RESEARCH ARTICLE

Occurrence of endocrine disrupters and selected pharmaceuticals in Aisonas River (Greece) and environmental risk assessment using hazard indexes

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Abstract

Purpose The presence of four phenolic endocrine disrupting compounds (EDCs: nonylphenol [NP], NP monoethoxylate [NP1EO], bisphenol A [BPA], triclosan, [TCS]) and four nonsteroidal anti-inflammatory drugs (NSAIDs: ibuprofen [IBF], ketoprofen [KFN], naproxen [NPX], diclofenac [DCF]) in a Greek river receiving treated municipal wastewater was investigated in this study.

Methods Samples were taken from four different points of the river and from the outlet of a sewage treatment plant (STP) during six sampling campaigns, and they were analyzed using gas chromatography–mass spectrometry.

Results According to the results, EDCs were detected in almost all samples, whereas NSAIDs were detected mainly in wastewater and in the part of the river that receives wastewater from the STP. Among the target compounds, the highest mean concentrations in the river were detected for NP (1,345 ng L^{-1}) and DCF (432 ng L^{-1}). Calculation of daily loads of the target compounds showed that STP seems to be the major source of NSAIDs to the river, whereas other sources contribute significantly to the occurrence of EDCs. The environmental risk due to the presence of target compounds in river water was estimated, calculating risk quotients for different aquatic organisms (algae, daphnids, and fish). Results denoted the possible threat for the aquatic environment due to the presence of NP and TCS in the river.

1 Introduction

Several micropollutants are commonly detected in surface water, and they are considered to be potential threats to environmental ecosystems. Among these threats, surfactants, personal care products, and pharmaceuticals are three groups of compounds that transferred to the environment mainly due to urban runoff and municipal wastewater discharge and present significant research interest due to their extensive use and their physicochemical and toxicological properties.

Nonylphenol (NP) and NP monoethoxylate (NP1EO) are biotransformation products of NP ethoxylates, an important group of nonionic surfactants that are widely used in many commercial and household functions, including detergents, cosmetic products, and textiles (Birkett and Lester 2003). Due to their formation in sewer system (Ahel et al. 1994) and their partial removal during wastewater treatment processes (Gonzalez et al. 2007; Stasinakis et al. 2008), these compounds are often detected in treated wastewater of sewage treatment plants (STPs) (Stasinakis et al. 2008) and surface water (Kolpin et al. 2002). NP has been reported to cause a number of estrogenic responses on aquatic organisms (Birkett and Lester 2003), and it has been listed as a priority substance in the Water Framework Directive (EU 2001). Bisphenol A (BPA) is widely used for the production of flame retardants, polycarbonate, and epoxy

resins. These products are used in food and drink packaging as additives in thermal paper and in dental fillings. As a result, BPA is often detected in municipal and industrial wastewater (Gomez et al. 2007; Stasinakis et al. 2008). It has been shown that BPA is slightly-to-moderately toxic to fish and invertebrates (Staples et al. 1998), whereas it possesses weakly estrogenic activity, as well as antiandrogenic activity (Birkett and Lester 2003). Triclosan (TCS) is a broad-spectrum antimicrobial and preservative agent that is widely used in personal care products. In Europe, approximately 350 tons of TCS are produced annually for commercial applications (Singer et al. 2002), whereas in United States, more than 300 tons year^{-1} of TCS is estimated to be disposed into wastewater (Halden and Paull 2005). Concentrations of TCS up to few micrograms per liter have been detected in treated wastewater of STPs (Stasinakis et al. 2008) and surface water (Peng et al. 2008). Nonsteroidal anti-inflammatory drugs (NSAIDs) are a significant group of pharmaceuticals. Among them, ibuprofen (IBF), naproxen (NPX), ketoprofen (KFN), and diclofenac (DCF), due to their partial removal in STPs, are frequently detected in treated wastewater and transferred to the aquatic environment (Gomez et al. 2007; Kasprzyk-Hordern et al. 2008; Zhou et al. 2009; Samaras et al. 2010).

During the last years, several papers have been published worldwide, indicating the occurrence of these compounds in surface water. A recent survey in European river waters revealed that DCF, NPX, IBF, BPA, and NP were detected in 83%, 69%, 62%, 34%, and 29% of collected samples, respectively (Loos et al. 2009). Moreover, a survey in US streams showed that TCS, NP, NP1EO, and BPA were detected in 58%, 51%, 46%, and 41% of the samples, respectively. Despite the above fact, so far, there is a lack of data for the occurrence of these compounds in Greek surface waters. Recently, Arditsoglou and Voutsa (2010) detected NP, NP1EO, and BPA in inland waters of Northern Greece, whereas there are no data on the occurrence of TCS and NSAIDs in Greek aquatic environment. In addition, it is important to estimate the environmental risk from these compounds in the aquatic environment. From this point of view, several recent studies try to estimate the environmental risk from certain categories of synthetic organic compounds using hazard indexes (Kim et al. 2007; Gros et al. 2010; Garcia-Galan et al. 2011).

The objectives of this study were to investigate the presence of NP, NP1EO, BPA TCS, IBF, KFN, NPX, and DCF in a Greek river receiving municipal and industrial wastewater (Aisonas River) and to estimate the amounts of target compounds that are daily transferred to the Aegean Sea through the river. Samples were taken during six sampling campaigns at four sampling points along the river as well as from the effluents of a STP discharging into the river. Concentrations of the target compounds were determined using gas chromatography–mass spectrometry. The presence of target compounds was correlated with conventional water quality parameters; factor analysis was applied for identification of the sources, whereas hazard indexes were calculated based on literature data for estimating the effects of these compounds.

2 Materials and methods
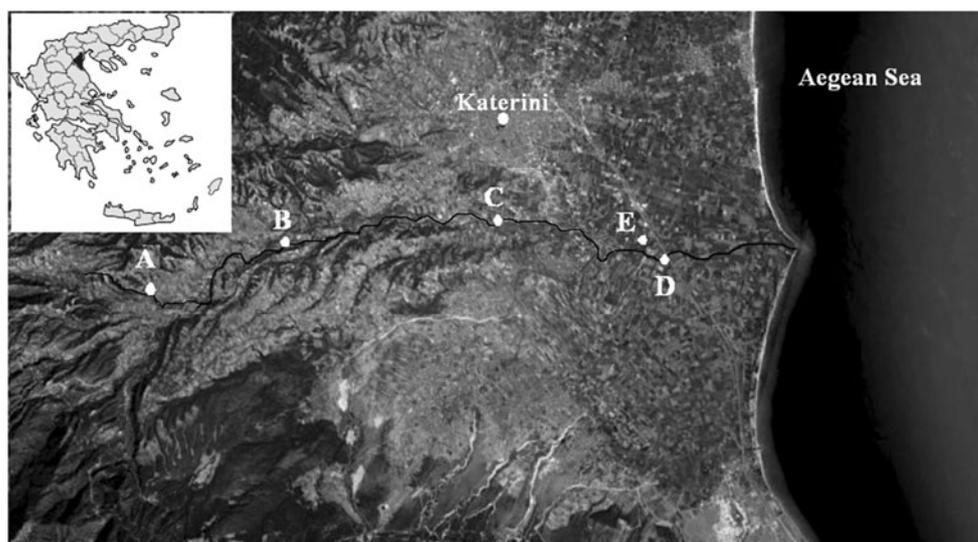
2.1 Chemical and standards

Methanol, dichloromethane, and ethyl acetate were of high-performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany) and were used as received. Bis(trimethylsilyl)trifluoroacetamide (BSTFA)+1% trimethyl chlorosilane (TMCS) and pyridine, used for silylation, were purchased by Supelco (Bellefonte, PA, USA) and Carlo Erba-SDS (Peypin, France), respectively. BPA (>97%) was purchased from Fluka (Buchs, Switzerland), whereas TCS (>97%) and deuterated BPA (BPA-16) were purchased from Fluka (Heidelberg, Germany). Analytical standards of NP, NP1EO, IBF, NPX, KFN, DFC, and meclofenamic acid (MFC) were supplied by Dr Ehrenstorfer (Germany). All compounds were used without further purification (minimum purity >99%). Stock solutions of individual compounds were prepared in methanol at 1,000 mg L^{-1} and kept at −18°C. HPLC-grade water was prepared in the laboratory using a MilliQ/MilliRO Millipore system (Millipore, Billerica, Massachusetts USA).

2.2 Study area, sampling, and water quality characterization

The Aisonas River is situated in the north part of Greece (Fig. 1). With a distance from source to mouth of 50 km, an average water flow of 2.2 m^3 s^{-1} at the mouth (during the sampling period of this study) and a residence time of 4 to 10 h, Aisonas is a small river that is impacted by human activities as it receives treated wastewater from a STP serving the city of Katerini and industrial wastewater from dairies and other food industries. The average sewage flow in the STP is 0.2 m^3 s^{-1}, and it is equipped with secondary treatment (activated sludge process). The relative amount of municipal treated wastewater in this river ranged from 4% to 12% of its total flow rate during the sampling period of this study.

Samples were taken during six sampling campaigns in May and June 2008 at four sampling points along the river (sampling stations A, B, C, and D) as well as from the effluents of STP discharging into the river (sampling station E) (Fig. 1). During these months, precipitation is low, so dilution of the river water is minimal and the concentrations of any compound found would therefore represent a "worst case scenario."

River and wastewater samples were collected in 1-L precleaned amber glass bottles (0.5 L for endocrine disrupting compounds (EDCs)/NSAIDs and 0.5 L for water quality parameters), and they were transported to the laboratory in a cooler. Samples were filtered through preashed glass-fiber filters (GF/F; Whatman, Kent, UK), stored in the dark at 4°C until solid-phase extraction (SPE) (normally 24 h after filtration), and analyzed within a period of 5 days.

To characterize water and wastewater samples, determination of various water quality parameters such as Chemical Oxygen Demand (COD), NH_4–N, NO_3–N, and Total Suspended Solids (TSS) was performed according to Standard Methods (APHA 1998). Moreover, temperature, conductivity, and pH values were measured using portable instruments during samples' collection.

2.3 Sample preparation and analysis of target compounds

The analysis of target compounds in water and wastewater samples was performed using an analytical method developed and optimized by the authors (Samaras et al. 2011). Water and wastewater samples were filtered, acidified to pH 2.5, and extracted using C18 SPE cartridges. The eluates of the extraction were evaporated to dryness, and the dried residues were subjected to derivatization reaction using BSTFA (1% TMCS) and pyridine. For the qualitative and quantitative analyses, a Hewlett-Packard Gas Chromatograph 5890 Series II connected to a Hewlett-Packard Mass Spectrometer HP5971 MSD was used (Palo Alto, California, USA). The separation of target compounds was achieved using a DB5MS capillary column (60 m) with a film thickness of 0.25 μm and an internal diameter of 0.32 mm (Supelco).

Detailed information about the quality parameters of the analytical methods have been reported by Samaras et al. (2011). In brief, this analytical method presented satisfactory

precision with relative standard deviations less than 12% for all the tested compounds. Satisfactory recoveries were obtained, ranging from 91.8% (IBF) to 117% (NPX), whereas limits of detection (LODs) of the target compounds varied from 0.37 ng L^{-1} (KFN) to 14 ng L^{-1} (BPA) (Samaras et al. 2011).

2.4 Calculations and statistical analysis

In cases that sample concentrations were below the LOD, a concentration equal to half of the detection limit was used for the calculations (Stasinakis et al. 2008). In order to identify possible sources of the target analytes, Spearman correlation coefficients were calculated. In addition, factor analysis with varimax rotation was applied to the whole data for the same purpose. Moreover, principal component analysis (PCA) was used in this work to reveal the similarities and dissimilarities of the sampling sites and the critical parameters that define the spatial variations. Data was treated by Statistica 7.0.

3 Results and discussion

3.1 Physicochemical parameters in river water and effluent wastewater

Physicochemical characteristics of river water (points A to D) and wastewater (point E) are presented in Table 1. According to the results, values of pH and conductivity were similar in points A to C, whereas a decrease of pH and an increase of conductivity were observed in point D due to the discharge of treated wastewater. The discharge of wastewater seems to increase also the concentrations of COD, TSS, and NH_4–N in point D compared with other sampling points.

Table 1 Physicochemical characteristics of river water and treated wastewater ($n=6$ for each sampling station)

Substance	Sampling points				
	A (River), mean±SD	B (River), mean±SD	C (River), mean±SD	D (River), mean±SD	E (STP), mean±SD
	(minimum–maximum)	(minimum–maximum)	(minimum–maximum)	(minimum–maximum)	(minimum–maximum)
Flow rate ($m^3\ s^{-1}$)	0.80±0.34	4.72±3.43	2.59±2.14	2.44±1.61	0.23±0.01
	(0.60–1.48)	(2.14–9.21)	(1.04–6.92)	(1.34–5.62)	(0.21–0.24)
pH	7.92±0.27	8.16±0.11	8.17±0.74	7.65±0.21	7.49±0.32
	(7.58–8.21)	(7.96–8.27)	(6.83–8.83)	(7.27–7.91)	(6.85–7.65)
T (°C)	18.2±1.9	19.2±1.9	22.3±3.9	24.0±0.9	23.5±0.9
	(16.5–21.0)	(17.4–21.7)	(17.6–28.0)	(22.5–25.3)	(22.3–24.8)
Conductivity ($\mu S\ cm^{-1}$)	246±40	254±50	263±39	471±90	1,062±156
	(202–319)	(175–305)	(197–309)	(366–599)	(752–1,164)
COD ($mg\ L^{-1}$)	9±5	7±6	12±9	23±6	43±12
	(4–18)	(2–16)	(2–24)	(16–35)	(25–57)
TSS ($mg\ L^{-1}$)	22±20	25±26	34±35	42±39	37±24
	(3–51)	(1–63)	(1–91)	(6–97)	(14–69)
NH_4–N ($mg\ L^{-1}$)	1.1±0.4	<0.2	<0.2	4.0±2.6	16.9±8.4
	(0.4–1.6)			(1.4–7.4)	(8.4–26.9)
NO_3–N ($mg\ L^{-1}$)	1.2±0.4	0.4±0.1	0.4±0.1	0.8±0.3	0.8±0.2
	(0.7–1.7)	(0.3–0.4)	(0.3–0.4)	(0.5 –1.2)	(0.6–1.1)

3.2 EDCs and NSAIDs occurrence in the river water and effluent wastewater

During the six sampling campaigns, all 24 river water samples and 6 treated wastewater samples were collected. The data obtained from all the analyzed samples are presented in Table 2. Regarding the river water, EDCs were found in more samples compared with pharmaceuticals. Specifically, NP and BPA were detected in all samples, whereas NP1EO and TCS were found in 21 and 23 samples, respectively. On the other hand, IBF, KFN, NPX, and DCF were detected in 5, 6, 15, and 5 samples, respectively (Table 2). The highest value of the means and the maximum concentration were 1,345 ng L^{-1} (point D) and 2,704 ng L^{-1} (point D), respectively, and they were due to NP (Table 2). With the exception of DCF in one sampling point (point D), the concentrations of the other target compounds ranged up to few hundreds of nanograms per liter.

Comparison of the concentrations detected in this study with literature data shows that the concentrations of all the target compounds fall into the range reported in the literature (Bendz et al. 2005; Kasprzyk-Hordern et al. 2008; Jonkers et al. 2009; Arditsoglou and Voutsa 2010), except those of NP whose concentration was higher than those usually reported for European rivers. In a recent study investigating the presence of EDCs in Greek rivers, concentrations of NP, NP1EO, and BPA ranging from 152 to 388, 70 to 337,and 15 to 138 ng L^{-1} have been reported, respectively (Arditsoglou and Voutsa 2010). Monitoring data in Hoje river (Sweden) and Glatt river (Switzerland)

have shown NP concentration levels ranging up to 195 and 200 ng L^{-1}, respectively (Bendz et al. 2005; Jonkers et al. 2009). In the same studies, BPA ranged between 2 and 46 ng L^{-1} (Jonkers et al. 2009), whereas TCS did not exceed 70 ng L^{-1} (Bendz et al. 2005). Regarding NSAIDs, Kasprzyk-Hordern et al. (2008) detected concentrations up to 100 ng L^{-1} for IBF, 261 ng L^{-1} for DCF, 14 ng L^{-1} for KFN, and 146 ng L^{-1} for NPX in two UK Rivers, whereas Möder et al. (2007) reported concentrations up to 33.6, 92.9, and 245.3 ng L^{-1} for IBF, NPX, and DCF, respectively, in Saale River (Germany). In addition, similar NPX, IBF, DCF, BPA, and TCS concentrations have been detected in Han River (South Korea) (Yoon et al. 2010).

Regarding wastewater samples (point E), target compounds were not totally eliminated by the applied treatment processes, and as a result, they were detected in most effluent samples (Table 2). The highest mean concentrations were detected for NP (3,514 ng L^{-1}) and DCF (3,328 ng L^{-1}), whereas significant lower concentrations were determined for the other compounds (Table 2). The concentration levels of most target compounds were similar or lower than those previously reported for treated wastewater originating from Greek and European STPs (Gomez et al. 2007; Stasinakis et al. 2008; Pothitou and Voutsa 2008; Gros et al. 2010; Samaras et al. 2010). On the other hand, concentrations of DCF exceeded concentrations levels, which are usually determined world-wide for this compound (Zhang et al. 2008).

Average daily loads of the target compounds were calculated for the river (point D), and the contribution of STP in total amounts of target compounds discharged to the

Table 2 Occurrence of the target EDCs and NSAIDs in river water and treated wastewater (in ng L^{-1}) ($n=6$ for each sampling station)

Substance	Sampling points A (River)		B (River)		C (River)		D (River)		E (STP)	
	[N]>LOD[a]	Mean±SD (minimum– maximum)	[N]>LOD[a]	Mean±SD (minimum– maximum)	[N]>LOD[a]	Mean±SD (minimum– maximum)	[N]>LOD[a]	Mean±SD (minimum– maximum)	[N]>LOD[a]	Mean±SD (minimum– maximum)
NP	6	1,236±405 (558–1,709)	6	984±431 (594–1,500)	6	1,309±419 (742–1,830)	6	1,345±825 (641–2,704)	6	3,514±757 (2,581–4,552)
NP1EO	6	149±85 (63–308)	6	104±23 (78–131)	6	159±72 (66–261)	3	52±63 (<2–145)	4	194±136 (<2–335)
BPA	6	96±18 (80–122)	6	82±28 (55–114)	6	122±29 (80–162)	6	94±30 (61–135)	6	292±279 (151–790)
TCS	6	28±8 (18–38)	6	25±3 (22–29)	5	32±35 (<3–98)	6	22±7 (22–39)	6	101±18 (75–120)
IBF	2	17±28 (1–67)	0	<1	0	<1	3	22±29 (<1–67)	6	313±167 (138–504)
KFN	1	66±161 (<0.37–395)	0	<0.37	0	<0.37	5	54±31 (<0.37–85)	6	743±554 (200–1,574)
NPX	5	146±97 (<3–243)	3	136±161 (<3–322)	2	70±106 (<3–222)	5	72±70 (<3–197)	6	331±186 (183–654)
DCF	0	<0.8	0	<0.8	0	<0.8	5	432±440 (<0.8–1,043)	6	3,328±3,026 (459–7,003)

[a] [N]>LOD: number of samples with concentrations higher than the LOD of the method

Aegean Sea was estimated using mass balances (Fig. 2). According to the results, more than 250 g of NP seems to be discharged daily to the sea, whereas amounts ranging from 4 gday^{-1} (IBF) to 82 gday^{-1} (DCF) are estimated to be discharged for the other target compounds. STP is the main source of IBF, KFN, and DCF to the river (Fig. 3). On the other hand, the contribution of STP to the total amounts of EDCs calculated in point D ranged from 20% (NP) to 30% (NP1EO), indicating the coexistence of other sources of these compounds along the river. It should be mentioned that according to the results presented in Table 2, significant concentrations of EDCs were detected in points A, B, and C in all sampling campaigns, and they are possibly due to the existence of food industries and smaller settlements in the wider area of study.

3.3 Correlation between target compounds and physicochemical parameters

In order to derive correlations between target compounds and physicochemical parameters, Spearman nonparametric correlation coefficients were calculated for all parameters at all sampling points. As it can be shown in Table 3, NSAIDs were all significantly correlated, and NP was significantly

Fig. 2 Contribution of STP and river in daily loads of target compounds discharged to the Aegean Sea (mass balance in point D of Aisonas River)
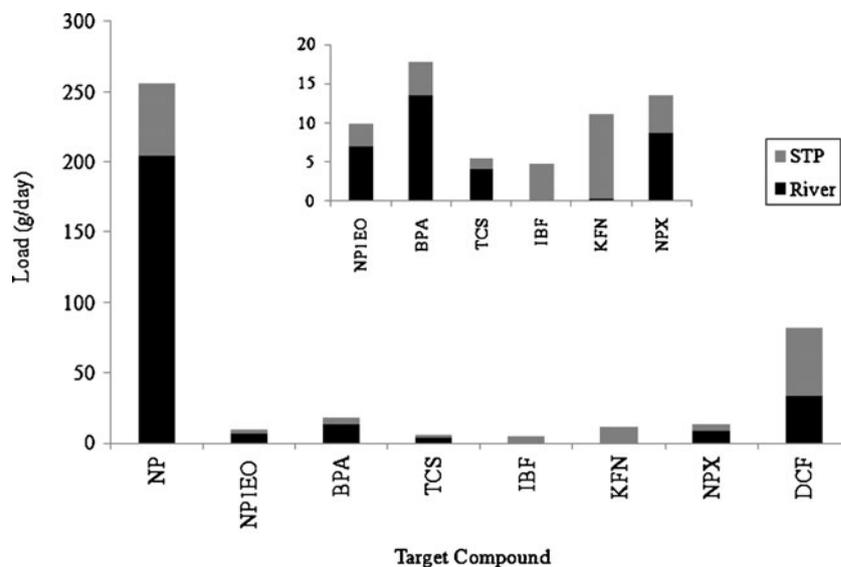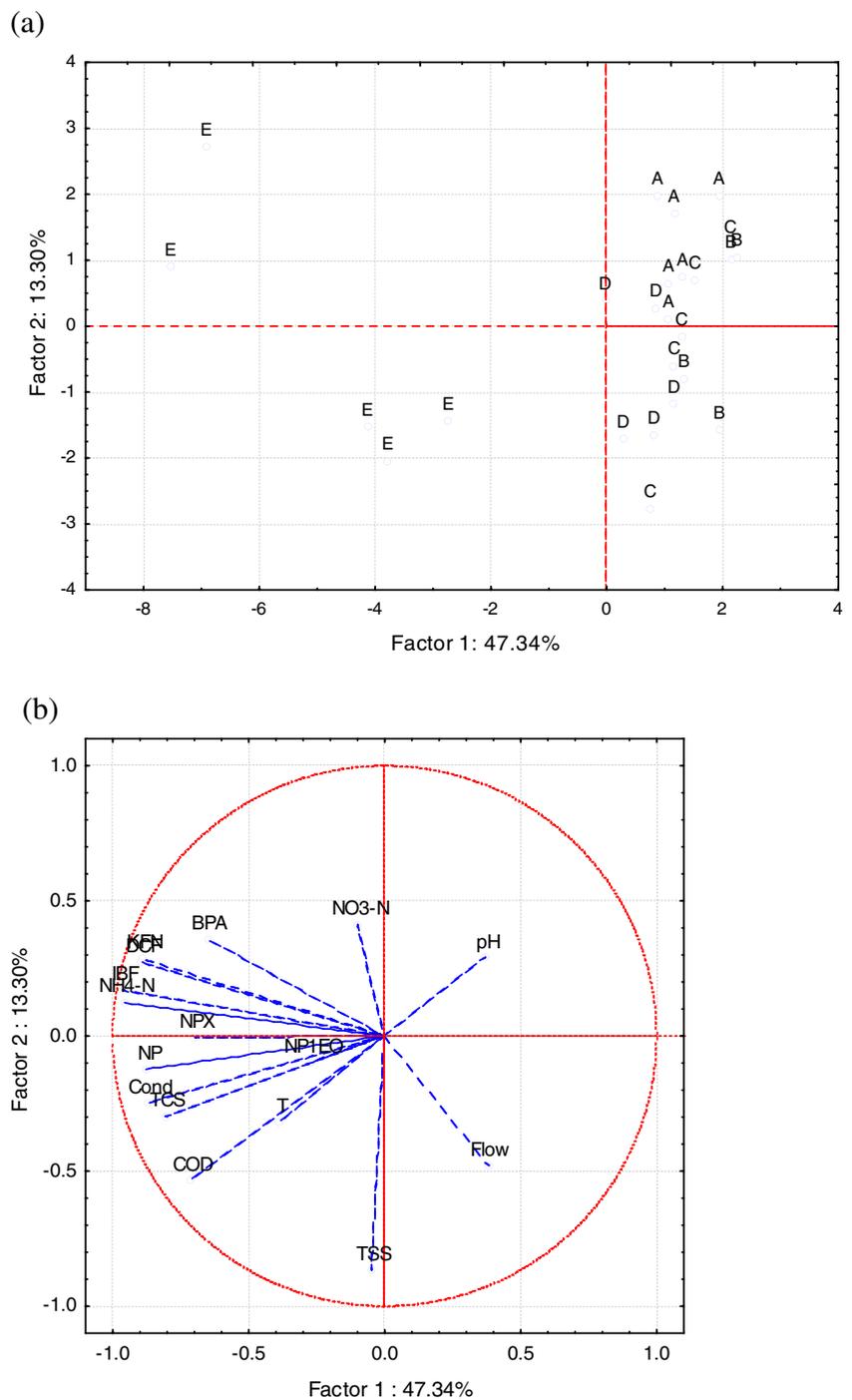
(a)

(b)

correlated with all the target analytes, whereas NP1EO was correlated significantly only with NP and BPA. TCS was correlated with all target analytes, except NPX. All target analytes were correlated significantly with NH_4–N, COD, and conductivity, whereas flow was negatively correlated with all the analytes as it was expected. To further exploit the correlation between analytes and the other parameters, factor analysis was performed, and the varifactors are given in Table 4. Four factors could explain 80% of the data variance. The results of factor analysis showed that all target analytes, except NP1EO, present high loadings for the first factor, which indicates the presence of a common source (here, the STP). However, NPX, NP, and TCS could have an additional source(s), as their loadings for factors 3 and 4 denote. NP1EO correlates significantly with TCS and NP (Tables 3 and 4), implying a second common source that could identified as runoff waters. The results of factor analysis and Spearman correlation verify

Table 3 Spearman rank order correlations between the target analytes, physicochemical parameters, and flow of the river in all sampling points

	IBF	NPX	DCF	KFN	TCS	BPA	NP	NP1EO	pH	T	Conductivity	COD	SS	NH$_4$–N	NO$_3$–N	Flow
IBU	1.0000															
NPX	**0.5221**	1.0000														
DCF	**0.8016**	**0.4040**	1.0000													
KFN	**0.8181**	**0.3869**	**0.8858**	1.0000												
TCS	**0.6129**	0.2406	**0.6087**	**0.6239**	1.0000											
BPA	**0.5418**	0.2304	**0.5073**	**0.5117**	**0.4676**	1.0000										
NP	**0.4803**	**0.5158**	**0.5560**	**0.5896**	**0.5904**	**0.3983**	1.0000									
NP1EO	0.1492	0.0559	0.0469	0.1458	0.2706	**0.3500**	**0.3911**	1.0000								
pH	**−0.4609**	−0.0889	**−0.6106**	**−0.5952**	**−0.5420**	−0.1902	**−0.4015**	−0.1147	1.0000							
T	0.3362	0.2379	**0.5300**	**0.3999**	0.0064	0.2242	0.1320	−0.2508	−0.1132	1.0000						
Conductivity	**0.6538**	0.2301	**0.8172**	**0.7467**	**0.5684**	**0.5250**	**0.3723**	−0.0287	**−0.4973**	**0.6414**	1.0000					
COD	**0.6165**	**0.5440**	**0.7525**	**0.6461**	**0.4477**	0.3012	**0.5853**	−0.0593	**−0.5561**	**0.7301**	**0.7317**	1.0000				
TSS	0.0624	**0.3949**	0.1776	0.1546	0.0629	−0.2208	**0.4080**	−0.0546	−0.2947	**0.3502**	0.0815	**0.5689**	1.0000			
NH$_4$–N	**0.8279**	**0.4594**	**0.8829**	**0.8804**	**0.6197**	**0.4114**	**0.5337**	0.0351	**−0.7106**	**0.4155**	**0.7716**	**0.7497**	0.2090	1.0000		
NO$_3$–N	0.2798	0.2678	0.2550	**0.3540**	0.1470	0.0539	0.1895	−0.0083	**−0.4023**	−0.0299	0.2587	0.2485	0.0566	**0.5845**	1.0000	
Flow	**−0.7328**	**−0.4054**	**−0.5095**	**−0.5608**	**−0.3944**	**−0.5505**	**−0.4182**	−0.3067	**0.4171**	−0.1765	**−0.4489**	**−0.4624**	0.1164	**−0.6579**	**−0.5634**	1.0000

Correlations marked in bold font are significant at $p < 0.050$

Table 4 Factor loadings (varimax normalized) of the factor analysis

	Factor 1	Factor 2	Factor 3	Factor 4
IBF	**0.900976**	0.150816	0.207156	0.303393
NPX	0.571278	0.078890	0.157336	0.372539
DCF	**0.918323**	0.160184	0.113992	0.185098
KFN	**0.958799**	0.111330	0.121401	0.104630
TCS	0.514670	−0.062818	**0.687125**	0.374784
BPA	**0.889009**	−0.013943	−0.173161	−0.066519
NP	**0.744198**	−0.092308	0.388955	0.318552
NP1EO	0.003133	0.207494	**0.718638**	0.041499
pH	−0.220038	0.210025	**−0.690274**	0.027975
T	0.182084	−0.098989	−0.402649	**0.839311**
Conductivity	0.512199	0.103273	0.393373	**0.693810**
COD	0.319506	−0.150913	0.282872	**0.841997**
TSS	−0.143806	**−0.760837**	0.266277	0.351002
NH$_4$–N	**0.832545**	0.192261	0.251566	0.378806
NO$_3$–N	0.031457	0.558237	0.325657	−0.191270
Flow	−0.185885	**−0.811931**	0.022739	−0.351899
Explained Variance	5.705150	1.796785	2.379374	2.846798
Propability Total	0.356572	0.112299	0.148711	0.177925

Significant correlations are denoted using bold font

the aforementioned conclusion from the calculation of daily loads. In addition, PCA was applied to the data set. The score plot (Fig. 3(a)) revealed distinct groups of the water samples. Although PCA is not always an optimal procedure for classification purposes, the sites' groups of A, B, C, and D seem to be completely discriminated from E sites. The significant discrimination of this site (STP) is related to the high loadings of all NSAIDs and EDCs (Fig. 3(b)), denoting that STP is the main common source for these compounds, which, in turn, affect the quality of water of site D indicative of the proximity of the respective points (Fig. 3(a)). In addition, the loading plot (Fig. 3(b)) shows groupings and relation-

ships between the variables. The group of all NSAID and EDC variables and their close relation is visible in the left hemisphere of the plot. Such a solid grouping pattern signifies the strength of their mutual correlation. Thus, whereas this group seem to differentiate E sites from the rest ones, NO$_3$–N, pH, flow, and TSS are the critical parameters for the "internal" discrimination of A to D sites.

3.4 Calculation of hazard indexes

Regarding the effects of target compounds in the aquatic environment, measured environmental concentrations (MECs) in river water were used together with predicted no-observed-effect concentrations (PNECs) obtained from peer-reviewed literature, to calculate risk quotients expressed as MEC/PNEC ratios (Lindberg et al. 2007). For the determination of PNECs, EC$_{50}$ values for fish, daphnids, and algae were divided by an assessment factor of 1,000 (EU 2003; Gros et al. 2010). According to the literature, if the exposure concentration exceeds the effect concentration (MEC>PNEC), then an ecological risk is suspected (Lindberg et al. 2007). Risk quotients and EC$_{50}$ values used for their calculation are presented in Table 5. It should be mentioned that for the selection of EC$_{50}$ values, search for ecotoxicity data was performed in the literature and the lowest acute EC$_{50}$ value was selected. Based on the results, no risk on aquatic organisms could be associated to the presence of NSAIDs and BPA in river waters. In this study, risk quotients were estimated for individual compounds. However, it should be mentioned that pharmaceuticals are usually present in the environment as mixtures. Based on the above fact, several studies have shown that their toxicity to nontarget organisms may be occurring at environmentally relevant concentrations due to combined and synergistic effects (Pomati et al. 2008; Quinn et al. 2009).

Table 5 EC$_{50}$ values and ratios of measured environmental concentrations (MECs) to PNECs of target compounds detected in river water. (Maximum concentrations detected in river were used; an assessment factor of 1000 was used for the determination of PNECs from EC$_{50}$ values)

Substance	EC$_{50}$ (μg L^{-1})			MEC/PNEC		
	Fish	Daphnids	Algae	Fish	Daphnids	Algae
NP	128[1]	85[1]	56.3[2]	21	32	48
BPA	4600[3]	3900[4]	2730[3]	0.035	0.042	0.059
TCS	260[5]	390[5]	1.4[5]	0.377	0.251	70
IBF	5000[6]	9060[7]	4000[8]	0.013	0.007	0.017
KFN	32000[6]	248000[6]	164000[6]	0.012	0.002	0.002
NPX	34000[6]	15000[6]	22000[6]	0.009	0.021	0.015
DCF	10100[9]	22430[10]	14500[10]	0.103	0.047	0.072

[1] Brooke (1993), [2] Kopf (1997), [3] Alexander et al. (1988), [4] Stephenson (1983), [5] Orvos et al. (2002), [6] Sanderson et al. (2003), [7] Halling-Sorensen et al. (1998), [8] Pomati et al. (2004), [9] Nassef et al. (2009), [10] Ferrari et al. (2004)

On the other hand, risk quotient values higher than 1 were obtained for NP (fish, daphnia, algae) and TCS (algae), indicating the risk of environmental threat due to the presence of these compounds in Aisonas River. The relative risk susceptibility for NP was estimated to be algae>daphnids>fish. Environmental quality standards have been proposed for the occurrence of NP in surface water (EC 2006). These criteria refer to the total (dissolved+ particulate) concentration of NP. Beside the fact that dissolved concentrations of this compound were determined in this study, the mean concentrations of NP in different points of Aisonas River (Table 2) were higher than the annual average concentration (300 ng L^{-1}) proposed by European Union (EC 2006). In addition, the maximum detected concentration was higher than the maximum allowable concentration (2000 ng L^{-1}) proposed by European Union (EC 2006). Having in mind that a part of NP can be accumulated in the particulate phase (Arditsoglou and Voutsa 2010), the excess of NP limit proposed by EU is expected to be more significant than that estimated in the present study.

4 Conclusions

The monitoring of selected EDCs and NSAIDs in Aisonas River showed that these compounds were frequently detected in river water. For most compounds, their concentrations were similar to those reported in the literature; however, elevated concentrations were detected for NP, reaching up to 2,704 ng L^{-1} in downstream river samples. As a result, almost 250 g of NP is estimated to be daily discharged through the river to the Aegean Sea. STP seems to be the major source of NSAIDs to the river, whereas other sources contribute significantly to the detection of EDCs. Preliminary risk assessment using risk quotients showed that potentially adverse effects on aquatic organisms should not be excluded for NP and TCS.

References

Ahel M, Giger W, Koch M (1994) Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment—I. Occurrence and transformation in sewage treatment. Water Res 28:1131–1142

Alexander HC, Dill DC, Smith LW, Guiney PD, Dorn P (1988) Bisphenol-A: acute aquatic toxicity. Environ Toxicol Chem 7:19–26

APHA (1998) Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Port City Press, Baltimore, Maryland, USA

Arditsoglou A, Voutsa D (2010) Partitioning of endocrine disrupting compounds in inland waters and wastewaters discharged into the coastal area of Thessaloniki, Northern Greece. Environ Sci Poll Res 17:529–538

Bendz D, Paxéus NA, Ginn TR, Loge FJ (2005) Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Höje River in Sweden. J Hazard Mat 122:195–204

Birkett JW, Lester JN (2003) Endocrine disrupters in wastewater and sludge treatment processes, CRC Press LLC

Brooke LT (1993) Acute and chronic toxicity of nonylphenol to ten species of aquatic organisms. USEPA Draft Report, EPA Contract Nº 68-C1-0034

European Union (2001) Decision No 2455/2001/EC of the European Parliament and of the council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending directive 2000/60/EC, Off. J. L331, 15/12/2001

European Union (2003) Technical Guidance Document on Risk Assessment (Part II), European Chemicals Bureau

European Union (2006) Proposal for a Directive of the European Parliament and of the Council on environmental quality standards in the field of water policy and amending Directive 2000/60/EC, 2006/0129, Brussels, 17.7.2006

Ferrari B, Mons R, Vollat B, Fraysse B, Paxeus N, Lo Gindice R, Pollio A, Garric J (2004) Environmental risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? Environ Tox Chem 23:1344–1354

Garcia-Galan MJ, Diaz-Cruz MS, Barcelo D (2011) Occurrence of sulfonamide residues along the Ebro river basin: removal in wastewater treatment plants and environmental impact assessment. Environ Int 37:462–473

Gomez MJ, Martinez Bueno MJ, Lacorte S, Fernandez-Alba AR, Aguera A (2007) Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. Chemosphere 66:993–1002

Gonzalez S, Petrovic M, Barcelo D (2007) Removal of a broad range of surfactants from municipal wastewater—comparison between membrane bioreactor and conventional activated sludge treatment. Chemosphere 67:335–343

Gros M, Petrovic M, Ginebreda A, Barcelo D (2010) Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ Int 36:15–26

Halden RU, Paull DH (2005) Co-occurrence of triclocarban and triclosan in U.S. water resources. Environ Sci Technol 39:1420–1426

Halling-Sorensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Lutzhoft HC, Jorgenses SE (1998) Occurrence, fate and effects of pharmaceutical substances in the environment—a review. Chemosphere 36:357–393

Jonkers N, Kohler HPE, Dammshauser A, Giger W (2009) Mass flows of endocrine disruptors in the Glatt River during varying weather conditions. Environ Poll 157:714–723

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2008) The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res 42:3498–3518

Kim Y, Choi K, Jung J, Park S, Kim PG, Park J (2007) Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. Environ Int 33:370–375

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones and other organic wastewater contaminants in U.S. Streams, 1999–2000: a national reconnaissance. Environ Sci Technol 36:1202–1211

Kopf W (1997) Wirkung endokriner stoffe in biotests mit wasserogranismen. In Stoffe mit endokriner wirkung in wasser. Bayerisches landesamt fur wasserwitschaft, Institut fur Wasserforschung Munchen (ed) Oldenbourg

Lindberg RH, Bjorklund K, Rendahl P, Johansson MI, Tysklind M, Andersson BAV (2007) Environmental risk assessment of antibiotics in the Swedish environment with emphasis on sewage treatment plants. Water Res 41:613–619

Loos R, Gawlik BM, Locoro G, Rimaviciute E, Contini S, Bidoglio G (2009) EU-wide survey of polar organic persistent pollutants in European river waters. Environ Pollut 157:561–568

Möder M, Braun P, Lange F, Schrader S, Lorenz W (2007) Determination of endocrine disrupting compounds and acidic drugs in water by coupling of derivatization, gas chromatography and negative-chemical ionization mass spectrometry. Clean 35:444–451

Nassef M, Matsumoto S, Seki M, Kang IJ, Moroishi J, Shimasaki Y, Oshima Y (2009) Pharmaceuticals and personal care products toxicity to Japanese medaka fish (*Oryzias latipes*). J Fac Agr Kyushu U 54:407–411

Orvos DR, Versteeg DJ, Inauen J, Capdevielle M, Rothenstein A, Cunningham V (2002) Aquatic toxicity of triclosan. Environ Toxicol Chem 21:1338–1349

Peng X, Yu Y, Tang C, Tan J, Xuang Q, Wang Z (2008) Occurrence of steroid estrogens, endocrine-disrupting phenols, and acid pharmaceutical residues in urban riverine water of the Pearl River Delta, South China. Sci Total Environ 397:158–166

Pomati F, Netting AG, Calamari D, Neilan BA (2004) Effects of erythromycin, tetracycline and ibuprofen on the growth of *Synechocystis* sp. and *Lemna minor*. Aquat Toxicol 67:387–396

Pomati F, Orlandi C, Clerici M, Luciani F, Zuccato E (2008) Effects and interactions in an environmentally relevant mixture of pharmaceuticals. Toxicol Sci 102:129–137

Pothitou P, Voutsa D (2008) Endocrine disrupting compounds in municipal and industrial wastewater treatment plants in Northern Greece. Chemosphere 73:1716–1723

Quinn B, Gagné F, Blaise C (2009) Evaluation of the acute, chronic and teratogenic effects of a mixture of eleven pharmaceuticals on the cnidarian, Hydra attenuate. Sci Total Environ 407:1072–1079

Samaras VG, Thomaidis NS, Stasinakis AS, Gatidou G, Lekkas TD (2010) Determination of selected non-steroidal anti-inflammatory drugs in wastewater by gas chromatography–mass spectrometry. Int J Environ Anal Chem 90:219–229

Samaras VG, Thomaidis NS, Stasinakis AS, Lekkas TD (2011) An analytical method for the simultaneous trace determination of acidic pharmaceuticals and phenolic endocrine disrupting chemicals in wastewater and sewage sludge by gas chromatography–mass spectrometry. Anal Bioanal Chem 399:2549–2561

Sanderson H, Johnson DJ, Wilson CJ, Brain RA, Solomon KR (2003) Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening. Toxicol Lett 144:383–395

Singer H, Muller S, Tixier C, Pillonel L (2002) Triclosan: occurrence and fate of a widely used biocide in the aquatic environment: field measurements in wastewater treatment plants, surface waters, and lake sediments. Environ Sci Technol 36:4998–5004

Staples CA, Dorn PB, Klecka GM, O'Block ST, Haris LR (1998) A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere 36:2149–2173

Stasinakis AS, Gatidou G, Mamais D, Thomaidis NS, Lekkas TD (2008) Occurrence and fate of endocrine disrupters in Greek sewage treatment plants. Water Res 42:1796–1804

Stephenson RR (1983) Diphenylol propane: acute toxicity to *Daphna magna* and *Selenastrum capricornutum*. Shell group research report SBGR.83.216

Yoon Y, Ryu J, Oh J, Choi BG, Snyder SA (2010) Occurrence of endocrine disrupting compounds, pharmaceuticals, and personal care products in the Han River (Seoul, South Korea). Sci Total Environ 408:636–643

Zhang Y, Geiben SU, Gal C (2008) Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. Chemosphere 73:1151–1161

Zhou JL, Zhang ZL, Banks E, Grover E, Jiang JK (2009) Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water. J Haz Mater 166:655–661