in maternity hospital, which might be justified with the higher consumption rate of metformin among Portuguese population comparatively to glibenclamide (INFARMED, 2012).

Among β-blockers, atenolol had the highest detected concentrations, reaching levels up to 8037 ng L^{-1} in pediatric hospital. These values were higher than concentrations previously reported, which showed the presence of atenolol in hospital effluents of Italy (Verlicchi et al., 2012a), USA (Nagarnaik et al., 2010) and Switzerland (Kovalova et al., 2012) at levels up to 6600, 3166 and 2315 ng L^{-1}, respectively.

Ranitidine was the most abundant histamine H_2 receptor antagonist, being found in the general hospital at concentrations up to 19,840 ng L^{-1}, which are one order of magnitude higher than those reported in Switzerland (Kovalova et al., 2012), Italy (Verlicchi et al., 2012a) and Spain (Gómez et al., 2006).

Relatively to psychiatric drugs, they are one of the therapeutic groups with highest frequency of detection, though with low concentrations (Table 1). Among them, carbamazepine, venlafaxine, lorazepam and citalopram were the most representative compounds, being detected at concentrations up to 2042 ng L^{-1} (pediatric hospital), 1914 ng L^{-1} (maternity hospital), 1325 ng L^{-1} (university hospital) and 888 ng L^{-1} (pediatric hospital), respectively. The levels of carbamazepine reported in this study are in agreement with previous findings reported in Greece (up to 1900 ng L^{-1}) (Kosma et al., 2010). However, higher concentrations (up to 14,400 ng L^{-1}) were detected in the effluents of four general hospitals in Korea (Sim et al., 2011), while in Italy the levels of carbamazepine ranged from 640 to 1200 ng L^{-1} (Verlicchi et al., 2012a) and in USA did not exceed 37 ng L^{-1} (Nagarnaik et al., 2011). In what concern to lorazepam, lower concentrations (from 170 to 790 ng L^{-1}) were reported in Italy (Verlicchi et al., 2012a).

3.2. Occurrence of pharmaceuticals in urban wastewaters: loads, impact of hospital effluents and removal efficiency of WWTP

In order to evaluate the individual contribution of each hospital to the load of pharmaceuticals into the public sewer and the capability of the WWTP to remove them, wastewaters from the receiving WWTP were analyzed.

The occurrence of pharmaceuticals in WWTP influent and effluent followed a similar pattern to that one observed in hospitals, embracing 65 and 61 compounds, respectively. However, pharmaceuticals belonging to histamine H_1 receptor antagonists were never detected in WWTP wastewaters. Summarily, a total of 10 out of 78 pharmaceuticals were never detected in these matrices, namely tenoxicam, meloxicam, fluvastatin, acridone, loratadine, desloratadine, torasemide, glibenclamide, xylazine and azaperol (Table 1). The total daily loads of pharmaceuticals per 1000 inhabitants for the most representative therapeutic groups, for WWTP influent and effluent, is depicted in Fig. 1. Boxplots correspond to the addition of individual concentrations of each pharmaceutical belonging to a certain therapeutic group found in WWTP influent or effluent and includes the seven sampling campaigns performed. Total mass loads detected were between 10 and 25 g/d/1000 inhabitants for WWTP influent and from 5 to 14 g/d/1000 inhabitants for WWTP effluent (Table 2). These values are higher than those reported for WWTPs in Italy (Castiglioni et al., 2006), Spain (Gros et al., 2007) and Sweden (Zorita et al., 2009), where total mass loads in WWTP influent ranged from 1.5 to 6.7 g/d/1000 inhabitants, while in effluents the levels went from 0.32 to 3 g/d/1000 inhabitants. Nevertheless, a much larger number of pharmaceuticals was included in this study (78 against 30, 28 and 13 in Italy, Spain and Sweden, respectively), which embraced most of the compounds previously reported in literature, together with the fact that the WWTPs studied in Italy, Spain and Sweden may not be influenced by discharges from hospitals. They only treat domestic and/or industrial wastewaters. The highest total mass loads observed in the present study were mainly due to the X-ray contrast agent iopromide, since from all the studied pharmaceuticals this compound had a greater impact in the total mass load both in WWTP influent and effluent (around 80-85% of the total mass load) (Fig. 1 and Table 2). Nevertheless, the differences pointed out in mass loads of WWTP effluents are also related with different consumption patterns of pharmaceuticals among countries, as well as differences in wastewater treatment processes employed in WWTPs or culture habits. Comparatively to the highest average daily mass loads of pharmaceuticals in WWTP effluents ranked by Verlicchi et al. (2012b), in general, the mass loads found in this study were lower than those reported in literature, with the exception of sulfamethoxazole, lorazepam and pravastatin. However the highest average mass loads found in this study belong to iopromide and valsartan (7241 and 276 mg/d/1000 inhabitants, respectively), two pharmaceuticals not included in the cited work (Verlicchi et al., 2012b).

Other therapeutic classes having high mass loads were antihypertensives, antibiotics, namely fluoroquinolones and sulfamethoxazole, diuretics, non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics (Table 2). Within each group, pharmaceuticals showing higher loads in WWTP influent corresponded to ibuprofen and naproxen for NSAIDs (loads up to 661 and 250 mg/d/1000 inhabitants, respectively); acetaminophen for analgesics (12 to 1058 mg/d/1000 inhabitants); valsartan and irbesartan for antihypertensives (up to 1146 and 157 mg/d/1000 inhabitants, respectively); ofloxacin and sulfamethoxazole for antibiotics (up to 1292 and 199 mg/d/1000 inhabitants, respectively); and furosemide for diuretics (from 194 to 614 mg/d/1000 inhabitants), whereas in effluents the most representative pharmaceuticals were also ibuprofen and ketoprofen for NSAIDs, with loads up to 88 mg/d/1000 inhabitants; codeine and acetaminophen for analgesics (highest loads of 41 and 22 mg/d/1000 inhabitants, respectively); valsartan and irbesartan for antihypertensives (up to 578 and 90 mg/d/1000 inhabitants); sulfamethoxazole and ciprofloxacin for antibiotics (from 57 to 201 and from 20 to 87 mg/d/1000 inhabitants,

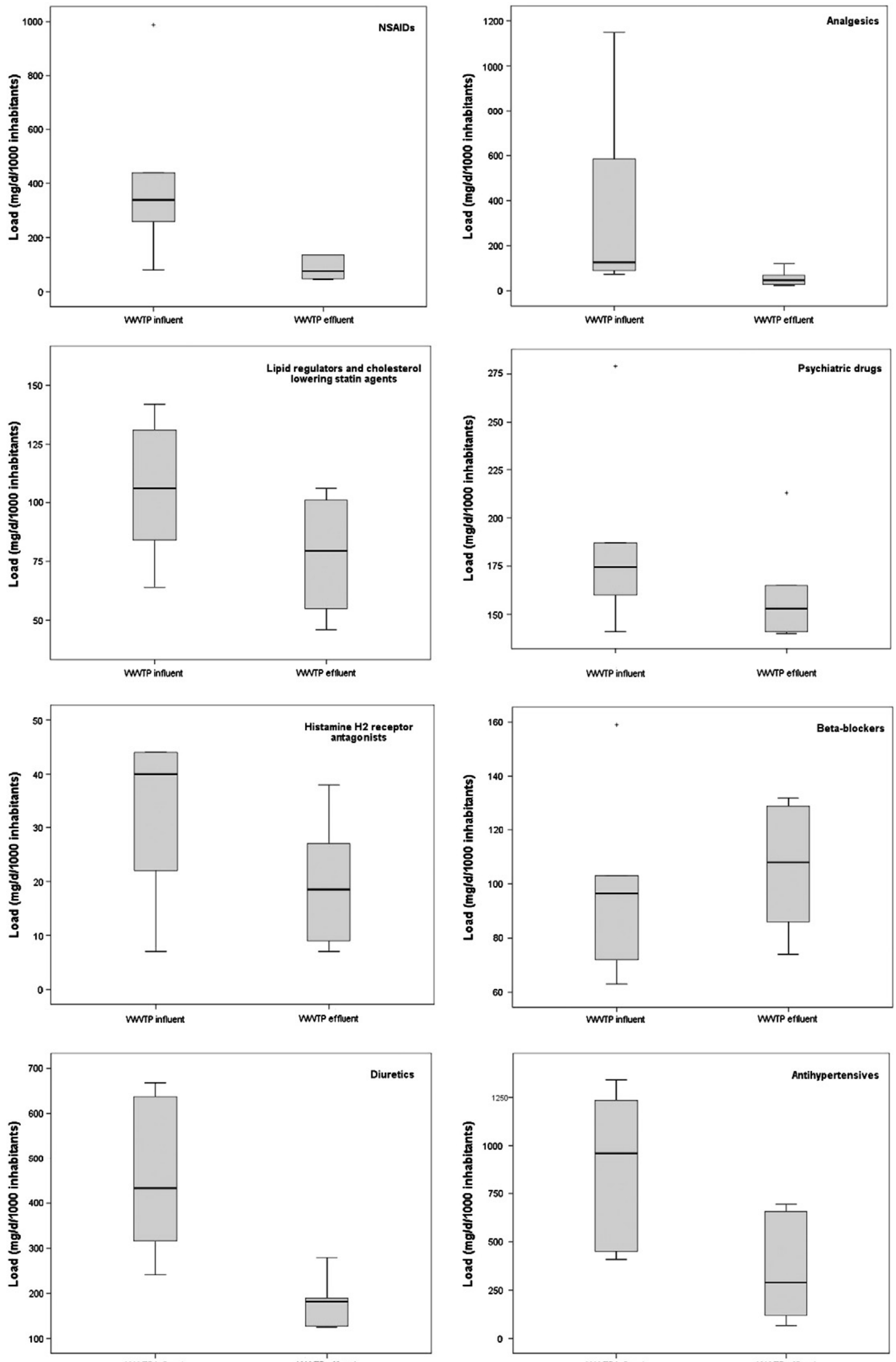

Fig. 1. Boxplots indicating total mass load values, expressed in mg/day/1000 inhabitants, of some of the most representative therapeutic groups in WWTP influent and effluent.

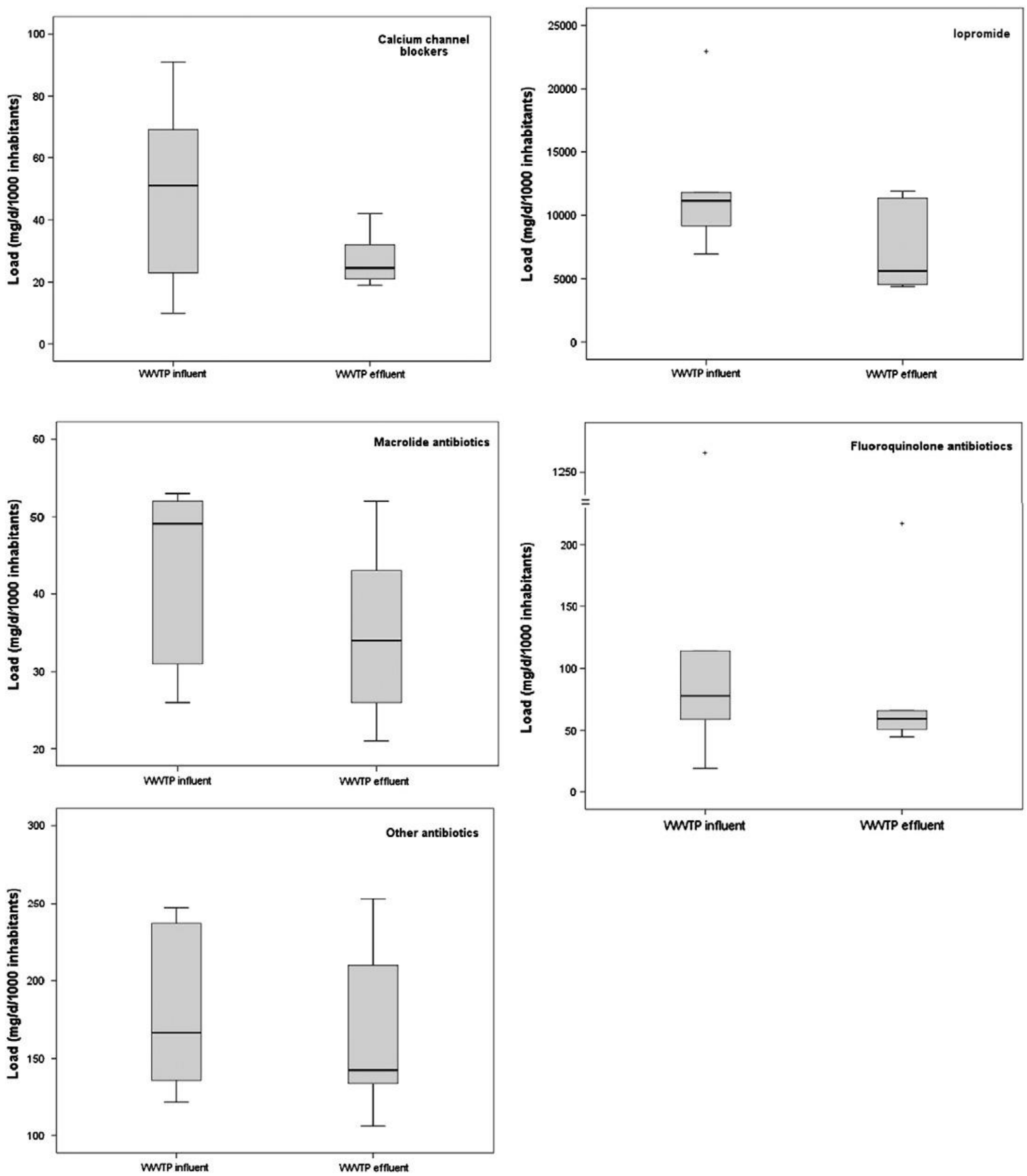

Fig. 1 (*continued*).

respectively); and furosemide for diuretics (from 69 to 252 mg/d/1000 inhabitants). More detailed information can be found in Supporting information.

Since the effluents of the four hospitals are discharged to the same WWTP, their individual impact in the receiving urban wastewaters was evaluated. The estimated total mass loading of the most representative therapeutic groups across hospitals based on the seven sampling campaigns is presented in Fig. 2. In general, the estimated total mass loadings were approximately 306 g d^{-1} for university hospital, 155 g d^{-1} for general one, 14 g d^{-1} for pediatric and 1.5 g d^{-1} for maternity hospital. Higher daily loads of pharmaceuticals from university and general hospitals into urban wastewaters might be explained by their dimension comparatively to the other two hospitals (1456 and 350 beds, respectively), since they have a high consumption rate of pharmaceuticals and higher water consumption (Table S2, Supporting information), which is reflected in an increased volume of effluents entering the public sewer system as well as their greatest contribution into the input of pharmaceuticals to the WWTP influent (Table 3). However, as the WWTP has higher flow rates than hospitals (Tables S1 and S2, Supporting information); the daily mass loads of pharmaceuticals from urban wastewater would be greater than those from hospital effluents even that its concentrations were, in general, lower.

The total contribution of hospital effluents into the load of pharmaceuticals to urban wastewaters was calculated for the different therapeutic groups taking into account the seven sampling campaigns performed. Table 3 summarizes the contribution to WWTP influent originated from each hospital relatively to the most representative therapeutic groups and more detailed information is given in Supporting Information (Tables S9–S12).

On the whole, the four hospitals contribution varied from approximately 3.3% for lipid regulators and cholesterol lowering statin agents load entering the WWTP to 74% for histamine H_2 receptors antagonists (Table 3). In general, the highest input of pharmaceuticals into WWTP influent was observed in the university hospital (bed density = 3.4, Table S3 Supporting information), while the contribution of the maternity hospital (bed density = 0.2, Table S3 Supporting information) represented less than 1% for all the therapeutic groups.

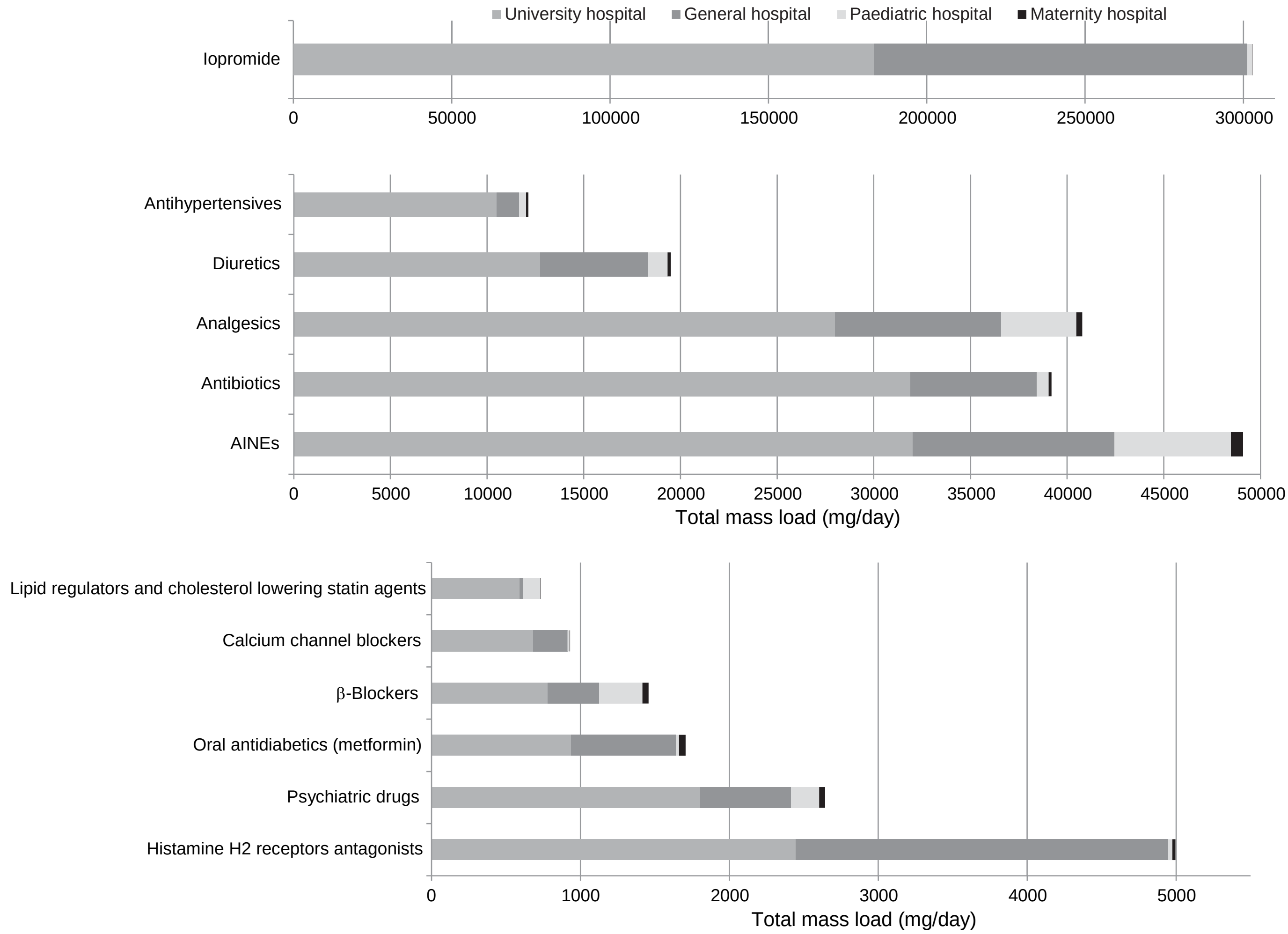

Fig. 2. Estimated total mass loadings of the most representative therapeutic groups to a WWTP influent from different hospital effluents. Please note that the scale for the *x*-axis (total mass load in mg d^{-1}) change between boxes.

These findings might be justified by the capacity of these two hospitals, since the former has 1456 beds and the latter only 96, which would be reflected in their consumption rate of pharmaceuticals as well as in their production of wastewaters. However, there was an exception for pediatric hospital that had a more marked contribution for antihelmintics (20% of the load entering the WWTP). Analgesics, antibiotics and NSAIDs were among the therapeutic groups with highest contributions to the total load of pharmaceuticals originating from hospital effluents, corresponding to 51, 41 and 32%, respectively. In fact, more pronounced contributions were described in literature for antibiotics (Beier et al., 2011; Ort et al., 2010; Thomas et al., 2007; Verlicchi et al., 2012a), reaching, in some cases, contributions as high as 272% (ciprofloxacin) (Thomas et al., 2007), 94% (clarithromycin) (Beier et al., 2011) or 67% (azithromycin) (Verlicchi et al., 2012a). On the other hand, for some of the most consumed analgesics/NSAIDs (for instance, ibuprofen, diclofenac or acetaminophen) hospital contribution reported in literature did not exceed 15% (Beier et al., 2011; Langford and Thomas, 2009; Thomas et al., 2007; Verlicchi et al., 2012a), which is in agreement with our results in what concern to ibuprofen and diclofenac (contribution up to 4.2 and 9.5%, respectively) (data not shown), however for acetaminophen, the contribution of university and general hospitals went to 483 and 115%, respectively (data not shown).

Nevertheless, the X-ray contrast agent iopromide, which had a mean total mass load of approximately 303 g d^{-1} coming from hospital effluents, only contributed with approximately 13% of its total mass load found in WWTP influent, though Ort et al. (2010) reported a minor contribution (less than 5%). These might be explained by the fact that iopromide is administered to patients to help in diagnostic exams and it would be mainly excreted in their houses entering directly in WWTP by urban wastewaters. Nevertheless, it has to be taken into account that the concentration of X-ray contrast agents widely varies over the day and from one day to another, influencing the amount of iopromide found in hospital effluents and urban wastewaters.

It is clear that for the most consumed therapeutic classes (analgesics, antibiotics and NSAIDs), hospital effluents are an important source of input of pharmaceuticals into WWTP, reaching in some cases more than 50% of total mass load. However, in general, hospitals contribution to the load of pharmaceuticals into urban wastewaters has not a great impact, being most of the total load owing to public wastewaters.

Removal efficiency of pharmaceuticals were evaluated by comparing the load of each pharmaceutical in WWTP influent and effluent. Table 2 shows the total mass loads found for the different therapeutic groups (range and mean value), expressed as the sum of all pharmaceuticals belonging to each therapeutic group, as well as their removal rates in the studied WWTP, taking into account the seven sampling campaigns carried out during this study. Results obtained proved that WWTP was not able to completely remove pharmaceuticals. Indeed, a great variation in removal efficiencies, between the different therapeutic groups as well as within each group, was observed, going from not eliminated to 99%. However, in terms of mean values, removal efficiency did not exceed 84%. Analgesics, NSAIDs, the oral antidiabetic metformin and fluoroquinolone antibiotics were among the most efficiently removed, showing removal efficiencies higher

Table 3

Contribution, expressed in percentage, to WWTP influent originating from hospital effluents for the most representative therapeutic groups.

Therapeutic group	Input to WWTP influent (%)				
	University hospital	General hospital	Pediatric hospital	Maternity hospital	Total
NSAIDs	21	6.9	4.0	0.4	32
Analgesics	35	11	4.8	0.4	51
Lipid regulators and cholesterol lowering statin agents	2.7	0.1	0.5	0.01	3.3
Psychiatric drugs	4.7	1.6	0.5	0.1	6.9
Histamine H_2 receptor antagonists	36	37	0.4	0.3	74
Diuretics	13	5.8	1.1	0.2	20
Oral antidiabetics (metformin)	4.3	3.3	0.1	0.2	7.9
Antihypertensives	5.7	0.6	0.2	0.05	6.6
Calcium channel blockers	7.3	2.5	0.2	0.03	10
Antibiotics	33	6.8	0.6	0.2	41
- Fluoroquinolone antibiotics	40	8.3	0.3	0.03	49
- Macrolide antibiotics	41	11	1.0	1.0	54
- Other antibiotics	21	3.8	1.1	0.1	26
Antiplatelet agent (clopidogrel)	26	6.3	0.9	0.2	33
Prostatic hyperplasia (tamsulosin)	7.2	2.7	1.1	0.2	11
Anticoagulant (warfarin)	6.0	2.3	0.2	0.05	8.6
X-ray contrast agent (iopromide)	7.9	5.1	0.07	0.001	13
Antihelmintics	1.7	10	20	0.4	32

than 70%, in contrast to β-blockers, antihelmintics, the antibiotic trimethoprim and the β-agonist salbutamol that were not eliminated at all. Relatively to antibiotics, differences in their removal efficiency were observed depending on their group. For instance, fluoroquinolones had the highest removal efficiency (70%), while all the other groups of antibiotics did not exceed 19%, or were not removed at all as was the case of trimethoprim. Our findings are in agreement with previous studies found in the scientific literature, where incomplete removal of a wide range of pharmaceuticals in conventional WWTPs has been described (Castiglioni et al., 2006; Jelic et al., 2011). Moreover, this was expected since removal of pharmaceuticals in conventional WWTP is generally due to the biological treatment, where biodegradation/biotransformation and sorption are the two main mechanisms occurring in the biological reactors. Therefore, the physico-chemical properties of pharmaceuticals, the origin and composition of wastewaters (urban, industrial, hospital, etc.), and the operational conditions of WWTP, such as biomass, concentration, sludge retention time (SRT), hydraulic retention time (HRT), pH, temperature, configuration (aerobic, anaerobic and/or anoxic reactors) and type of plant are determinant factors for the removal of pharmaceuticals in conventional WWTPs (Verlicchi et al., 2012b).

3.3. Environmental risk assessment

Nowadays the majority of prioritization lists of pharmaceuticals are based on the concept of risk assessment, which takes into account the potential effect of a given pharmaceutical and its exposure level (Guillén et al., 2012). For that hazard quotients (HQ), which establish the ratio between Predicted Environmental Concentration (PEC) and Predicted No-Effect Concentration (PNEC), could be a useful tool, as was proved by some authors (Ginebreda et al., 2010; Gros et al., 2010; Verlicchi et al., 2012a). However, the replacement of PEC for Measured Environmental Concentration (MEC) allows evaluating risks posed by pharmaceuticals in a more realistic scenario.

In this work, HQs were evaluated according to EU guidelines in both hospital and WWTP effluents. An approach of "worst case scenario" was followed, that is HQs were calculated using the highest level detected for each pharmaceutical as MEC. HQs were evaluated using three different trophic levels representative of aquatic ecosystem, namely algae, daphnids and fish, in order to despite differences between the complex mixture of species present in natural ecosystems (von der Ohe et al., 2011).

Figs. 3 and 4 summarizes the HQs obtained for algae, daphnids and fish in hospitals and WWTP wastewaters, respectively. According to the results, algae appeared to be the most sensitive species followed by daphnids and fish, which is in agreement with data reported in literature for surface waters (Ginebreda et al., 2010). As expected, higher HQs were obtained in hospital effluents than in WWTP wastewaters. Pharmaceuticals like ciprofloxacin, ofloxacin and ibuprofen showed HQs higher than one for all trophic levels, posing a risk to algae, daphnids and fish, therefore it is expected that they might be a threat for all aquatic ecosystem. Besides those, iopromide, diclofenac, dexamethasone and gemfibrozil also pose a risk to fish, while acetaminophen, metronidazole, ketoprofen, thiabendazole, salbutamol and propranolol pose an ecotoxicological risk to daphnids. On the other hand, besides the above mentioned fluoroquinolone antibiotics, algae showed high sensitivity to others antibiotics, such sulfamethoxazole, azithromycin and clarithromycin, as well as other pharmaceuticals like iopromide, naproxen, ketoprofen, fluoxetine, and propranolol. Regarding WWTP effluent, only the antibiotics ciprofloxacin, ofloxacin, sulfamethoxazole, azithromycin and clarithromycin revealed to pose an ecotoxicological risk for algae (Fig. 4). Nevertheless, most of the pharmaceuticals that revealed to pose a risk for algae in WWTP effluent had low removal efficiencies or, in some cases, where not removed at all, as in the case of ciprofloxacin (HQ = 279) (Fig. 4). These results indicate that more attention should be paid to the receiving waters of WWTP effluents, since pharmaceuticals are being discharged into the environment at concentrations that are able to pose a threat to aquatic ecosystems, at least to a lower trophic level. However, if a lower level of the food chain would be affected, this could have a negative impact in the entire aquatic ecosystem.

In accordance with these findings, it could be concluded that due to the incomplete removal of pharmaceuticals in WWTPs, especially some antibiotics, their effluents would represent a threat to aquatic ecosystems and probably the dilution of wastewaters in receiving surface waters may be not enough to mitigate their ecotoxicological risk. Indeed, the mitigation of the risk posed by the occurrence of pharmaceuticals in the treated effluent is due to not only dilution of the receiving water body but also to auto-depurative processes occurring within the water phase in the bulk of the receiving water body, as well as photocatalytic processes once pharmaceuticals reach the environment and remain in the free water systems (rivers, lakes, sea, etc.).

It was also observed that the detection of high concentrations of a pharmaceutical in the environment did not necessarily imply an environmental risk. For example, a high concentration of acetaminophen was found in WWTP effluent (Table 2), but did not pose a risk for daphnids (HQ b 1) (Fig. 4). Therefore, besides the consumption rate of pharmaceuticals, risk assessment studies should be taken into account, in order to prioritize the compounds to be monitored.

Based on the analytical and ecotoxicological data reported in this study, a list of 10 pharmaceuticals potentially dangerous for the aquatic organisms could be delineated for hospital and WWTP effluents, based on HQs, in order to being considered for further inclusion in monitoring programs or even in future regulations. The proposed list for WWTP effluents should include the antibiotics ciprofloxacin, ofloxacin, sulfamethoxazole, azithromycin and clarithromycin, since they showed to pose an ecotoxicological risk to algae (HQ > 1); the X-ray contrast agent iopromide due to its high concentration in WWTP effluents (34–85 μg L^{-1}) together with HQs close to the unit to algae and fish; the NSAIDs ibuprofen and diclofenac given that they may be potential harmful to fish, especially diclofenac (HQ = 0.9); and finally the SSRI fluoxetine and its human metabolite norfluoxetine,

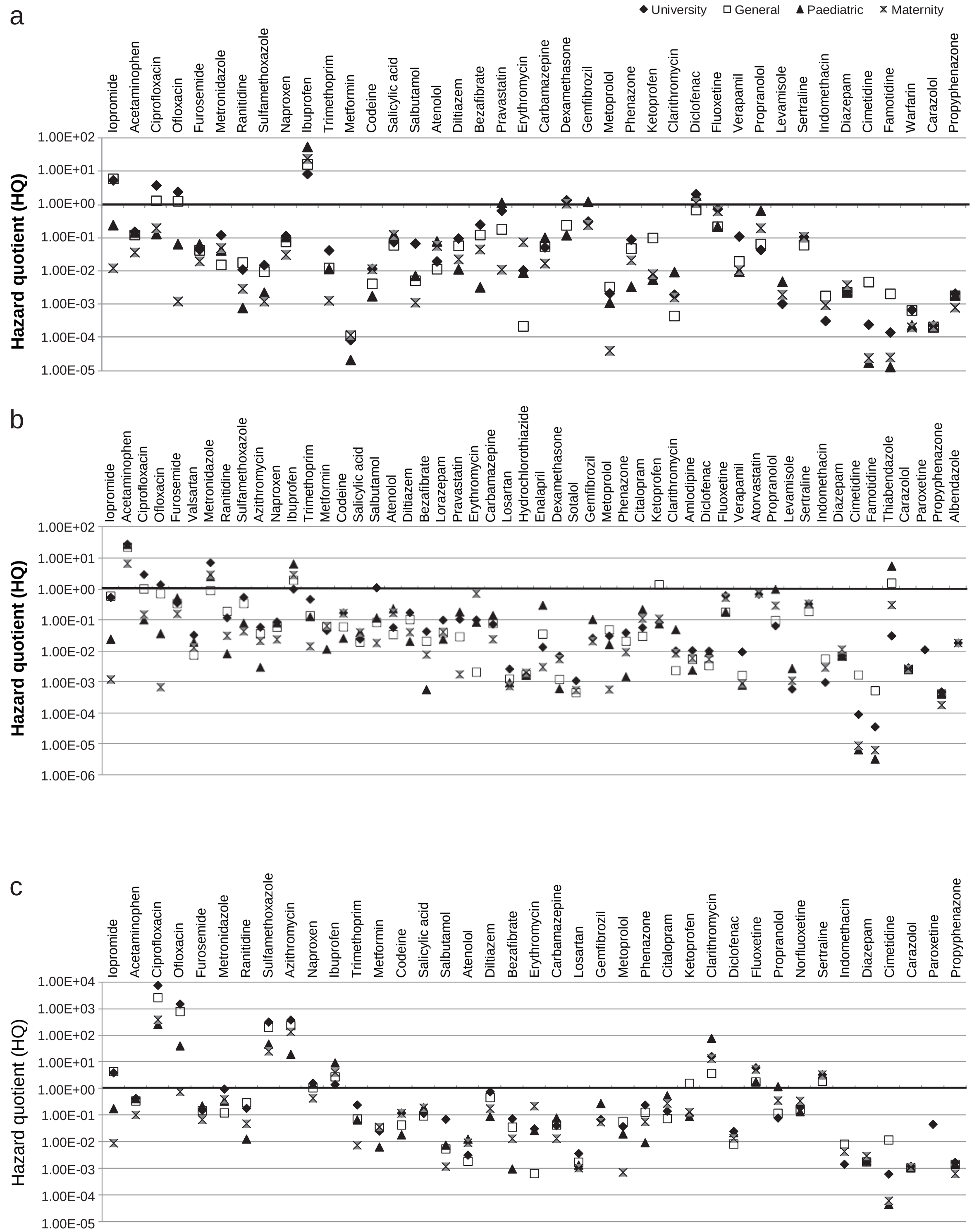

Fig. 3. Hazard quotients of hospital effluents for three different trophic levels. a) Fish; b) Daphnid; c) Algae.

since norfluoxetine posed a higher risk to aquatic organisms than the parent compound, pointing out the importance to extend the monitoring studies to metabolites. On the other hand, the proposed list for hospital effluents should embrace the antibiotics ciprofloxacin, ofloxacin, sulfamethoxazole, azithromycin, clarithromycin and metronidazole; the NSAID ibuprofen; the analgesic acetaminophen; the SSRI fluoxetine; and the X-ray contrast agent iopromide, since their HQs exceeded the unit in a great extent for most of the pharmaceuticals. Moreover, those pharmaceuticals showed, in general, high concentrations in hospital effluents as well as high frequency of detection among the different hospitals.

The proposed lists of pharmaceuticals potentially dangerous for the environment include some compounds that have been already identified as high priority (sulfamethoxazole, diclofenac, ibuprofen and ciprofloxacin) or priority pharmaceuticals (acetaminophen, iopromide, ofloxacin and clarithromycin) (de Voogt et al., 2009). On the other hand, fluoxetine and norfluoxetine have been classified as pharmaceuticals of lower priority (de Voogt et al., 2009); however fluoxetine was the

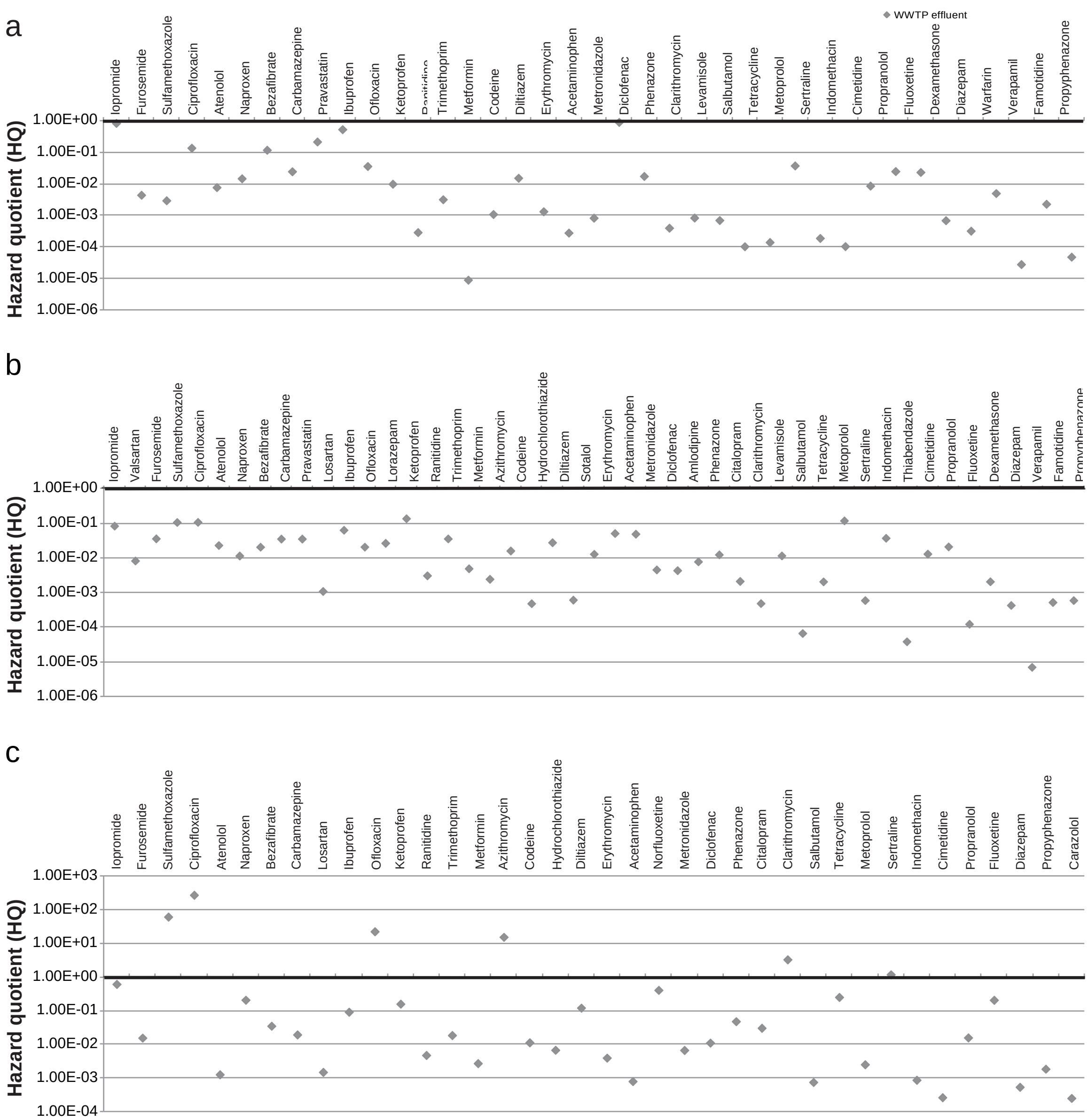

Fig. 4. Hazard quotients of WWTP effluent for: a) Fish; b) Daphnid; c) Algae.

only pharmaceutical included in a top 20 priority ranking based on ecological effects defined by Kumar and Xagoraraki (2010). Another study that ranking the potential impact of 98 frequently detected pollutants, including 38 pharmaceuticals and 10 metabolites, also showed that the impact of WWTP effluent in receiving surface waters is mainly due to fluoxetine and ciprofloxacin (Muñoz et al., 2008), two of the pharmaceuticals included in our proposed list for WWTP effluents.

Nevertheless, it should bear in mind that the type of water has also effect in the ranking of pharmaceuticals (Kumar and Xagoraraki, 2010), justifying the development of different prioritization lists of pharmaceuticals in agreement with the kind of water sample that is being considered.

The approach followed in this work is only focused on the ecotoxicity that individual pharmaceuticals may cause to aquatic organisms. However, in the aquatic environment they are present as a mixture of different therapeutic groups, their metabolites and transformation products, which may have synergic or additive effects, exhibiting higher toxicities than single compounds, even at lower concentrations, as was shown by some authors (Cleuvers, 2003, 2004; DeLorenzo and Fleming, 2008; Quinn et al., 2009).

4. Conclusions

Higher concentrations of pharmaceuticals were found in hospital effluents than in WWTP influent; however such high levels in hospital effluents did not imply the same high contribution in terms of mass loads due to the much lower flow of hospital effluents compared to total WWTP influent flow.

The contribution of hospital effluents entering the receiving WWTP influent varied in a wide range among the different therapeutic groups and from hospital to hospital, reaching in some cases more than 50% of total input. NSAIDs, analgesics and antibiotics are amongst the groups with highest loads coming from hospitals, whereas antihypertensives, psychiatric drugs or lipid regulators do not have a very significant contribution (b 10%), being most of the input of these kind of pharmaceuticals attributed to public wastewaters. Contribution of hospitals with a higher number of beds is also more pronounced comparatively to small hospitals.

Removal efficiencies of pharmaceuticals in WWTP varied from more than 90% for compounds like the analgesic acetaminophen and the NSAIDs salicylic acid and ibuprofen, to no removal at all for β-blockers, antihelmintics and salbutamol, proving that the wastewater treatment applied is not able to efficiently remove a large number of pharmaceuticals. As a consequence, WWTP effluents are discharging pharmaceuticals into receiving surface waters, being one of the most important contributors to their environmental load. In the present work, a total mass load between 5 and 14 g/d/1000 inhabitants in WWTP effluent was reported.

Environmental risk assessment posed by the pharmaceuticals found in hospital effluents and WWTP wastewaters was evaluated at three different trophic levels (algae, daphnids and fish). In hospital wastewaters, ciprofloxacin, ofloxacin and ibuprofen revealed to pose a risk to all trophic levels, which is related to their high measured concentrations. In terms of high risk for the environment, more attention should be paid to antibiotics (fluoroquinolones, macrolides and sulphonamides), given that they showed HQs higher than the unit in WWTP effluent for algae, which were the most sensitive species for the majority of pharmaceuticals. Prioritization of environmental risk assessment stated in this work was only established taking into account individual acute toxicity data. Nevertheless, synergic or additives effects should be considered, since this is a more realistic scenario.

Furthermore, two lists of pharmaceuticals potentially dangerous for the environment were proposed, taking into account both hospital and WWTP effluents. For the former, pharmaceuticals like ciprofloxacin, ofloxacin, sulfamethoxazole, azithromycin, clarithromycin, metronidazole, ibuprofen, acetaminophen, fluoxetine and iopromide, which have HQs higher than the unit should be considered, while for WWTP effluents, the list embraces pharmaceuticals such as ciprofloxacin, ofloxacin, sulfamethoxazole, azithromycin, clarithromycin, fluoxetine and its human metabolite norfluoxetine, iopromide, ibuprofen and diclofenac, which are potentially dangerous to aquatic organisms, and should be included in further monitoring programs. The proposed list of pharmaceuticals highlights the importance of extending, in the future, the monitoring studies to metabolites.

This data suggests that authorities and scientific community should improve the co-treatment of hospital and urban wastewaters, since the former have a high concentration of contaminants and conventional WWTPs are unable to efficiently remove pharmaceuticals and evaluate the use of alternative treatments for a better management of hospital wastewaters.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.04.077.

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