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Occurrence and removal of PPCPs in municipal and hospital wastewaters in Greece

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ABSTRACT

A monitoring study was carried out for the four seasons over 1-year monitoring period (March 2006–March 2007) to investigate the residues of 11 pharmaceuticals and personal care products (PPCPs) belonging to various therapeutic categories. The selected areas of the study were the municipal and hospital wastewater treatment plants (WWTPs) of Ioannina city, located in Western Greece. The most common pre-treatment technique for pharmaceuticals, solid-phase extraction (SPE), was used for the isolation and pre-concentration of the target analytes. The samples were screened using gas chromatography mass spectrometry (GC–MS). The results of the monitoring study, showed the occurrence of all target compounds in the wastewater samples. Concentrations in the municipal WWTP ranged between 0.3 and 164.4 μg/L in the influent and between 0.5 and 13.9 μg/L in the effluent. In the hospital WWTP concentrations ranged between 0.6 and 70.1 μg/L in the influent and between 0.5 and 14.6 μg/L in the effluent. Mean removal efficiencies ranged between 13% and 97% and between 9% and 87% for municipal and hospital WWTPs, respectively. Removal efficiencies were higher in the municipal WWTP than in the hospital WWTP.

1. Introduction

Interest in the presence of pharmaceuticals and personal care products (PPCPs) in the environment has increased significantly over the last decade. Public interest is quickly increasing because of their potential impact on the environment and possibly human health, even at trace concentrations [1]. The political community has also noticed the danger of pharmaceutical pollution in the environment. Two directives have been written (2001/83/EC for human pharmaceuticals, 2001/82/EC for animal pharmaceuticals) in order to challenge an environmental assessment for the acceptance of new pharmaceuticals on the market [2]. Despite these legislations pharmaceuticals are not yet included in any priority list either in Europe or in US. Moreover, limits and regulation on PPCPs and new compounds have not yet specifically been made for water and wastewater treatment criteria.

There are several direct and indirect pathways through which PPCPs can be introduced into the aqueous environment. Insufficiently treated municipal wastewater discharge is identified as the major route responsible for surface water contamination with PPCPs. As it well known, common conventional methods of treatment (i.e., biological, physical, and chemical methods) have limited success and do not efficiently remove all of the PPCPs [3–6]. Many WWTPs include only two treatment steps (physical and biological) while few of them use a tertiary treatment or an advanced sewage treatment (e.g. ultrafiltration, flocculation, ozonation, advanced oxidation, or osmosis). The later treatments are seldom used because of their high cost. Hence, variable amounts of PPCPs are released to surface, ground and coastal waters depending on the elimination rates in the WWTPs and induce adverse effects in terrestrial and aquatic ecosystem [7].

The removal efficiencies of pharmaceuticals in WWTPs vary depending on the nature of the target pharmaceuticals, the type of wastewater treatment technology implemented, age of the activated sludge, hydraulic retention time, environmental conditions (e.g. dilution of wastewater effluent, rainfall, temperature and level of sunlight) and physical properties including the adsorption capacity of compound on sludge [4,8–10]. Generally, systems with efficient removal of conventional wastewater constituents (BOD_5 and NH_4^+) also have good removal of many PPCPs.

The ubiquitous occurrence of pharmaceuticals as environmental contaminants is demonstrated by reports of their presence in WWTPs influents and effluents around the world at concentrations ranging from a few ng/L to the high μg/L (see Table 1).

Despite the great majority of studies on the occurrence of PPCPs in wastewaters of many European countries only a few references may be found in the literature concerning findings of PPCPs in wastewaters of Greece with most being related to sporadically

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Table 1
Levels of PPCPs in different countries.

Pharmaceutical class	Influent (μg/L) concentration	Effluent (μg/L) concentration	Removal (%)	Reference
Non steroidal/anti-inflammatory				
Salicylic acid	5.866 (mean)/12.674 (mean)	0.164 (mean)/0.075 (mean)	>98	[5]
		0.05/3.6	99	[11]
		0.57–13		[12]
		0.9–12		[13]
		0.554–2.178		[14]
	88.99 (max)	6.73 (max)		[15]
		0.14 (max)		[16]
	0.62/29.06			[17]
	2.566 (mean)	34 (mean)		[18]
	3.125 ± 0.172/1.601 ± 0.105/1.825 ± 0.047	3.522 ± 0.097/1.675 ± 0.070/1.955 ± 0.111		[19]
Ibuprofen	1.681 (mean)/2.294 (mean)	0.263 (mean)/0.143 (mean)		[5]
		7.11 (max)		[10]
	0.54–38.7	0–3.8	12–99	[11]
		1.2–95		[13]
		3.4 (max)		[16]
	4.38/5.09			[17]
	13.228 (mean)	3.090 (mean)		[18]
	1.171 ± 0.064/1.132 ± 0.043/0.827 ± 0.069	0.858 ± 0.040/1.060 ± 0.051/0.609 ± 0.032		[19]
	0.027–7.741	1.979–4.239		[20]
		0.37–0.6	65–90	[21]
	13.1	1.3	92	[22]
	34–168	0.24–28		[23]
	n.d.–0.9	0.04–0.8		[24]
	1.9	0.25	87	[25]
		0.01–0.137		[26]
		01212 (median)		[27]
	6.900 ± 0.9	0.0475 ± 0.0035	99	[28]
	0.381–1.130		>90	[29]
			≥90	[30]
	0.711–17.933	0.313–3.777	22–56	[31]
	9.922 ± 1.177	0.038 ± 0.002	>99	[32]
			>98	[33]
Paracetamol	211.380 (mean)/178.116 (mean)	11.733 (mean)/0.353 (mean)	92/100	[5]
	6.9	0	100	[11]
	0.26 (max)	0.16 (max)/0.42 (max)		[15]
		6.0 (max)		[16]
	10.899 (mean)	0.276 (mean)		[18]
	0.069–6.924	<0.02		[20]
	29–246	<LOD–4.3		[23]
	0.130–26.09	<LOD–5.990		[24]
	0.96	n.d.	>99	[25]
		0.0018–0.019		[26]
	39.300 ± 0.685	0.01 ± 0.001		[32]
		1.78 (max)		[34]
Naproxen	0.838 (mean)/1.173 (mean)	0.370 (mean)/0.170 (mean)	<58/>74	[5]
		1.847 (mean)		[7]
		5.22 (max)		[10]
	0.6–40.7	n.d.–12.5	15–100	[11]
		0.633–7.962		[14]
		0.52 (max)		[16]
	15.22/16.65			[17]
	3.249 (mean)	0.598 (mean)		[18]
	3.934 ± 0.357/0.449 ± 0.040/0.348 ± 0.025	2.579 ± 0.188/0.382 ± 0.044/0.217 ± 0.015		[19]
	4.9	0.84	80	[22]
	<LOD–0.190	<LOD–0.160		[24]
	3.2	0.38	88	[25]
		0.02–0.438		[26]
	4.900 ± 0.48	0.290 ± 0.0010	94	[28]
	0.038–0.230		0–80	[29]
			50–80	[30]
	10.418 ± 1.530	0.090 ± 0.010		[32]
	0.44	0.08/0.30	66–78	[35]
Diclofenac	0.069 (mean)/0.260 (mean)	0.098 (mean)/0.179 (mean)	Not removed	[5]
		0.273–2.134		[7]
	0.35–5	0.17–2.5	0–71	[11]
		0.032–0.457		[14]
		2.1 (max)		[16]
	1.72/6.36			[17]
	0.726 (mean)	0.323 (mean)		[18]
	0.216 ± 0.005/0.026 ± 0.003/0.020 ± 0.001	0.214 ± 0.005/0.020 ± 0.003/0.013 ± 0.001		[19]
	0.901–1.036	0.261–0.598		[20]
		0.06–0.81	69–75	[21]

Table 1 (*Continued*)

Pharmaceutical class	Influent (μg/L) concentration	Effluent (μg/L) concentration	Removal (%)	Reference
	0.35	0.26	26	[22]
	0.2–3.6	0.14–2.2		[23]
	0.050–0.540	<LOD–0.390		[24]
	0.11	0.09	18	[25]
		0.0088–0.127		[26]
	0.230 ± 0.009	0.490 ± 0.055	Not removed	[28]
			20–40	[30]
	0.003–0.437	0.004–0.101	14–80	[31]
			Not removed	[33]
Antihyperlipidemic				
Gemfibrozil		2.366 (mean)		[7]
		4.76 (max)		[10]
	0.3/0.7	0.18–1.3	16–75	[11]
		0.080–0.478		[14]
		1.5 (max)		[16]
	0.68/0.64			[17]
	0.219 (mean)	0.101 (mean)		[18]
		0.31–0.40	46–69	[21]
	<LOD–0.360	LOD–0.320		[24]
	0.41	0.13	68	[25]
		0.0039–0.017		[26]
	1.652 ± 0.112	0.600 ± 0.036		[32]
			>84	[33]
		0.07/0.40	46–96	[35]
Fenofibrate		0.110–2.353		[7]
		0.16 (max)		[10]
		0.03 (max)		[16]
	<LOD	<LOD		[18]
	n.d.	0.38	45–64	[35]
Antiepileptic				
Carbamazepine	1.694 (mean)/0.950 (mean)	2.499 (mean)/0.826 (mean)	Not removed	[5]
	0.7/1.5	0.7/1.5	4–93	[11]
	9.42 (max)	0.97 (max)/2.30 (max)		[15]
		6.3 (max)		[16]
	0.157 (mean)	0.198 (mean)		[18]
	0.701 ± 0.044/0.098 ± 0.011/0.098 ± 0.002	0.656 ± 0.028/0.100 ± 0.005/0.091 ± 0.004		[19]
	0.3–0.5	<LOD–0.3	20	[23]
	<LOD–0.950	LOD–0.630		[24]
		0.073–0.729		[26]
		0.2911 (median)		[27]
	0.015–0.270		<45	[29]
			Not removed	[30]
	0.082–0.357	0.093–0.214	26–40	[31]
	0.057 ± 0.004	0.11 ± 0.007		[32]
			9	[33]
		0.27 (max)		[34]
	0.160–0.820	0.290–2.440	Not removed	[36]
Analgesic/antipyretic				
Phenazone		n.d.–0.37		[10]
		0.41 (max)		[16]
	<LOD	<LOD		[18]
	0.92	0.16/0.52	33	[35]
Sychomotor stimulant				
Caffeine		1.742–8.132		[14]
		3.60 (max)		[15]
	52–192	1.4–44	85/99	[23]
		0.023–0.776	99	[26]
	5.173–17.500	0.019–1.727	85–100	[31]
	25.567 ± 5.710	0.028 ± 0.006		[32]
		7.99 (max)		[34]
Disinfectant				
Triclosan	2.04/0.66			[17]
	0.811 ± 0.054/0.126 ± 0.007/0.102 ± 0.002	0.662 ± 0.59/0.113 ± 0.11/0.055 ± 0.005		[19]
		0.07–0.650	44–92	[21]
	0.39–4.2	0.08–0.40		[23]
	0.8	0.25	69	[25]
		0.0013–0.032		[26]
	0.511 ± 0.243 (mean)		45–93	[29]
		1.6 (max)		[34]
	1.3–37.8	0.4–22.1		[37]

Table 2
Overview of physicochemical properties of studied PPCPs.

Pharmaceutical class	Compound	Molecular formula	MW	pKa	LogK_{ow}	Pv (mm Hg)
Non steroidal/anti-inflammatory	Salicylic acid	$C_7H_6O_3$	138.12	2.97	1.13	8.20E − 05
	Ibuprofen	$C_{13}H_{18}O_2$	206.28	4.91	3.97	1.86E − 04
	Paracetamol	$C_8H_9NO_2$	151.17	9.38	0.46	7.00E − 06
	Naproxen	$C_{14}H_{14}O_3$	230.26	4.15	3.50	1.89E − 06
	Diclofenac	$C_{14}H_{10}C_{l2}NO_2K$	334.23	4.15	4.51	6.14E − 08
Antihyperlipidemic	Gemfibrozil	$C_{15}H_{22}O_{13}$	250.34	4.7	4.77	n.d.
	Fenofibrate	$C_{20}H_{21}ClO_4$	360.83	4.46	5.19	n.d.
Antiepileptic	Carbamazepine	$C_{15}H_{12}N_2O$	236.27	7	2.47	1.84E − 07
Sychomotor stimulant	Caffeine	$C_8H_{10}N_4O_2$	194.20	10.4	−0.07	15
Analgesic/antipyretic	Phenazone	$C_{11}H_{12}N_2O$	188.23	1.5	0.38	3.06E − 05
Disinfectant	Triclosan	$C_{12}H_7Cl_3O_2$	289.50	4.5	4.80	6.45E − 07

detections with no considerations on the WWTP efficiency. For this last point, it is important to study more extensively the occurrence and fate of the most widespread PPCPs in Greek conventional wastewater treatment plants. Furthermore there is a real need for complementary studies such as the PPCP occurrence in wastewater based on a similar wastewater treatment technology.

The primary objective of the research presented in this paper was to verify the occurrence and fate of different classes of PPCPs during wastewater treatment. These were: pharmaceuticals (analgesic/anti-inflammatory drugs, antibiotics, antiepileptics, lipid regulating agents, etc.), personal care products (disinfectant/antiseptics) and the sychomotor stimulant caffeine. The target PPCPs (Table 2) were mainly chosen by their high annual consumption and concern over their possible effects on human and aquatic organisms [38].

An SPE/GC–MS multi-residue analytical method for routine use was developed and validated to facilitate simultaneous determination of these compounds in both influent and effluent wastewater. Two contrasting WWTPs (municipal and hospital WWTPs of Ioannina city, located in Western Greece) utilizing different wastewater treatment processes have been selected for the research in order to better understand factors affecting the occurrence and fate of PPCPs in the environment. An assessment of the efficiency of PPCPs removal for both wastewater treatment technologies was attempted. The impact of treated wastewater hospital effluent on the quality of receiving waters of the WWTP of the city was also investigated. To the author's knowledge, the range of these PPCPs has not been investigated in Greece before, so extensively and comprehensively. The paper also aims to provide a better understanding of the factors affecting the levels of concentration of PPCPs in surface water: i.e. surrounding area and proximity to wastewater effluents. Our results will help to identify the eventual sinks and fates of these compounds and will contribute to an assessment of the human and ecological risk resulting from the unintended presence of these compounds in our environment.

2. Experimental

2.1. Chemicals and materials

All pharmaceutical standards were purchased from Promochem (Wesel, Germany). Individual stock standard solutions were prepared in methanol (1000 mg/L) and stored at −20 °C. For GC–MS analysis, standard mixtures, at different concentrations, were prepared by appropriate dilution of the stock solutions in ethyl acetate. Methanol, ethyl acetate and acetone were supplied from Pestiscan (Labscan, Ltd., Dublin, Ireland) and anhydrous sodium sulfate from Merck (Darmstadt, Germany). Cartridges HR-P (highly cross-linked polystyrenedivilbenzene) were purchased from Chromabond.

2.2. Wastewater sampling and preparation

2.2.1. Sampling

Wastewater samples used in this study were collected from the municipal and hospital wastewater treatment plants (WWTPs) of Ioannina city located in Western Greece. The municipal plant is connected to a sewage system servicing a municipal area with 100,000 inhabitants. This system, as many others in Greece with old infrastructure, still have "combined" sewers that collect both domestic sewage and stormwaters, often deliver sewage volumes during rainstorm events that exceed the treatment capacity of the municipal WWTP. This system can therefore has numerous direct discharges of untreated effluents (Fig. 1). Primary treatment, known as mechanical treatment, consists of a screen, an aerated grit-removal tank and a primary sendimadation tank. The next step is the biological treatment that separates and breaks down organic contaminants, with the aid of microorganisms. After the primary treatment the effluent is directed to the activated sludge system for the removal of phosphorus, denitrification and nitrification. The activated sludge process is used involving recycling of ca. 60% of the sludge giving an average solid retention time (SRT) of 11 days. The HRT of the WWTP, calculated from the flow and the volume of the treatment tanks, varies between 1.52 and 4.05 h depending on seasonal variation and precipitation. The biological step consists of an anaerobic step followed by an anoxic and a larger aerobic decomposition. Phosphorus removal is achieved first with the biological anaerobic step and then with a chemical treatment. In the chemical removal of phosphorus a simultaneous precipitation takes place with the addition of $FeCl_2$ (500–600 lt/day) after the biological step. Then the water is passed through the secondary sedimentation and as a final polishing step the water is passed through a sand filter and is disinfected before it reaches the recipient Kalamas River.

The hospital plant has a capacity of 800 beds and applies a pretreatment (grit-removal), a mix tank, and a biological secondary treatment concluding with disinfection (Fig. 2). The HRT of the WWTP is 6 h. This plant discharges the untreated wastewater into the urban network which eventually makes their way to municipal WWTP and thus the evaluation of its efficiency in order to predict environmental loads on the municipal WWTP has substantial interest. Bearing in mind that in the case examined here the WWTP of Ioannina city discharges the treated water directly into the Kalamas River and WWTP effluents were also major contributors to Kalamas River's flows it is important to assess plant's effectiveness in the context of the effect of WWTP effluent on the quality of river water.

During the study, a monitoring program was carried out for the four seasons over 1-year monitoring period (March 2006–March 2007). Thirty two influent and effluent wastewater samples were collected during the sampling period from both WWTPs. Samples

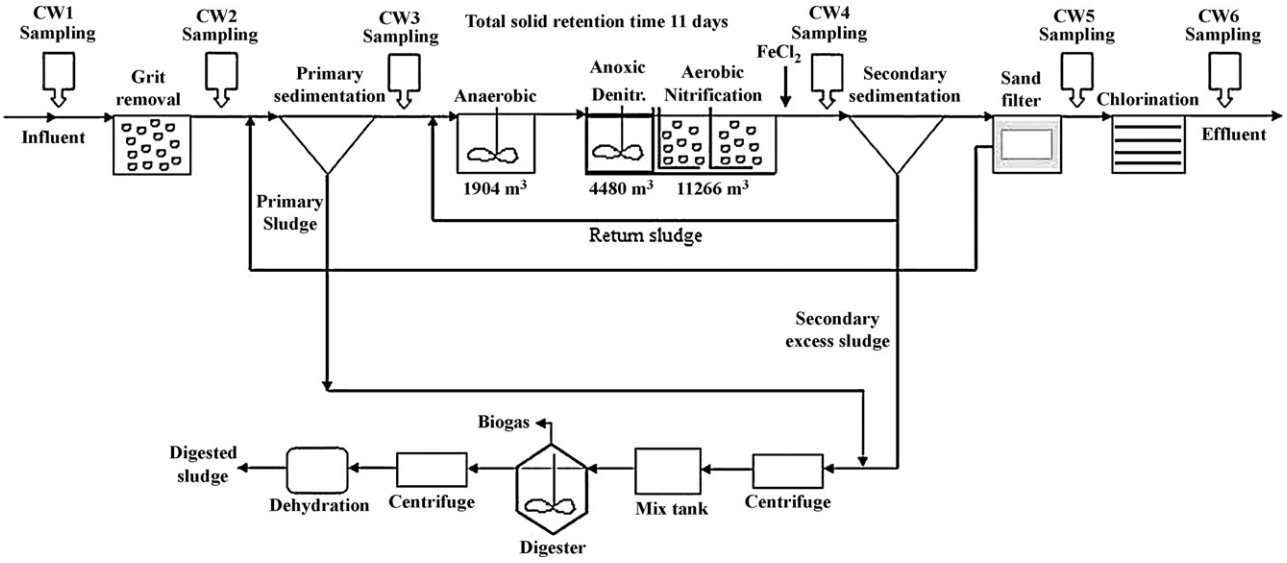

Fig. 1. Scheme of the municipal WWTP of Ioannina city.

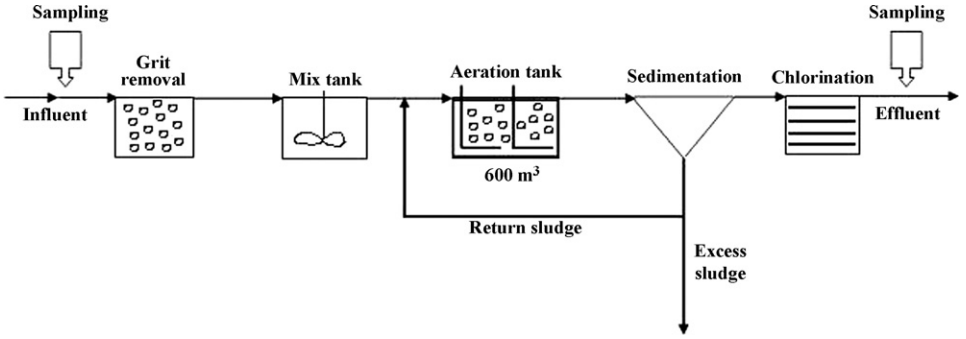

Fig. 2. Scheme of the hospital WWTP of Ioannina city.

have been taken from sewage pipes from hospital inlet and outlet leading to the treatment plant, as well as at the inlet, the outlet, and between different treatment steps within the municipal WWTP, giving additional and useful information on the effects of the different treatment steps on the removal of PPCPs. Overall rates were monitored as well. There were six sampling stations at the municipal WWTP and two at the hospital WWTP. The sampling stations of the municipal WWTP were: (1) influent (CW1), (2) after grit-removal – before primary sedimentation (CW2), (3) after primary sedimentation – before biological step (CW3), (4) after biological step – before secondary sedimentation (CW4), (5) before chlorination (CW5), (6) effluent (CW6). The sampling stations of the hospital WWTP were influent and effluent. In order to estimate the removal efficiencies of the PPCPs in the municipal WWTP, composite samples were collected in three consecutive days of May, July, October and December and the removal efficiencies were calculated as a mean value. Daily composite samples were obtained by mixing sample volumes collected every 6 h during 24 h. Sample volumes collected each 6-h period were proportional to influent and effluent flows. For the hospital WWTP the raw influents and final effluents were collected as grab samples taking into account the hydraulic retention time of this WWTP. Weeks without significant rainfall were chosen in order to avoid dilution by rainwater below the limit of quantification (LOQ). All samples were collected in 1 L amber clean glass bottles and kept cooled during the transportation to the laboratory, where they were stored at 4 °C until analysis. The sample holding time was less than 48 h. The samples were filtrated and acidified to pH 3 to enhance trapping of the acidic compounds on the solid-phase extraction (SPE) sorbent.

Some of the operational parameters of the WWTPs studied and the values of wastewater characterization parameters measured during the monitoring period for both WWTPs are depicted in Table 3.

2.2.2. Sample preparation

Wastewater samples were collected by using pre-rinsed amber glass bottles. After collection, samples were filtered through a 0.7 μm glass fibre filters (Whatman, UK) prior to analysis, in order to remove particles that may interfere during the extraction procedure and the pH was adjusted to 3. Subsequent extraction of solid matter retained by the 0.7 μm filter with dichloromethane did not show any presence of analytes of interest [39]. Filters and samples were stored in the dark at 4 °C and extracted within 48 h in all the cases.

Isolation of the pharmaceuticals from the water samples were performed off-line, using a standard SPE-system connected to a vacuum pump. Extraction cartridges were pre-conditioned with 6 mL of ethyl acetate, 6 mL of methanol and 6 mL of distilled water adjusted to pH <3 and without letting the cartridge become dry, sample aliquots of 100 mL were passed through the cartridges at a flow rate of 10 mL/min. At the end of percolation, Erlenmeyer flasks were washed with 3 mL × 15 mL of acidified water, which are also passed through the cartridge. Next the cartridges were dried under vacuum for 10 min. The analytes were eluted with 2 mL × 5 mL of ethyl acetate at 1 mL/min. The extracts were dried over anhydrous sodium sulphate and then under a gentle stream of nitrogen. The final volume extract was 100 μL. After that, they were stored at −20 °C until being analyzed by GC–MS.

Table 3
Characterization parameters (mg/L), in influent and effluent wastewater from each of the evaluated WWTPs over the monitoring period.

WWTP	Influent			Effluent		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
City						
pH	7.11 ± 0.10	7.58 ± 0.13	7.36 ± 0.10	7.25 ± 0.19	7.77 ± 0.24	7.55 ± 0.15
BOD5 (mg/L)	567 ± 319	3130 ± 1203	1636 ± 576	4 ± 2	24 ± 10	12 ± 4
COD (mg/L)	840 ± 472	4719 ± 1818	2441 ± 883	7 ± 4	35 ± 12	17 ± 6
NO_3 (mg/L)	1.3 ± 0.64	7.4 ± 3.11	3.0 ± 0.49	1.2 ± 0.52	5.4 ± 1.10	3.2 ± 1.05
PO_4 (mg/L)	1.7 ± 0.49	7.8 ± 2.36	3.9 ± 1.18	0.2 ± 0.18	1.6 ± 0.48	0.7 ± 0.31
TSS (mg/L)	1071 ± 584	3729 ± 552	2364 ± 779	3 ± 2	16 ± 6	8 ± 2
Hospital						
pH	7.56 ± 0.12	7.92 ± 0.17	7.80 ± 0.10	7.23 ± 0.17	7.62 ± 0.20	7.40 ± 0.15
BOD5 (mg/L)	121 ± 42	160 ± 72	139 ± 57	4 ± 1	11 ± 4	6 ± 2
COD (mg/L)	278 ± 103	588 ± 197	414 ± 253	8 ± 3	41 ± 19	21 ± 8
NO_3 (mg/L)	15 ± 4	28 ± 9	21 ± 4	14.2 ± 2.3	38.4 ± 6.8	20.6 ± 4.3
PO_4 (mg/L)	1.8 ± 0.78	7.43 ± 2.95	4.65 ± 1.98	2 ± 1	7 ± 2	5 ± 2
TSS (mg/L)	562 ± 201	1093 ± 435	1023 ± 560	147 ± 54	512 ± 197	312 ± 135

2.3. GC–MS analysis

A GC–MS, QP 5000 Shimadzu equipped with capillary column DB-5-MS, 30 × 0.25 mm × 0.25 μm, contained 5% phenylmethylpolysiloxane (J&W Scientific) was used at the following chromatographic conditions: Injector temperature 240 °C, oven temperature program: 70 °C (2 min) to 250 °C (5 min) at 10 °C/min and finally from 250 to 280 °C (10 min) at 6 °C/min. Helium was used as the carrier gas at 1.0 mL/min. The interface was kept at 290 °C and the spectra were obtained at 70 eV. To achieve better detection limits and enhanced selectivity subsequent SPE analyses were performed in the selected ion monitoring mode (SIM). Three ions (*m*/*z*) were selected from the spectrum to quantify the response under SIM mode. The presence of these three characteristic masses at the correct retention time and with correct relative ion intensities was considered as valid confirmation criterion. Diagnostic ions and quantification masses used in the study are shown in Table 4.

2.4. Analytical determination of PPCPs in wastewaters

One significant drawback in water analysis is what is known as matrix effects [24]. In order to achieve low detection limits when we have to deal with complex matrices, we must develop sensitive and selective analytical methods which are usually time and labor consuming procedures. The two most common methods of quantifying pharmaceuticals in wastewaters involve the use of GC–MS and LC–MS. In both cases, the pharmaceuticals usually are extracted from water by SPE using one (or sometimes two) of several different commercially available sorbents. For LC–MS methods sample clean-up or selective extraction may be used to control interference from organic matter. For GC–MS methods, derivatization is usually applied in polar analytes prior to analysis in order to reduce their polarity [40]. However, in the context of routine work the usual but extensive and time-consuming derivatization step is one of the major drawbacks of GC–MS, especially when working with complex matrices such as wastewater samples. Some of the major drawbacks of derivatization are: the dependence of various experimental parameters, the incompleteness of derivatization reactions, the analyte degradation, the prolonged analysis time, the reduction of resolving power of the column and the additional cost for derivatization system and reagents. Therefore, methods that employ GC–MS without derivatization are attractive to many researchers since the instruments are usually less expensive and easier to operate compared to LC–MS systems. Moreover, GC–MS systems often are available in private or research laboratories that routinely perform other environmental analyses. Its high resolution and the fact that allows exact identification combined with structural information can be considered as other advantages.

Table 4
Diagnostic (*m*/*z*) ions of the 11 PPCPs in the GC–MS system.

Pharmaceuticals	Diagnostic (*m*/*z*) ions		
Salycilic acid	92	120	138
Ibuprofen	161	163	107
Paracetamol	109	151	80
Caffeine	194	109	55
Phenazone	188	281	96
Gemfibrozil	122	107	129
Naproxen	185	230	
Triclosan	288	289	218
Fenofibrate	121	232	139
Diclofenac	214	242	295
Carbamazepine	193	236	165

In the present study, the SPE/GC–MS analytical method allowed the simultaneous determination of the 11 selected compounds in wastewaters. The method provided precise identification and quantification of the target compounds in a simple and rapid way, in order to be easily used in routine analysis. Although polar drugs were included in the analysis, the usual derivatization step was avoided. Lower sensitivity which derives from the direct injection of the samples without derivatization was compensated by using 3 μL sample injection volumes [23]. As a consequence of thermal degradation under injection conditions, a typical degradation of carbamazepine to iminostilbene was observed. This factor cannot be avoided and has as a result the degradation of the chromatographic analysis. However, validation studies showed an acceptable repeatability and reproducibility of the carbamazepine peak (around 12%), despite degradation, and the LOD (16.7 ng/L) was low enough to reach the concentration levels present in the samples. There so, results of carbamazepine were finally included in the paper [23]. In addition to carbamazepine, diclofenac and gemfibrozil were also showed analytical discrepancies during the preparation and analysis step. An artifact, identified as 1-(2,6-dichlorophenyl) indolin-2-one (main ions with *m*/*z*, 214, 242, 277) was observed for diclofenac. The formation of this artifact in water samples has been first reported by Reddersen and Heberer [41], who suggested that is matrix-dependent and is likely formed in the acidification step of the sample preparation. Apart from the matrix sample, other processes, such as natural occurring photochemical reactions would also contribute to the conversion of the parent drug to the above compound [42]. Although, according the work of Reddersen and Heberer [41], the additional quantification of this artifact and the addition of the respective result to the total

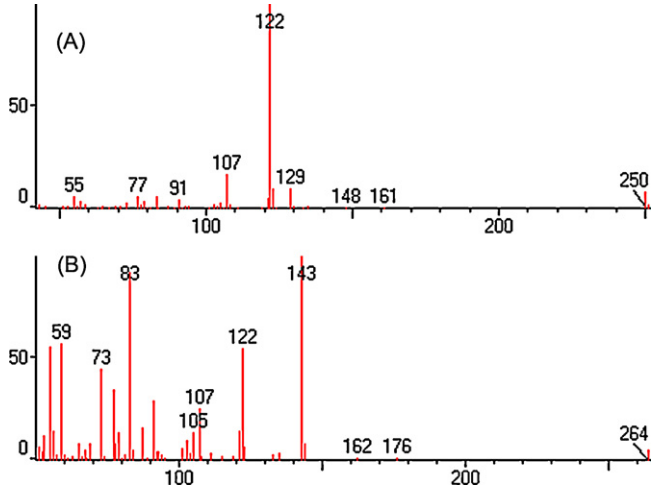

Fig. 3. Mass spectrum of the gemfibrozil (A) and gemfibrozil artifact (B) recorded with GC–MS in the full scan mode using electron impact ionization at 70 eV.

amount of diclofenac is suggested for more precise quantification of the parent compound, this was not followed in the present study in order to supersede overload problems of the total determined amount of diclofenac by concentrations come from artifact which additionally formed in the environment via sunlight irradiation. As far as gemfibrozl is concerned, a second analyte peak was detected in some of the chromatograms. The mass spectra of the unknown peak and of gemfibrozil is depicted in Fig. 3. Further study is needed in order to clarify the conditions under of which this artifact is formed. However, as in the case of carbamazepine, validation studies showed an acceptable repeatability and reproducibility of the parent peak and thus results of gemfibrozil were finally included in the paper.

2.5. Quality control

Identification and confirmation of the target compounds was based on the quality control procedures established by the EU. Thus, identifications of pharmaceuticals in wastewater samples were made by comparing the retention time, identifying the target and qualifier ions, and determining the qualifier-to-target ratios of the peak in the wastewaters with that of a pharmaceutical standard. Acceptance criteria for positive identification consisted of retention times within (0.50 min of the expected) value and % qualifier-to-target ratios within 20% of the standard (0.1 mg/L) for qualifier-to-target abundance percentages greater than 50%. For less than 50%, the criterion for the qualifier-to-target ratios was set at 30% of the calibration standard.

Internal quality control was applied in every batch of samples in order to check if the system is under control. This quality control implies a matrix-matched calibration, a reagent blank, a matrix blank and a spiked blank sample at 0.5 μg/L in order to evaluate stability of the proposed method with time.

With each batch of 12 samples, a five-point calibration curve was prepared for analytes concentrations between the LOQs and 10 LOQs by injections before and after those of the sample extracts. In addition, two quality control (QC) samples were injected in every batch of samples. The QC samples were blank wastewater sample fortified at LOQ level and 10 times the LOQ level. Blanks were subtracted and recoveries taken into account for concentration calculations.

3. Results and discussion

3.1. Validation studies

The limit of detection (LOD) and limit of quantification (LOQ) of the methods were determined from the injection of spiked water samples and calculated as the minimum detectable amount of analyte with a signal-to-noise ratio of 3:1 and 10:1, respectively. Any peak above the LOQ was quantified (Table 5) [17]. LODs in distilled water were between 12.9 and 143.0 ng/L and in wastewaters from 14.5 to 184.1 ng/L.

Precision of the chromatographic method, determined as relative standard deviation (RSD), was obtained from the repeated injection (five times) of a spiked extract during the same day (repeatability) and in different days (reproducibility) [23] (Table 5). The recovery studies ($n = 3$) were carried out by spiking samples at two concentration levels of 0.5 and 5 μg/L. Recoveries were determined for distilled water and wastewaters (Table 6). Mean recoveries in distilled water ranged from 75.2 to 101.3% at 0.5 μg/L and from 87.2 to 107.2% at 5 μg/L. In the wastewaters, recoveries varied in the influent from 44.3 to 101.2% and in the effluent from 47.1 to 95.2% at 0.5 μg/L. At 5 μg/L recoveries varied in the influent from 45.7 to 95.6% and in the effluent from 51.6 to 105.2%. Paracetamol presented lower recoveries than the other compounds. Despite these low recoveries, the other validation data, such as repeatability and limit of detection are good, and therefore a reliable determination of this compound is feasible. Quantification was performed by using matrix-matched calibration solutions prepared by spiking sewage extracts.

3.2. Concentrations of PPCP compounds in wastewater samples

The target analytes were investigated attempting to evaluate their occurrence and fate in municipal and hospital wastewater treatment plants (WWTP) of Ioannina city, located in Western Greece.

Table 5
Limits of detection (LOD), limits of quantification (LOQ), RSD in the same day (RSD_r) and RSD in different days (RSD_R) in distilled water and wastewater (effluent of municipal WWTP).

Compound	LOD (ng/L)	LOQ (ng/L)	RSD_r (%) ($n = 5$)	RSD_R (%) ($n = 5$)	LOD (ng/L)	LOQ (ng/L)	RSD_r (%) ($n = 5$)	RSD_R (%) ($n = 5$)
	Distilled water				Wastewater			
Salicylic acid	143.0	429.1	5.2	12.2	184.1	552.3	8.3	14.2
Ibuprofen	18.2	54.5	4.1	8.7	19.3	57.9	6.7	11.4
Paracetamol	21.8	65.4	4.9	5.6	35.0	105.0	7.3	8.6
Caffeine	12.9	38.8	2.3	9.2	14.5	43.5	5.8	7.3
Phenazone	32.0	96.0	9.8	10.1	43.4	130.2	12.4	13.1
Gemfibrozil	17.9	53.8	5.6	9.6	21.4	64.2	8.3	11.2
Naproxen	30.3	90.9	3.2	7.5	43.7	131.1	5.4	9.1
Triclosan	43.0	129.0	7.4	8.2	43.6	130.8	9.3	11.5
Fenofibrate	101.0	303.0	9.3	10.6	109.7	329.1	8.9	16.3
Diclofenac	90.8	272.3	6.3	13.3	126.2	378.6	11.3	12.4
Carbamazepine	16.2	48.6	4.7	9.4	16.7	50.1	6.3	11.4

Table 6
Mean recoveries (%) and RSD (%) obtained for each water sample matrix after spiking with 0.5 and 5 μg/L (n = 3) in distilled water and wastewater (influent and effluent of municipal WWTP).

Compound	Recoveries after spiking with 0.5 μg/L (RSD%)			Recoveries after spiking with 5 μg/L (RSD%)		
	Distilled water	Wastewater influent	Wastewater effluent	Distilled water	Wastewater influent	Wastewater effluent
Salicylic acid	99.2 (9.3)	85.2 (6.4)	77.2 (6.7)	107.2 (4.2)	90.4 (8.3)	85.5 (6.3)
Ibuprofen	87.7 (6.5)	95.6 (7.3)	84.6 (7.1)	99.3 (4.1)	71.4 (9.8)	105.2 (7.0)
Paracetamol	79.2 (7.4)	44.3 (8.2)	47.1 (9.6)	87.2 (4.3)	45.7 (10.1)	51.6 (7.2)
Caffeine	87.4 (5.3)	91.2 (7.1)	82.3 (7.5)	99.1 (6.4)	95.6 (10.8)	85.3 (5.3)
Phenazone	91.5 (4.2)	60.3 (12.4)	65.9 (10.9)	89.7 (4.8)	65.2 (9.7)	74.9 (8.4)
Gemfibrozil	94.0 (8.3)	76.1 (8.6)	81.4 (7.4)	91.3 (6.7)	79.4 (8.5)	84.5 (9.1)
Naproxen	75.2 (6.1)	101.2 (3.5)	50.8 (3.5)	89.8 (7.2)	69.3 (11.2)	64.1 (9.3)
Triclosan	86.2 (7.6)	97.3 (6.8)	88.3 (5.4)	94.7 (5.6)	90.1 (5.6)	91.2 (6.8)
Fenofibrate	78.4 (7.3)	70.5 (6.1)	73.2 (9.4)	87.2 (7.3)	75.4 (9.3)	84.6 (11.7)
Diclofenac	101.3 (6.6)	82.4 (9.7)	72.4 (9.6)	96.3 (6.3)	92.1 (5.4)	85.0 (9.4)
Carbamazepine	80.8 (9.4)	99.3 (8.6)	95.2 (7.2)	99.5 (4.0)	90.6 (6.3)	87.3 (4.8)

Generally, the frequency of quantification in the influents of the two WWTPs was above 50% for the majority of the target PPCPs. Salicylic acid, ibuprofen, caffeine, paracetamol and gemfibrozil were detected in 100% of the analyzed samples for both WWTPs. Mean dissolved concentrations in the influent range from 0.3 μg/L for phenazone (quantified in 6% of the influent samples) to 86.8 μg/L for salicylic acid (quantified in 100% of the influent samples) in the case of municipal WWTP and from 0.6 μg/L for fenofibrate (quantified in 6% of the influent samples) to 45.3 μg/L for salicylic acid (quantified in 100% of the influent samples) in the case of hospital WWTP. These influent concentrations depend mainly on the degree of prescription and human metabolization.

For naproxen, hospital influent concentrations (mean 11.6 μg/L) were significantly higher than in the influent (mean 1.5 μg/L) of the municipal WWTP. For gemfibrozil, diclofenac and carbamazepine hospital influent concentrations were higher or ranged in the same order as for municipal influent concentrations. Comparison of influent concentrations of both WWTP demonstrate that for salicylic acid, ibuprofen, paracetamol and caffeine the municipal influent concentrations were higher than those of hospital influent concentrations indicate that municipal WWTP, a large wastewater plant serving over 100,000 inhabitants, receives sewage with higher loads of these PPCPs than hospital WWTP.

Salicylic acid, paracetamol and ibuprofen presented the highest concentrations in the influents, with mean concentrations of 86.8, 20.6 and 12.5 μg/L, respectively for municipal WWTP and 45.3, 9.3 and 7.8 μg/L, respectively for hospital WWTP. Paracetamol is excreted mainly as conjugates which can undergo hydrolysis during wastewater treatment resulting in the release of the parent compound. Levels of the PPCP detected in the influents (Tables 7 and 8) are consistent with the ones found in other raw wastewaters in the European Union [15,11,23,39].

Santos et al. [1] presented higher mean concentrations for ibuprofen (69.7–115 μg/L) in Spain, while Kasprzyk-Hordern et al. [43] presented lower concentrations for salicylic acid (17.461 μg/L) and higher concentrations for paracetamol (492.340 μg/L) in Wales. Salicylic acid may be a metabolite of acetylsalicylic acid (aspirin), but there are several other possible sources of salicylic acid, since is also a widely used additive in cosmetics and foodstuff and occurs naturally in the environment [14]. High concentrations of salicylic acid and paracetamol can be explained by the high number of these pharmaceuticals dispensed in Greece. Apart from the high application level their ubiquitous persistence can be explained by the fact that most of them can be purchase without prescription. In Greece, aspirin and paracetamol are freely available over-the-counter and are two of the most popular first line analgesics.

Ibuprofen showed a variability of influent concentrations (i.e., 2.8–25.4 μg/L for municipal WWTP and 7.0–8.9 μg/L for hospital WWTP), but these concentrations were slightly higher or in the same order than those reported in other European countries such as Sweden [28,44] and Finland [22], but much lower than the ibuprofen concentration reported in Spain [1,39,45]. Discrepancies in ibuprofen concentration could be associated with different consumption rates from country to country. Furthermore, variations in excretion rates, strongly affected by an individual's sex, age, hypoxaemia, nutrition, and thyroid function [46], among other factors, further embarrass comparison. Ibuprofen has a high metabolic rate in humans (low excretion rate as parent compound) and exhibits half-live of less than 1–2 days depending on external factors (e.g., temperature and radiation) [47,10] and these factors could also affect its occurrence.

Another compound also identified as a major constituent in influents is the stimulant caffeine with the highest concentration level of 74.9 and 25.8 μg/L, for municipal and hospital WWTP, respectively. Caffeine was finally included in our survey because

Table 7
Range and mean concentrations (μg/L) detected for PPCPs in the influents and effluents of municipal WWTP.

Pharmaceuticals	Influent		Effluent	
	Range	Mean	Range	Mean
Salicylic acid	34.0–164.4	86.8	2.9–10.1	5.4
Ibuprofen	2.8–25.4	12.5	0.5–2.6	1.5
Paracetamol	4.7–52.5	20.6	0.5–1.7	0.9
Caffeine	17.1–113.2	74.9	1.9–13.9	7.9
Phenazone	n.d.–0.3	0.3	–	–
Gemfibrozil	0.7–3.3	1.6	Bql–1.3	0.7
Naproxen	n.d.–2.0	1.5	n.d.–0.7	0.5
Triclosan	n.d.–1.0	0.8	n.d.–bql	–
Fenofibrate	n.d.	–	n.d.	–
Diclofenac	n.d.–3.9	2.0	n.d.–2.6	1.3
Carbamazepine	n.d.–1.1	0.8	n.d.–1.1	0.9

Table 8
Range and mean concentrations (μg/L) detected for PPCPs in the influents and effluents of the hospital WWTP.

Pharmaceuticals	Influent		Effluent	
	Range	Mean	Range	Mean
Salicylic acid	23.4–70.1	45.3	4.9–14.6	9.4
Ibuprofen	7.0–8.9	7.8	0.5–0.9	0.6
Paracetamol	3.1–21.2	9.3	1.3–7.4	3.6
Caffeine	12.3–42.0	25.8	3.1–10.6	6.5
Phenazone	n.d.–2.5	0.8	n.d.–0.7	0.7
Gemfibrozil	1.1–7.3	2.7	0.5–1.7	1.0
Naproxen	n.d.–21.8	11.6	n.d.–10.0	3.8
Triclosan	n.d.	–	n.d.	–
Fenofibrate	n.d.–0.6	0.6	n.d.	–
Diclofenac	n.d.–6.3	2.9	n.d.–6.5	3.4
Carbamazepine	n.d.–1.7	1.0	n.d.–1.9	0.7

it is ranked number one drug worldwide and is commonly found in coffee, tea, cocoa, soft drinks, chocolate, dairy desserts and it is also a component of hundreds of prescription and over-the-counter drugs [48]. Its widespread occurrence in wastewater, surface water and groundwater worldwide has led it to be considered as a human-derived marker for wastewater contamination of natural water. From coffee alone, an average human consumes 131 mg of caffeine per day. However, only 3.9 mg caffeine excreted unchanged in the urine since is extensively metabolized by humans [49]. The concentration levels detected are, in general, similar with those presented in other reports [39]. In contrast, Santos et al. [1] reported lower mean concentrations for caffeine (4.87–7.37 μg/L) than those discussed here.

Gemfibrozil was also one of the most often detected compounds, present in all samples and found in concentrations up to 3.3 and 7.3 μg/L, in municipal and hospital WWTP, respectively. As far as triclosan is concerned only limited data is available for the occurrence and fate of this personal care product during wastewater treatment. It was detected only in the municipal influents with concentrations up to 1.0 μg/L. Recent surveys on the occurrence of triclosan in wastewaters have shown the compound to be present at detectable concentrations in the investigated samples. For triclosan, similar concentrations have been reported by Kasprzyk-Hordern et al. [43], while higher concentrations have been reported by Agüera et al. [37].

As reported elsewhere, we found that carbamazepine, which is metabolized to a very high degree in humans and has a logK_{ow} value of 2.47, is relatively survived the treatment process and sometimes emerged with even higher effluent concentrations. These findings for carbamazepine may be attributed to the excretion of glucuronides which may act as a reservoir from which a later yield of the parent substance can occur. Similar carbamazepine concentrations have been reported in Finland [36], lower in Spain [1] and higher in Wales [43].

Mean influent concentrations of naproxen were 1.5 and 11.6 μg/L, for municipal and hospital WWTPs, respectively. Similar results have been reported by Fent et al. [11]. The lower concentration levels and the less frequent detection of naproxen in the municipal influents is possibly due to its vulnerability to biodegradation and photodegradation in the environment [50,51].

Diclofenac concentrations appear to be greater than those previously reported for Greek WWTPs (up to 560 ng/L) by Koutsouba et al. [52] and mean concentrations were 2.0 and 2.9 μg/L for municipal and hospital WWTPs, respectively. These data may reflect differences in sampling time campaign or in the type of the sewage treatment process among Greek municipalities. Elimination rates were found to be sensitive to changes in operating conditions (temperature, flow rate, etc) and can vary significantly from plant to plant and in one plant at different time periods. Recent surveys on the occurrence of diclofenac in WTW influents have shown the compound to be present at relative high concentrations in most of the samples collected [17]. A decrease of diclofenac concentration in municipal WWTP effluents was observed for the sampling campaign in summer period, probably due to the high light intensities at this season and consequently the higher photodegradation efficiency [50].

Fenofibrate was detected in only one influent sample of hospital WWTP system in concentration of 0.6 μg/L. The absence of this compound in wastewater samples is attributed to its rapid hydrolysis to fenofibric acid which is further accelerated by acidification of wastewater samples [53]. Phenazone showed the lowest concentrations (i.e., 0.3 μg/L) among the pharmaceuticals analyzed. As in the case of fenofibrate it was detected only in sample of hospital WWTP at sampling campaign of winder period. Similar concentrations have been reported for phenazone (0.92 μg/L) by Beausse [35]. The absence of phenazone in the municipal influents may suggest its limited use in Greece. Overall, the difference in the occurrence of the acid pharmaceutical residues in this work from reports in literature may mirror the different usage patterns of these drugs in Greece from Europe and US.

The target compounds were not entirely eliminated by the applied treatment processes and many of them were ubiquitous in the effluent samples. As for the influents, salicylic acid, ibuprofen, caffeine, paracetamol and gemfibrozil were detected in 100% of the effluents of the two WWTPs (Tables 7 and 8). In spite of the fact that salicylic acid, ibuprofen, caffeine and paracetamol exhibited high (>75%) removal efficiency, yet due to the high influent concentrations of these PPCPs, appreciable amounts of these compounds were found in the municipal WWTP effluent. The lowest effluent concentrations quantified (<1.0 μg/L level) are found for fenofibrate, triclosan, phenazone, gemfibrozil and paracetamol and the highest measured concentration (above 5 μg/L) are recorded for ibuprofen, salicylic acid and caffeine. Similar concentrations for the latter compounds have been reported by other authors [12,34,23]. Paracetamol was detected in maximum municipal effluent concentration of 1.7 μg/L similar to those reported by Glassmeyer et al. [34]. Concentrations of diclofenac (0.4–6.5 μg/L) and naproxen (0.3–10.0 μg/L) in hospital effluents are consistent with previously reported concentrations by Andreozzi et al. [10] in WWTPs of France and Italy. Carbamazepine was present in the WWTP influent at relatively low concentrations (compared with the previous mentioned compounds), but its low biodegradability and poor elimination rate throughout the WWTP (<19%) makes its presence recurrent in treated effluents, at concentrations between 0.7 and 1.1 μg/L and 0.4 and 1.9 μg/L for municipal and hospital WWTP, respectively [23,26,16]. Gemfibrozil was detected in lower concentrations with mean concentrations of 0.7 and 0.1 μg/L, for municipal and hospital WWTP, respectively [16,26].

Among the PPCPs that have been detected in hospital wastewater effluent, phenazone was detected in very low concentrations in October (below LOQ) and December (0.7 μg/L). The concentrations of this substance in winter period were in the same order of magnitude than published by other authors [10]. Triclosan was detected in only two samples at concentrations of below LOQ, which is a result of the biodegradation and its adsorption in the sludge (relatively high partition coefficient logK_{ow} = 4.8) [39]. Relative high degradation efficiencies (58–93%) during activated sludge treatment have been reported for triclosan by many researchers [44,25,39,54] which is consistent with the concentration levels presented in this study.

Taking into account the concentration levels in effluents we can conclude that anthropogenic impact in Kalamas River and streams receiving effluents from WWTP of Ioannina city is evidenced by the occurrence of PPCPs [55,56]. Although several of the studied compounds are removed with relatively high efficiency in WWTP, their occurrence in surface waters maybe related to leaking sewers and combined sewer overflows. Thus, the combined sewer overflows and leaking sewers should be considered in the evaluation of surface water quality and additional research is needed on attenuation mechanisms to related measured concentrations in surface waters.

3.3. Temporal evolution of the PPCP compounds during the sampling period

The results of the four sampling campaigns at the WWTP of the city showed that during the spring and summer season two times higher loads of caffeine were observed than in autumn and winter. Concentration of caffeine increases from a mean value around 34.6 μg/L during October–December to concentration values in the range from 99.5 to 121.6 μg/L during May–July. This seasonal effect arises, because the consumption of coffee or beverages is

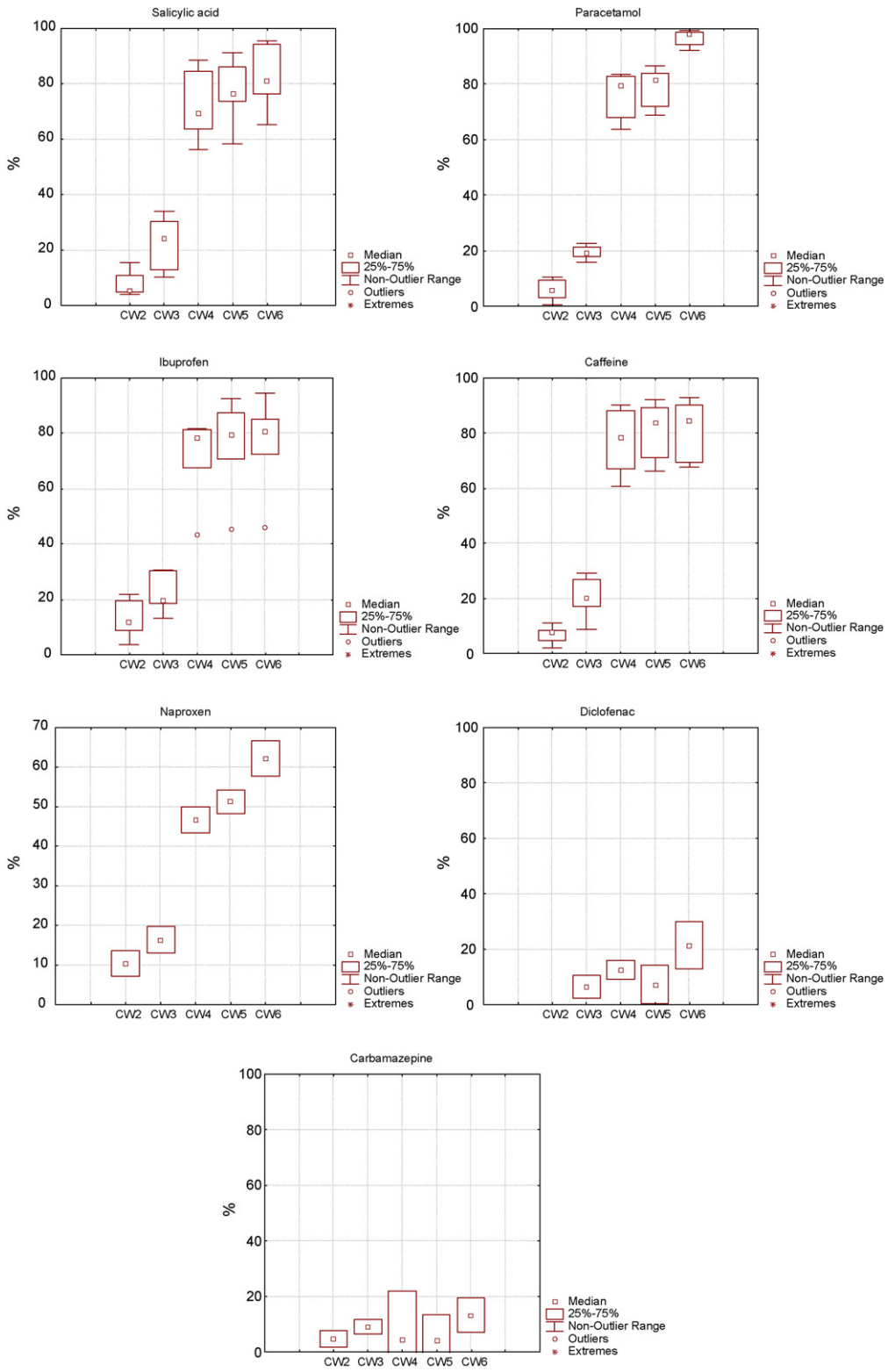

Fig. 4. Box-and-Whisker graphs of the removals of the PPCPs in each of the six sampling stations of municipal WWTP.

much higher during the summer period when the daylight conditions in Greece are elevated. Temporal trend was also observed for diclofenac the concentration levels of which were higher during the winter period, with the higher application rates. Based on the detected seasonal differences and eventual varying concentrations in influents of both WWTPs, we can infer that such variations must be considered for environmental exposure assessments.

As far as the seasonal variation of the rest pharmaceuticals is concerned there was not detected any significant difference between the sampling months. This may attributed to the fact that the analyzed pharmaceutical compounds belong to those thera-

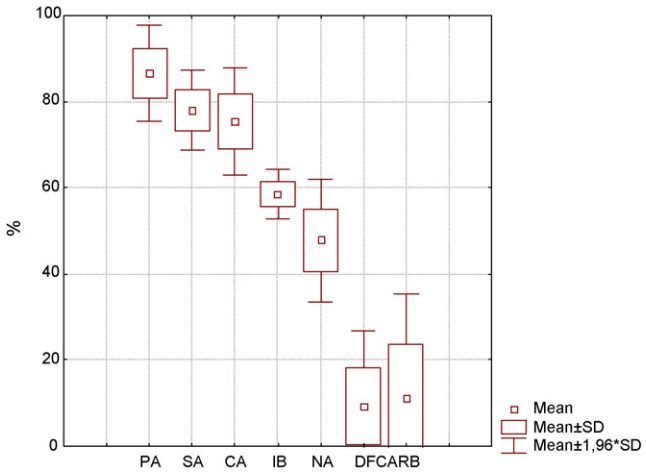

Fig. 5. Box-and-Whisker graphs of the removals of the PPCPs in hospital WWTP (PA = Paracetamol, SA = Salicylic acid, CA = Carbamazepine, IB = Ibuprofen, NA = Naproxen, DF = Diclofenac, CARB = Carmbamazepine).

peutic categories that are used for therapeutic reasons all over the year.

Application of the method was limited to target compounds only. Additional work should be performed to elucidate levels of conjugated or metabolic forms of the active compounds. It would also be advantageous to determine levels of parent compounds present in sewage sludge, in order to determine levels of sorption.

3.4. Removal of PPCP compounds

The extent to which organic micro-pollutants are removed in wastewater unit processes will be influenced both by the biodegradability and physicochemical properties (most notable their water solubility, hydrophobicity, and tendency to volatilize) of the compound in question and of the unit treatment process employed at the WWTP itself. Physicochemical properties of pharmaceutical residues are summarized in the Table 2. These properties will influence whether a compound will remain in the aqueous phase (like many of the acidic pharmaceuticals) or interact with solid particles, such as triclosan, which has a higher potential to be adsorbed to sewage sludge. The removal efficiencies in the present study were calculated as the percentage of reduction between the dissolved aqueous phase concentration of the contaminant in the influent and the dissolved aqueous phase concentration of the contaminant in the effluent.

Figs. 4 and 5 show a Box and Whisker graph of the removal efficiencies of the pharmaceutical compounds in the two WWTPs (municipal and hospital) during the monitoring period. Lines in each box show the first (lower) and the second (upper) quartile of the concentration values for each pharmaceutical compound. The box shows the mean ± SD concentration and the point inside each box shows the mean concentration. The whiskers or lines outside each box show the mean ± 1.96*SD concentrations of the pharmaceuticals. This study showed a high variability of concentrations (up to a factor 4) and of removal rates during the week. Thus, to obtain reliable results wastewater samples were analyzed for three consecutive days of May, July, October and December and the removal efficiencies were calculated as a mean value. However, in some cases there was not possible to estimate the removal efficiencies of some pharmaceuticals, due to the fact that they were not detected in the three consecutive days of analysis (fenofibrate, phenazone), or their concentration was below the LOQ (gemfibrozil, triclosan). In some sampling campaigns during the week, some of the compounds either were not detected in the influent (carbamazepine) or the sum of their effluent loads was greater than the load observed in the influent (naproxen, diclofenac, carbamazepine). Because of these problems, the removal efficiencies for these compounds were estimated based on the measured concentrations of one or the two days (carbamazepine, naproxen and diclofenac).

Mean removal efficiencies ranged between 13% and 97% and between 9% and 87% for municipal and hospital WWTPs, respectively. The highest mean removals were reported for paracetamol (97%) and salicylic acid (82%), while the lowest for carbamazepine (11%) and diclofenac (9%).

Paracetamol probably undergoes a rapid biodegradation during wastewater treatment. Furthermore, it readily reacts with free chlorine as revealed by controlled studies conducted by Boyd et al. [57] and probably due to these effects the concentration levels of this compound in effluents were drastically reduced leading to elevated elimination efficiencies. Significant removals (75–82%) can also be seen for salicylic acid and caffeine in both WWTPs.

Ibuprofen was removed in <77% in the case of municipal WWTP and in <58% in the case of hospital WWTP (Fig. 5). These results agree closely with those of other researchers [16,58,22,59], who also found relative high removal efficiencies in secondary WWTPs studied in Spain [60], Italy and Tokyo [29]. However, lower efficiencies of removal of ibuprofen (<30%) were reported at WWTPs with shorter solid retention time (SRT) [59] and hydraulic retention time (HRT) [9]. Findings derived from the previous reported studies [9,59] and from controlled laboratory studies revealed that SRT is one of the most critical factors for the removal of ibuprofen in WWTPs. Complete removal of ibuprofen have been reported for activated sludge facilities with longer SRTs (SRT > 50 days) [9,47]. Based on the literature data [30,61,62] and due to the low partition coefficient low sorption onto solid particles during primary treatment in sedimentation tanks or to the activated sludge would be expected for this compound [63,45]). Thus, removal due to its biodegradability appears to be as the most presumable method for its elimination [58].

It is worth emphasizing here that despite a fairly high removal rate (77%) of ibuprofen its possible effect on aquatic life cannot be underestimated as this compound was still easily detectable in all analyzed samples of effluents of municipal WWTP of Ioannina city (100% occurrence) and can enter surface water directly as a result of direct discharge of effluents to the Kalamas river. Ibuprofen has at least two-degradation products 1-(4-isobutylphenyl)-1-ethanol and 4-isobutylacetophenone (4-IBAP) [64,65] and therefore, the fate of this compound during the WWTP treatment process and it's monitoring in surface waters is of great importance. Presence of the 4-IBAP metabolite has been referenced in environment [66] and in a WWTP in Sweden at a relatively high concentration, ca. 500 ng/L [28]. Despite the elevated reduction in the 4-IBAP concentration reported in the final effluents of WWTP (removal rate >90%) [28], the presence of this compound in the environment cannot be ruled out, highlight the importance of investigating degradation products during the wastewater treatment process.

Diclofenac exhibited less than 21% removal, suggesting their persistence throughout the wastewater treatment processes. In addition, the removal of this pharmaceutical was not dependent on the sampling period. Thereby, the removal of this substance was very variable between different STPs (0–90%) studied by various authors and contradictory results are documented in the literature [67,39,30,68,22,69,70,16]. Given the physicochemical properties of diclofenac (higher logK_{ow} values exceeding 4) sorption to suspended matter would be a presumable removal mechanism. This finding is consistent with results from a laboratory study in controlled experiments using wastewater sludges in which diclofenac exhibited higher sorption characteristics leading to a 5–15% partitioning to particulate matter present in raw wastewater [61]. However, no or only slight removal was obtained for diclofenac

during wastewater treatment of other studies [47,71]. Another interesting consideration is that disinfection would be a possible removal mechanism for diclofenac. Under laboratory-scale controlled chlorination studies with surface water diclofenac exhibited a high degree of reactivity with chlorine to concentrations below the limit of detection [72]. As far as the biodegradability of diclofenac is concerned, no biotic transformation was observed in batch experiments performed by various authors [73,74], whereas Urase and Kikuta [75] reported slow biodegradation.

The reasons for the discrepancies of the diclofenac removal during wastewater treatment process require further study. The differences in sludge age, as well as the composition of sludge and wastewater would be considered as possible considerations for such results [76].

Carbamazepine was consistently about 7–19% removed across WWTPs, leaving measurable effluent concentrations. Carbamazepine is generally considered moderately hydrophilic ($\text{Log}K_{ow} = 2.47$) and could be partially removed by sorption during the wastewater treatment process. Effluent concentrations vary within the same range as the influent concentrations. Furthermore, in one sampling campaign, the sum of the output loads was greater than the load observed in the influent probably due to the hydrolysis of carbamazepine glucuronide conjugate and cleavage of free parent compound. These findings are consistent with the results presented in other reports [76]. Studies on the fate of carbamazepine during wastewater treatment process [44,29] demonstrated a much higher efficiency of carbamazepine removal (30% and >80%, respectively) than the one observed in the present study and reported by others [77,30,22,78–80].

Naproxen was consistently about 48–62% removed across WWTPs, leaving measurable effluent concentrations. The moderate removal efficiencies for naproxen can be partly attributed to their persistence under microbial attack [81]. Controlled studies conducted by Boyd et al. [81] also confirmed that naproxen readily reacts with free chlorine. Hence disinfection would be considered as another mechanism for removal of this compound. As in the case of diclofenac large variability in naproxen removal was also reported from WWTPs in different European countries (66% in Germany [16], 40–55% in Spain [45], 94% in Sweden [28], and between 55% and 98% in Finland [22]).

In general, for most of the compounds, the removal rates obtained in this study fall into the range reported in the literature. It is worthing to say that our study focused entirely on the water phase. This probably does not change results dramatically for hydrophilic molecules, but it cannot be overlooked for more hydrophobic compounds, such as triclosan for instance. An alternative route for the pharmaceuticals to reach the environment is through the sludge and more research on this question is certainly needed.

3.5. Municipal plant configuration

The type of treatment units utilized across WWTPs, the physicochemical properties of individual pharmaceuticals, and environmental factors can greatly affect the removal rates. The municipal WWTP in this study was a tertiary sewage treatment plant by using primary treatment, conventional activated sludge plants, oxidation steps, nitrification/denitrification to enhance the nitrogen removal, chemical treatment by flocculation–sedimentation and finally disinfection step.

The primary treatment in the municipal WWTP consists of a screen, an aerated grit-removal and a primary sedimentation tank. Typically, it is unlikely that many pharmaceutical compounds will be removed during grit-removal. Of the pharmaceutical residues screened in this study, no compound exhibited a removal of more than 14.5% during this step. The primary sedimentation utilized before the step of the activated sludge treatment process of the municipal WWTP is characterized by rather poor efficiency for hydrophilic pharmaceuticals such as paracetamol, salicylic acid and caffeine as had eliminated for less than 25.5%. As there is little biological activity, any PPCP removal at this stage will rely on both the tendency of the individual drug to adsorb to solids and the degree of suspended solid removal from the primary sedimentation tank [62]. Sorption onto primary sludges was only observed for acidic pharmaceuticals ibuprofen, naproxen and diclofenac which are characterized by higher $\log K_{ow}$ values exceeding 3.5, which might indicate a tendency to sorb to suspended matter.

As it was expected the highest removal for most of the investigated compounds was detected in the point CW4 (secondary biological treatment) where the hydraulic retention time of the pharmaceuticals is bigger and there so a big quantity of organic matter is removed. Nitrification and denitrification processes employed in the municipal WWTP and would be likely contribute to the PPCP removal efficiencies. It has been reported that these processes may act synergistically affecting biological treatment system and having potential on pharmaceutical removal [62]. This is an indication of an improve biological diversity and growth conditions which could increase biological transformation and thus lead to higher removal of the compounds. The results obtained clearly indicate that the activated sludge treatment is an efficient process for most of the PPCPs studied. In general, out of all the pharmaceuticals studied only a few were characterized by low removal efficiency (<50%) during activated sludge treatment. These were: diclofenac (12%) and carbamazepine (4%).

PPCPs left in the effluent after primary and secondary treatment may be eliminated by tertiary treatment. The tertiary treatment, consisting of a chemical step using $FeCl_2$ and a process with sand filter. Of the pharmaceutical residues screened in this study, it seems that flocculation, sedimentation and sand filter steps would likely contribute in removal of PPCPs with $\log K_{ow} > 3$. This is probably due to the flocculation process, in which these analytes may adsorb to flocs.

The last step of the municipal WWTP, includes the disinfection process. Chlorine doses of 10–20 mg/L are commonly applied with contact times often exceeding 10 min. Limited studies have focused on the removal of PPCPs during wastewater disinfection. Among the PPCPs investigated naproxen and paracetamol were efficiently removed in the tertiary effluent (disinfection accounted for about 20% of the degradation or removal of naproxen and paracetamol, from the water phase) confirming that are quite reactive with free chlorine. These findings are consistent with laboratory-scale simulations of their fates through the disinfection process.

Results of this study indicate wide variability in the effectiveness of each treatment among the studied PPCPs. Therefore, more extensive sampling campaigns in both WWTPs would have been needed to confirm the above results.

3.6. Comparison of WWTPs evaluated

According to our findings, not only a wide variability of the removal efficiency of one pharmaceutical compound to another was observed, but also different removal efficiencies in each WWTP. For example, some compounds such as carbamazepine and diclofenac are removed in the same degree in both WWTPs studied whereas others such as ibuprofen and naproxen are more efficiently removed in municipal WWTP than in the hospital one. The hospital WWTP operates only secondary treatment comprising of initial raw sewage screening, primary mix tank, and biological secondary treatment concluding with disinfenction. It is highly probable therefore that the removal of pharmaceuticals is less effective than the tertiary treatment works in the municipal WWTP. Despite the lower removal efficiencies observed in the hospital

WWTP the lower standard deviation observed for most of the investigated compounds suggesting that this system is more reliable and robust treatment system than the municipal WWTP. This may attribute to the fact that WWTP receives lower volume of the sewages.

Overall, the observed removal rates and influent and effluent concentrations detected agreed well with previously reported values for other secondary wastewater treatment plants around the world [1,11,16,22,23,25,30,39,59].

4. Conclusions

In this work we have identified and traced 11 most-detected compounds, all of which were present in all influent and effluent water samples from both WWTPs investigated. This group includes some of the most often used and environmentally persistent PPCPs in Greece.

A method including SPE and GC–MS analysis is proposed for simultaneous determination of different classes of pharmaceutical compounds in hospital and municipal wastewaters. The compounds investigated include frequently used pharmaceuticals belonging to various therapeutic categories. The application of the proposed method has allowed a simple, rapid and reliable evaluation of the reported compounds at mean concentration levels ranged from 0.3 to 164.4 μg/L in the influent and from 0.5 to 14.6 μg/L in the effluent. The removal efficiencies of the WWTPs for these compounds varied from 9% (diclofenac) to 97% (paracetamol). The highest removal rate was achieved during biological treatment, which was satisfactory except for diclofenac and carbamazepine. The survey conducted in wastewaters of N.W. Greece revealed levels and removal efficiencies of the pharmaceuticals comparable with those found elsewhere in Europe, proving once again that conventional wastewater treatment plants appear to be variably and incompletely effective in removing most of the target PPCP residuals. Therefore, the goal of decreasing the levels of PPCPs in ambient waters can only be achieved by reducing inputs from WWTPs. Consequently, more exploration of advanced wastewater treatment technologies that are able to eliminate these new unregulated micro-pollutants is highly desirable, not only to provide advantages to human, but also for the benefit of other living things. Anthropogenic impact in Kalamas River and streams receiving effluents from WWTP of Ioannina city is evidenced by the occurrence of PPCPs. Although, the dilution produced after releasing effluent wastewater to receiving water is expected to reduce concentration levels of PPCPs to concentrations with no toxicological effect to the aqueous environment, continuous release of pharmaceuticals into the aquatic environment may lead to chronic exposure of aquatic organisms and consequently higher effect concentrations.

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