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Occurrence, temporal evolution and risk assessment of pharmaceutically active compounds in Doñana Park (Spain)

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ABSTRACT

Doñana National Park (Southern Spain) is one of the most emblematic protected areas in Europe and is included in UNESCO's World Heritage List. A 1-year monitoring study was carried out to investigate the presence of 16 pharmaceutical compounds belonging to seven therapeutic groups in wastewater discharges, rivers and streams affecting Doñana Park. Fourteen pharmaceuticals were detected in effluent wastewater at concentration levels up to 26.8 $\mu g\,L^{-1}$ and thirteen were detected in surface water at concentration levels up to 4.55 $\mu g\,L^{-1}$. Ibuprofen was the compound at the highest concentration levels. An increase of the concentration levels in surface water was observed in summer months due to the reduction of the flow rates of the rivers. Nevertheless, risk quotient values estimated in surface water were lower than one so no toxicological effect is suspected to occur. The highest average risk quotients were obtained for ibuprofen (risk quotient 0.67 ± 0.28), gemfibrozil (risk quotient 0.52 ± 0.33), propranolol (0.13 ± 0.06) and naproxen (0.10 ± 0.09). Nevertheless, in summer months, risk quotient values up to 9.3 and 10.7 were estimated for the estrogenic compounds 17α-ethinylestradiol and 17β-estradiol.

1. Introduction

Doñana National Park (Southern Spain) constitutes a wintering site for miles of European and African birds and a place where to live for threatened species as the Iberian imperial eagle, the Iberian lynx (*Lynx pardinus*) and some bird species. In 1994, Doñana National Park was included in UNESCO's World Heritage List [1]. Moreover, Doñana National Park has been recognised as an "Important Bird Area" by BirdLife International [2] and has been included in the "Ramsar List of Wetlands of International Importance" [3]. Unfortunately, the presence of pharmaceutically active compounds in wastewater discharges affecting Doñana Park [4] could endanger its natural ecosystems due to their toxic effects [5]. The first aim of this study was to evaluate the occurrence of pharmaceutical compounds in wastewater and surface water affecting Doñana Park. Sixteen pharmaceutical compounds from seven therapeutic groups were monitored during 1-year period in wastewater effluents from eleven wastewater treatment plants (WWTP), in direct discharges of untreated wastewater (DD), and in the main river and streams flowing into Doñana National Park. The second aim was to evaluate the temporal evolution of pharmaceuticals in the main river and streams affecting Doñana Park. The third aim was to carry out a risk assessment of the presence of pharmaceutical compounds to Doñana aquatic media. Pharmaceuticals monitored were five anti-inflammatory drugs (diclofenac, ibuprofen, ketoprofen, naproxen and salicylic acid), a nervous stimulant (caffeine), two antibiotics (sulfamethoxazole, trimethoprim), two lipid regulators (clofibric acid and gemfibrozil), an antiepileptic drug (carbamazepine), a β-blocker (propranolol) and four hormones (17α-ethinylestradiol, 17β-estradiol, estriol and estrone).

2. Experimental

2.1. Studied area and sampling points

Fig. 1 shows the location of the sampling points of wastewater discharges from WWTPs, the point of direct discharges of untreated wastewater (DD) and the four surface-water (S) sampling points. Wastewater samples from the eleven WWTPs and from DD were collected in May 2008, September 2008 and January 2009, one sample per month and sampling point. Daily-flow proportional composite samples were collected every hour by an automatic device.

Twelve surface-water samples were collected monthly from May 2008 to April 2009 from each surface-water sampling point. Sampling points S1 and S2 were located at Guadiamar River, in the Northeast of Doñana Natural Park. S1 (N 37°14′39.1″; W 6°15′56.6″) was located below the confluence of *Ardachón* and *Alcarayón* streams, and S2 (N 37°6′21.1″; W 6°15′29.0″) was located

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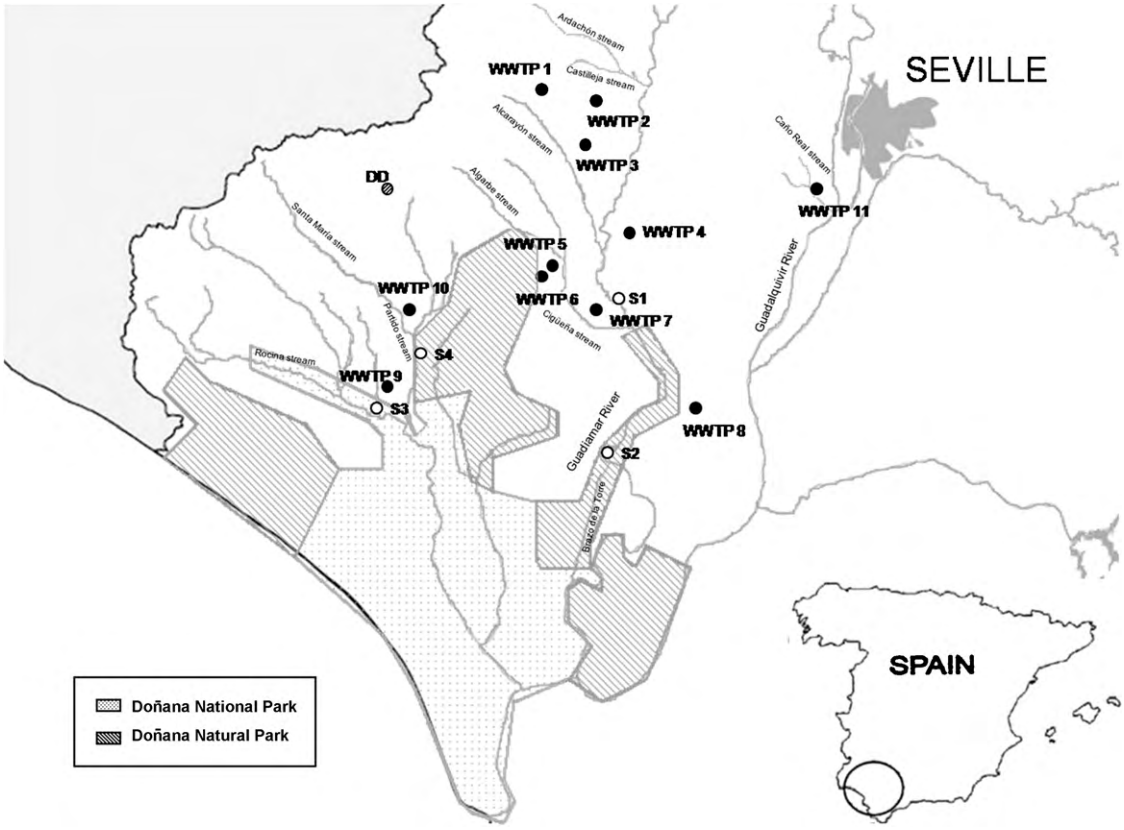

Fig. 1. Location of sampling points of wastewater discharges from wastewater treatment plants (WWTPs), direct discharges of untreated wastewater (DD) and surface water (S).

within the Natural Park, downstream from S1. Sampling point S3 (N 37°7′33.0″; W 6°29′38.4″) was located at *La Rocina* stream, in the west of Doñana National Park, downstream from discharges from WWTP 9. Sampling point S4 (N 37°10′27.7″; W 6°27′28.5″) was located at *El Partido* stream, in the North of Doñana Natural Park and downstream from WWTP 10 and untreated wastewater (DD) discharges. Surface-water samples were obtained by mixing five aliquots collected at the same depth across the river. Samples were collected at the same depth because of the low flow rates and small river basins of the streams evaluated. Samples were transferred to amber glass bottles and stored at 4 °C until analysis.

2.2. Materials and methods

HPLC-grade acetonitrile, methanol and water were supplied by Romil Ltd. (Barcelona, Spain). HPLC-grade acetone and hexane and analytical grade sulfuric acid were obtained from Panreac (Barcelona, Spain). Potassium dihydrogen phosphate of analytical grade was obtained from Scharlau (Barcelona, Spain). Carbamazepine, clofibric acid, diclofenac, ketoprofen, naproxen and salicylic acid (>97%) were purchased from Sigma–Aldrich (Steinheim, Germany). 17α-ethinylestradiol, 17β-estradiol, estriol, estrone, gemfibrozil, ibuprofen, propranolol hydrochloride, sulfamethoxazole and trimethoprim (>98.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Caffeine was obtained from Merck (Darmstadt, Germany). Stock solutions of 1000 μg mL^{-1} in each of the pharmaceutical compounds were prepared in methanol and stored at 4 °C. Working solutions and HPLC calibration standards were prepared by diluting stock standard solutions and working solutions, respectively, in methanol.

The analytical determination was carried out accordingly to a previous reported method [6] based on sample treatment by solid-phase extraction with cartridges packed with 60 mg of Oasis HLB (Waters, Milford, MA, USA) and determination by high-performance liquid chromatography with diode array and fluorescence detectors online. Chromatographic separation was carried out using a Zorbax Eclipse XDB-C18 (150 mm × 4.6 mm, 5 μm) cartridge column (Agilent, USA) protected by a XDB-C18 (4 mm × 4 mm, 5 μm) guard column (Agilent, USA) and thermostated at 30 °C, by gradient elution with acetonitrile and a 25 mM potassium dihydrogen phosphate solution at a flow rate of 1.2 mL min^{-1} [6].

2.3. Risk assessment

Potential risks of each pharmaceutical compound was assessed by means of the risk quotient values (RQ) calculated from the measured environmental concentrations (MEC) and the predicted no effect concentrations (PNEC). RQ values higher than one imply significant ecotoxicological risk to aquatic organisms. Average concentrations during the monitoring period discarding outlier values were used as MEC values. PNEC values were estimated from the toxicity data to several aquatic organisms: bacteria, algae, invertebrate and fish species reported in the literature (Table 1). PNEC values were estimated as 1000 times lower than the toxic concentration reported for the most sensitive specie assayed (marked in bold in Table 1) to consider the toxicity to other aquatic species more sensitive than those used in toxicity studies. This way of estimating PNEC values has been recommended by several authors [30,31] because it reduces the uncertainty associated with the extrapolation from the limited number of

Table 1
PNEC values calculated from ecotoxicological studies reported in the literature.

Pharmaceutical compound	Ecotoxicological study				PNEC value calculated ($\mu g\,L^{-1}$)
	Species	Test	Ecotoxicity ($mg\,L^{-1}$)	Reference	
Diclofenac	*Daphnia magna* (invertebrate)	EC_{50} (48 h)	22.4	[7]	
	Pseudokirchneriella subcapitata (algae)	EC_{50} (96 h)	16.3	[7]	
	Vibrio fischeri (bacterie)	EC_{50} (30 min)	11.4	[7]	
	***Vibrio fischeri* (bacterie)**	**EC_{50} (15 min)**	**9.70**	[8]	9.7
Ibuprofen	*Vibrio fischeri* (bacterie)	CE_{50} (15 min)	37.5	[8]	
	Vibrio fischeri (bacterie)	EC_{50} (15 min)	12.1	[9]	
	Daphnia magna (invertebrate)	EC_{50} (48 h)	9.06	[10]	
	***Hydra attenuata* (invertebrate)**	**EC_{50} (96 h)**	**1.65**	[11]	1.65
Ketoprofen	*Daphnids*	EC_{50} ECOSAR	248	[12]	
	Algaes	EC_{50} ECOSAR	164	[12]	
	Fish	EC_{50} ECOSAR	32.0	[12]	
	***Vibrio fischeri* (bacterie)**	**EC_{50} (15 min)**	**15.6**	[9]	15.6
Naproxen	*Desmodesmus subspicatus* (algae)	EC_{50}	626	[13]	
	Hydra attenuata (invertebrate)	LC_{50} (96 h)	22.4	[11]	
	Vibrio fischeri (bacterie)	EC_{50} (15 min)	21.2	[9]	
	***Hydra attenuata* (invertebrate)**	**EC_{50} (96 h)**	**2.62**	[11]	2.62
Salicylic acid	*Daphnia longispina* (invertebrate)	EC_{50} (48 h)	1147	[14]	
	Daphnia magna (invertebrate)	LC_{50} (48 h)	112	[15]	
	Scenedesmus subspicatus (algae)	EC_{50} (48 h)	>100	[16]	
	***Vibrio fischeri* (bacterie)**	**EC_{50} (15 min)**	**43.1**	[9]	43.1
Sulfamethoxazole	*Oryzias latipes* (fish)	EC_{50} (96 h)	563	[17]	
	Daphnia magna (invertebrate)	EC_{50} (48 h)	>100	[7]	
	Vibrio fischeri (bacterie)	EC_{50} (15 min)	78.1	[17]	
	***Pseudokirchneriella subcapitata* (algae)**	**EC_{50} (96 h)**	**0.15**	[7]	0.15
Trimethoprim	*Vibrio fischeri* (bacterie)	EC_{50} (15 min)	177	[17]	
	***Daphnia magna* (invertebrate)**	**EC_{50} (96 h)**	**121**	[17]	121
	Hydra attenuata (invertebrate)	LC_{50} (96 h)	>100	[11]	
	Oryzias latipes (fish)	EC_{50} (48 h)	>100	[17]	
Carbamazepine	*Desmodesmus subspicatus* (algae)	EC_{50} (3 days)	74.0	[18]	
	Vibrio fischeri (bacterie)	EC_{50} (15 min)	52.2	[17]	
	Oryzias latipes (fish)	EC_{50} (48 h)	35.4	[17]	
	***Daphnia magna* (invertebrate)**	**EC_{50} (48 h)**	**13.8**	[7]	13.8
Propranolol	*Vibrio fischeri* (bacterie)	EC_{50} (30 min)	61.0	[7]	
	Oryzias latipes (fish)	EC_{50} (48 h)	24.3	[19]	
	Ceriodaphnia dubia (invertebrate)	LC_{50} (48 h)	0.80	[19]	
	***Desmodesmus subspicatus* (algae)**	**EC_{50} (48 h)**	**0.70**	[20]	0.70
Caffeine	*Daphnia magna* (invertebrate)	EC_{50} (48 h)	182	[21]	
	Hydra attenuata (invertebrate)	LC_{50} (96 h)	>100	[11]	
	Desmodesmus subspicatus (algae)	EC_{50} (72 h)	>100	[21]	
	***Leuciscus idus* (fish)**	**EC_{50} (96 h)**	**87.0**	[21]	87.0
17α-Ethinylestradiol	*Brachionus calyciflorus* (invertebrate)	EC_{50} (72 h)	4.15	[22]	
	Nitocra spinipes (invertebrate)	LC_{50} (96 h)	0.51	[23]	
	Tisbe battagliai (invertebrate)	LC_{50} (10 days)	0.10	[24]	
	***Strongylocentrotus purpuratus* (invertebrate)**	**EC_{50}**	**0.03**	[25]	0.03
17β-Estradiol	*Daphnia magna* (invertebrate)	LC_{50} (48 h)	2.97	[26]	
	Americamysis bahia (invertebrate)	LC_{50} (96 h)	0.89	[26]	
	Tisbe battagliai (invertebrate)	LC_{50} (10 days)	0.10	[24]	
	***Strongylocentrotus purpuratus* (invertebrate)**	**EC_{50}**	**0.01**	[25]	0.01
Estriol	***Strongylocentrotus purpuratus* (invertebrate)**	**EC_{50}**	**1.52**	[25]	1.52
Estrone	*Strongylocentrotus purpuratus* (invertebrate)	EC_{50}	0.60	[25]	
	Acartia tonsa (invertebrate)	EC_{50}	0.41	[27]	
	***Tisbe battagliai* (invertebrate)**	**LC_{50} (10 days)**	**0.10**	[24]	0.10
Clofibric acid	*Pseudokirchneriella subcapitata* (algae)	EC_{50} (96 h)	94.0	[7]	
	Vibrio fischeri (bacterie)	EC_{50} (30 min)	91.8	[28]	
	Daphnia magna (invertebrate)	EC_{50} (48 h)	83.5	[29]	
	***Daphnia magna* (invertebrate)**	**EC_{50} (48 h)**	**72.0**	[18]	72.0
Gemfibrozil	*Vibrio fischeri* (bacterie)	EC_{50} (15 min)	35.3	[29]	
	Vibrio fischeri (bacterie)	EC_{50} (15 min)	18.8	[9]	
	Daphnia magna (invertebrate)	EC_{50} (48 h)	10.4	[15]	
	***Hydra attenuata* (invertebrate)**	**EC_{50} (96 h)**	**1.18**	[11]	1.18

EC_{50}: 50% Effective concentration; LC_{50}: 50% Lethal concentration.

Table 2
Ranges and mean concentration levels of pharmaceutically active compounds in wastewater affecting Doñana Park watersheds.

Pharmaceutical compound	Wastewater discharges									
	Discharges affecting S1 (WWTPs 1–4)		Discharges affecting S2 (WWTPs 5–8)		Discharges affecting S3 (WWTP 9)		Discharges affecting S4 (WWTP 10 + DD)		Discharges affecting Guadalquivir River (WWTP 11)	
	Range ($\mu g\,L^{-1}$)	Mean ($\mu g\,L^{-1}$)	Range ($\mu g\,L^{-1}$)	Mean ($\mu g\,L^{-1}$)	Range ($\mu g\,L^{-1}$)	Mean ($\mu g\,L^{-1}$)	Range ($\mu g\,L^{-1}$)	Mean ($\mu g\,L^{-1}$)	Range ($\mu g\,L^{-1}$)	Mean ($\mu g\,L^{-1}$)
Diclofenac	<LOD–1.31	0.16	<LOD–0.89	0.20	<LOD–0.71	0.24	<LOD	<LOD	<LOD–0.72	0.24
Ibuprofen	<LOD–18.7	4.35	<LOD–25.4	2.88	<LOD–2.40	0.80	<LOD–26.8	8.02	1.31–7.21	3.41
Ketoprofen	<LOD–1.94	0.16	<LOD–1.04	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD–2.95	1.48
Naproxen	0.14–2.11	0.78	<LOD–3.18	0.92	<LOD–0.15	0.09	0.05–6.34	2.57	0.33–1.05	0.74
Salicylic acid	<LOD–15.3	2.36	<LOD–3.24	0.55	<LOD–0.86	0.30	0.12–15.7	5.72	0.22–0.91	0.62
Sulfamethoxazole	<LOD–1.51	0.15	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD–1.62	0.54
Trimethoprim	<LOD–1.72	0.14	<LOD–1.72	0.14	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Carbamazepine	<LOD–17.3	1.67	<LOD–1.05	0.28	<LOD	<LOD	<LOD–2.13	0.39	<LOD–0.18	0.06
Propranolol	<LOD–0.70	0.32	<LOD–0.64	0.28	<LOD–0.40	0.13	0.04–0.45	0.32	0.33–0.64	0.51
Caffeine	<LOD–2.72	0.70	<LOD–2.10	0.46	<LOD–1.10	0.42	<LOD–2.50	0.47	<LOD–0.59	0.20
17α-Ethinylestradiol	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
17β-Estradiol	<LOD	<LOD	<LOD	<LOD	<LOD–0.24	0.08	<LOD	<LOD	<LOD	<LOD
Estriol	<LOD	<LOD	<LOD–0.16	0.01	<LOD	<LOD	<LOD–4.40	1.10	<LOD	<LOD
Estrone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Clofibric acid	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD–1.80	0.30	<LOD	<LOD
Gemfibrozil	<LOD–3.66	1.11	<LOD–4.48	1.76	<LOD–0.50	0.22	0.75–6.05	2.69	2.07–2.96	2.61

species tested to the diversity of species in the environment [32].

3. Results and discussion

3.1. Occurrence of pharmaceutical compounds in wastewater

The concentration levels of pharmaceutical compounds in WWTP effluents and in the untreated wastewater discharge affecting Doñana Park watersheds are shown in Table 2. All the studied pharmaceuticals, except the estrogenic compounds 17α-ethinylestradiol and estrone, were detected in the analysed wastewater samples. The anti-inflammatory drugs were found at the highest concentration levels in effluent wastewater, specially ibuprofen and salicylic acid (mean values 8.02 and 5.72 $\mu g\,L^{-1}$, respectively). These high concentrations can be caused by the high consumption of these pharmaceuticals for which no medical prescription is necessary. The antiepileptic carbamazepine was found only in 37% of effluent samples; however, it reached concentration levels up to 17.3 $\mu g\,L^{-1}$. Special attention should be paid to the presence of carbamazepine in wastewater due to its persistence in the environment. Moreover, it is used to treat an illness, epilepsy, which usually lasts for life so it will be continuously released into the environment. Clofibric acid was the compounds at the lowest concentration level (mean concentration: 0.05 $\mu g\,L^{-1}$), probably due to its low consume in Spain compared to the other pharmaceuticals, followed by the two antibiotics (mean concentration of antibiotics: 0.10 $\mu g\,L^{-1}$), the estrogenic compounds (mean concentration of estrogens: 0.13 $\mu g\,L^{-1}$) and propranolol, caffeine, and gemfibrozil (mean values: 0.30, 0.53 and 1.64 $\mu g\,L^{-1}$, respectively). These concentration levels were similar to those described in wastewater effluents discharging to unprotected areas in Finland [33], Spain [34–38], Switzerland [31] and United Kingdom [39]. The most contaminated wastewater effluents were those discharging to El Partido stream (S4), followed by discharges to Guadiamar River (S1 and S2), Guadalquivir River, and La Rocina stream (S3) (mean concentrations: S4: 1.35 $\mu g\,L^{-1}$; S1 and S2: 0.74 $\mu g\,L^{-1}$ and 0.47 $\mu g\,L^{-1}$, respectively; Guadalquivir River: 0.65 $\mu g\,L^{-1}$; S3: 0.14 $\mu g\,L^{-1}$). The most problematic discharges are those affecting El Partido and La Rocina streams, due to their low flow rates and, specially, discharges to El Partido stream because their high content in pharmaceutical compounds.

3.2. Occurrence of pharmaceutical compounds in surface water

Thirteen of the sixteen pharmaceutical compounds, involving all the therapeutic groups studied, were detected in Doñana Park watersheds (Table 3). Caffeine, gemfibrozil, ibuprofen, naproxen, propranolol and salicylic acid were the pharmaceuticals most frequently detected while clofibric acid, estrone and sulfamethoxazole, were not detected in any of the 48 surface-water samples analysed. The anti-inflammatory drugs and gemfibrozil were the compounds at the highest concentration levels in superficial surface water. Those pharmaceuticals were also the compounds at the highest concentration levels in wastewater (Table 2) where were present at concentration levels about 5–10 times higher than in surface water (Table 3). The concentration levels measured in Doñana Park watersheds are slightly higher than those reported by other authors in European rivers, such as Ebro (Spain) [40] or Elbe (Germany) [41], with higher water flows. The similar pattern of occurrence of pharmaceutical compounds in wastewater and surface water (Fig. 2) reveals that wastewater discharges are the main source of pharmaceuticals affecting Doñana Park. The considerable decrease of concentration from wastewater to surface water observed for some compounds as ibuprofen is probably

Table 3
Ranges and mean concentration levels of pharmaceutically active compounds in the main river and streams flowing into Doñana National Park.

Pharmaceutical compound	Surface-water sampling points							
	S1		S2		S3		S4	
	Range ($\mu g L^{-1}$)	Mean ($\mu g L^{-1}$)	Range ($\mu g L^{-1}$)	Mean ($\mu g L^{-1}$)	Range ($\mu g L^{-1}$)	Mean ($\mu g L^{-1}$)	Range ($\mu g L^{-1}$)	Mean ($\mu g L^{-1}$)
Diclofenac	<LOD	<LOD	<LOD–1.09	0.09	<LOD–0.54	0.05	<LOD	<LOD
Ibuprofen	<LOD–3.50	0.56	<LOD–0.64	<LOD	<LOD–4.55	1.11	<LOD–4.47	1.21
Ketoprofen	<LOD	<LOD	<LOD	<LOD	<LOD–1.56	0.13	<LOD–1.00	0.20
Naproxen	<LOD–0.51	0.16	<LOD–0.30	0.05	<LOD–0.21	0.03	0.09–2.01	0.64
Salicylic acid	0.04–0.99	0.45	0.03–1.40	0.44	<LOD–2.24	0.52	<LOD–2.04	0.46
Sulfamethoxazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Trimethoprim	<LOD–0.41	0.03	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Carbamazepine	<LOD–0.19	0.05	<LOD	<LOD	<LOD–1.11	0.15	<LOD–0.21	0.05
Propranolol	<LOD–0.45	0.08	<LOD–0.13	0.02	<LOD–0.15	0.02	0.02–0.21	0.14
Caffeine	<LOD–1.02	0.33	<LOD–0.73	0.15	<LOD–0.43	0.08	0.13–2.13	1.06
17α-Ethinylestradiol	<LOD–0.18	<LOD	<LOD	<LOD	<LOD–0.28	<LOD	<LOD	<LOD
17β-Estradiol	<LOD–0.15	<LOD	<LOD–0.12	<LOD	<LOD	<LOD	<LOD	<LOD
Estriol	<LOD	<LOD	<LOD	<LOD	<LOD–0.48	0.04	<LOD	<LOD
Estrone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Clofibric acid	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Gemfibrozil	<LOD–0.64	0.36	<LOD–0.62	0.23	<LOD–1.02	0.39	0.29–1.94	1.18

due to degradation processes under the aerobic conditions in surface water. The estrogenic compound 17α-ethinylestradiol was the only pharmaceutical compound not detected in wastewater but detected in surface water. It was detected in two of the 48 surface-water samples at concentration levels of 0.18 and 0.28 $\mu g L^{-1}$.

3.3. Temporal evolution of pharmaceutical compounds in surface water

Fig. 3 shows the temporal evolution of the pharmaceutical compounds at the highest concentration levels in the main river and streams flowing into Doñana National Park. An increase of the concentration of ibuprofen and salicylic acid, which were the compounds at the highest concentration levels, was observed in summer period (June–September) in most of the surface-water flows sampled. This fact can be explained by the decrease of the flow rates of rivers and streams flowing into Doñana Park during the summer period and, consequently, with a minor dilution of wastewater discharges. Only in sampling point S2 the concentration levels of salicylic acid measured in summer period were higher than concentration levels of ibuprofen. This fact could be to the longer distance of sampling point S2 with respect to wastewater discharges which could allow a degradation of ibuprofen since its discharge to the environment. No significant temporal evolution of the concentration levels of the other pharmaceuticals was observed probably due to their low concentration levels.

3.4. Risk assessment

Ecotoxicological risk assessment was carried out to estimate the potential risk of the presence of pharmaceutical compounds to aquatic organisms. Fig. 4 shows RQ values calculated from mean concentrations (12 samples measured from each surface-water sampling point) discarding outlier values. Under such conditions, none of the pharmaceutical compounds exceeded the RQ limit value for which no toxicological effect is expected to occur (RQ higher than 1). The highest average RQ values were obtained for gemfibrozil (RQ = 1 in S4) and ibuprofen (RQ = 0.90 in S3 and RQ = 0.88 in S4). Nevertheless, RQ values of the estrogenic compounds 17α-ethinylestradiol and 17β-estradiol, the anti-inflammatory drug ibuprofen and the lipid regulator gemfibrozil exceeded RQ limit value in some months and surface-water sampling points. Special attention should be paid to the environmental risk associated with estrogenic compounds not only because their high RQ values but also because of their endocrine disrupting effects. RQ values of 17α-ethinylestradiol were 6.0 in December in sampling point S1 and 9.3 in September in sampling point S3. RQ values of 17β-estradiol were 10.7 and 8.6 in June in

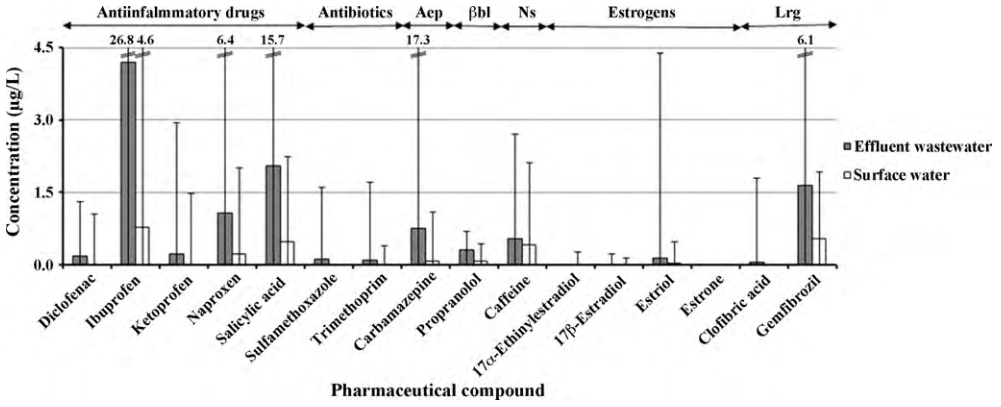

Fig. 2. Mean and highest concentrations of each pharmaceutical compound in wastewater and surface water (Aep: antiepileptic; βbl: β-blocker; Ns: nervous stimulant; Lrg: lipid regulators).

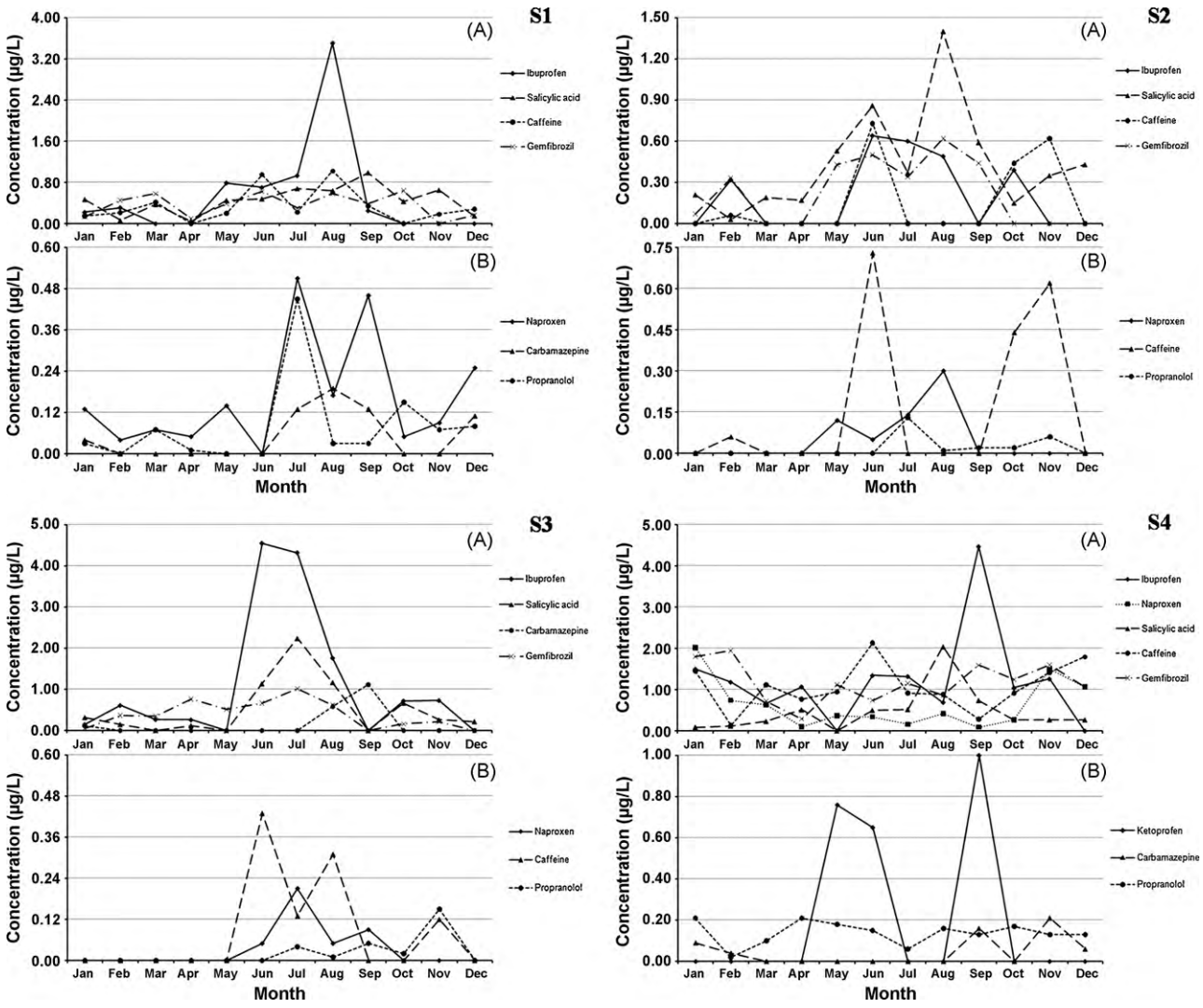

Fig. 3. One-year temporal evolution of the most concentrated pharmaceutical compounds in surface water.

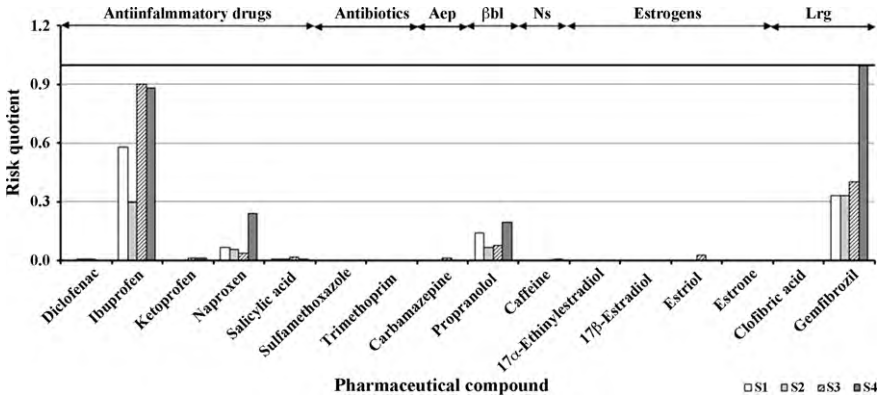

Fig. 4. Mean risk quotient values of the pharmaceutical compounds to the aquatic media (Aep: antiepileptic; βbl: β-blocker; Ns: nervous stimulant; Lrg: lipid regulators).

sampling points S1 and S2, respectively, and 4.3 in October in sampling point S1. The high removal rates of ibuprofen described in wastewater treatment plants [37] have demonstrated to be not enough to avoid environmental risk due to this compound. RQ values of ibuprofen were 2.1 in August in sampling point S1; 2.8, 2.6 and 1.1 in June, July and August, respectively, in sampling point S3 and 2.7 in September in sampling point S4. Additionally, the slight increase of gemfibrozil concentrations in S4 in winter months resulted in RQ values higher than 1 in January, February, September, October and November (RQ = 1.5, 1.6, 1.4, 1.1 and 1.4, respectively).

4. Conclusions

All the pharmaceutical compounds monitored, except estrone, were found in Doñana Park watersheds. The hormones 17α-ethinylestradiol and estrone were the only pharmaceutical

compounds not detected in wastewater effluents and the antibiotic sulfamethoxazole, the estrogenic compound estrone and the lipid regulator clofibric acid were the only ones not detected in surface water. The anti-inflammatory drugs were the pharmaceutical compounds at the highest concentration levels both in effluent wastewater and in surface water. An increase of ibuprofen and salycilic acid concentrations was observed in summer months whereas no significant temporal evolution was observed for the other compounds. Environmental risk assessment showed the existence of ecotoxicological risk to the aquatic organisms due to 17α-ethinylestradiol, 17β-estradiol, ibuprofen and gemfibrozil mainly in the summer period. Wastewater treatments applied in wastewater treatment plants affecting Doñana Park do not achieve enough removal rates of pharmaceutical compounds to ensure no environmental risk. Tertiary treatments, mainly those based on physical and chemical oxidation or ultrafiltration with membranes, should be implemented in wastewater treatment plants to eliminate, or at least reduce, the release of pharmaceutical compounds to Doñana Park protected area.

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