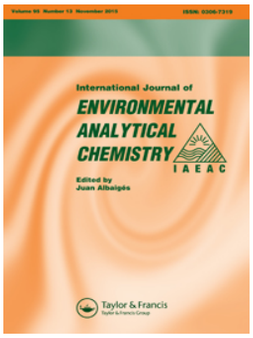

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Occurrence of pharmaceuticals in surface waters: analytical method development and environmental risk assessment

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The present work illustrates the development of an analytical method, based on offline solid phase extraction (SPE) followed by liquid chromatography–electron spray ionisation–single quadrupole mass spectrometry (LC-ESI-MS) for the simultaneous determination of pharmaceuticals belonging to various therapeutic classes (analgesics/anti-inflammatories, lipid regulators, antibiotics, antiepileptics, antipsychotics, psychomotor stimulants, glucocorticoid steroids, disinfectants, beta-blockers, H2 receptor antagonists and oestrogens) in surface waters. The method developed was applied for the analysis of pharmaceutical residues in surface waters from different sampling points along the aquatic systems of Lake Pamvotis and the River Kalamas, close to the city of Ioannina (Epirus, Greece), while the monitoring programme was carried out during the four seasons of the year. According to the results obtained, the majority of the compounds were detected in discrepant concentrations. The concentrations for all the compounds ranged from levels below quantification limit to 3506 ng/L, with caffeine and salicylic acid being the ubiquitous compounds. The results of the monitoring contributed substantially to the knowledge on the occurrence of pharmaceuticals in Greece and more specifically in surface waters of the region of Epirus. Regarding the environmental risk due to the presence of target compounds in surface waters, this was estimated calculating risk quotients (RQs) for different aquatic organisms (algae, daphnids and fish). The results denoted a possible threat for the aquatic environment, rendering in this way the RQ method as a helpful tool for a first approach. Extensive study is needed for triclosan, salicylic acid, sulfamethoxazole and erythromycin in order to better correlate their occurrence and potential toxic effects in aquatic life and humans.

Keywords: pharmaceuticals; surface waters; LC-MS; environmental risk assessment; Greece

1. Introduction

The focus for water pollution research has recently been shifted from the conventional organic priority pollutants to the so-called emerging contaminants; many of them are not regulated yet [1]. This category of pollutants includes very different substances with both industrial and domestic applications and varying potential harmful effects (e.g. endocrine disruption, carcinogenicity, etc.) [2]. Among others, they include pharmaceuticals, personal care products and disinfectants [1].

Pharmaceuticals are one of the most relevant groups of substances in aquatic ecosystems due to their universal use, physicochemical properties and known mode of action in aquatic organisms at low concentrations [3]. Despite these relatively low concentrations,

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pharmaceutically active compounds (PhACs) may pose a risk to aquatic organisms, because they are designed to modify biochemical pathways in the human body at low doses [4]. In Europe and the USA, around 4000 different PhACs are commercialised to be used as human and veterinary drugs [5,6].

Residues from industrial production, improper disposal of expired and unused medication via the toilet, landfills, leachates and accidental spills during manufacturing and distribution are possible sources of water pollution [7,8]. However, the main source of such pollution is that after administration many drugs and their transformation products (TP) are sometimes insufficiently retained in wastewater treatment plants (WWTPs), entering therefore the aquatic environment in considerable high amounts [3]. Human pharmaceuticals are excreted into the sewage system as a mixture of the parent compound and metabolites, comprising mostly of either TP or conjugated glucuronides [9]. In general, PhACs are rather hydrophilic, and their bioaccumulation potential might be considered irrelevant, particularly when compared to other persistent organic pollutants (POPs), such as non-polar pesticides [10]. More hydrophobic pharmaceuticals tend to accumulate in rivers and sea sediments. The persistence of some of them has also been confirmed in soils fertilised with contaminated sewage sludge [11]. Several studies worldwide investigated and reported on the occurrence of pharmaceuticals and personal care products (PPCPs) in surface waters (Table SD1).

A great number of toxicity studies have been carried out with the aim of assessing the potential risk that pharmaceuticals pose to the aquatic environment [12–17]. For human health, the resistance in bacteria due to the continuous presence of antimicrobials in the aquatic ecosystem is gaining concern. In addition, we are still far from knowing the toxic effect of the complex mixture of pharmaceuticals and their metabolites [11].

Since there are potentially adverse effects from pharmaceutical residues in the environment, data referring to their concentration levels, fate and behaviour is required. To achieve this requirement, rapid, sensitive, selective and robust analytical methods are developed for a variety of compounds in different environmental matrices.

The work presented in this manuscript describes the development, optimisation and validation of an analytical method based on offline solid phase extraction (SPE) followed by liquid chromatography–electron spray ionisation coupled to mass spectrometry (LC-ESI-MS) for the simultaneous determination of 23 multi-class human pharmaceutical residues in surface waters, including river and lake water.

Several studies report the occurrence of PPCPs in Greek wastewaters and surface waters [13,18–24]. To the best of our knowledge, this is the first study reporting the occurrence of pharmaceutical compounds in the aquatic region of Epirus and especially in the River Kalamas and Lake Pamvotis, so extensively and comprehensively. This region is intensely subjected to anthropogenic activity and our aim is to provide a better understanding of the eventual sinks and fates of these pharmaceuticals. Furthermore, the results of the present study will contribute to estimate the potential risk deriving from the WWTP of Ioannina City, the discharges of which drain into the River Kalamas. One-year monitoring study results from different sites along the lake and the river are given. Finally, an ecotoxicological study took place, by means of risk quotient (RQ), in order to assess the potential environmental risk in the aquatic organisms of the surface waters.

2. Experimental

2.1. *Standards and reagents*

All pharmaceutical analytical standards were purchased from Sigma-Aldrich (Steinheim, Germany) and were of high purity grade (>95%). Solvents used for sample preparation were

obtained from Pestiscan (Labscan, Ltd., Dublin, Ireland) while acetonitrile (ACN) and water (for chromatographic analysis, LC-MS grade) were received from Fisher Scientific (Leicestershire, UK). Formic acid (purity 98–100%) was acquired from Merck KGaA (Darmstadt, Germany). The disks used for SPE, namely SDB-RPS, SDB-XC and C18, were all purchased from Empore™, 3 M (Minnesota, USA). Glass fibre filters (1 μm) and nylon membrane filters (0.45 μm) were purchased from Whatman (United Kingdom).

Individual stock solutions of the selected pharmaceuticals, except for ciprofloxacin, were prepared in methanol (1000 mg/L) and stored at −20°C. Ciprofloxacin was dissolved in methanol by adding 100 μL of NaOH 1 M, because of its low solubility in methanol [25]. Mixtures of pharmaceuticals were prepared by diluting the appropriate volumes of individual stock solutions in methanol. Working standard solutions, containing all pharmaceuticals, were prepared in methanol/water 25:75 (v/v).

The selected pharmaceuticals were: the analgesic/antipyretic drug paracetamol, the analgesic/anti-inflammatory drugs phenazone, salicylic acid, diclofenac, ibuprofen, ketoprofen, indomethacin and mefenamic acid, the lipid regulators gemfibrozil, fenofibrate and bezafibrate, the antibiotics sulfamethoxazole, sulfamethazine, ciprofloxacin and erythromycin, the antiepileptic carbamazepine, the antipsychotic risperidone, the psychomotor stimulant caffeine, the glucocorticoid steroid budesonide, the disinfectant triclosan, the beta-blocker atenolol, the H2 receptor antagonist cimetidine and the oestrogen oestriol. Target compounds were selected according to the following criteria: (1) their high human consumption in Greece [22,26]; (2) their proven occurrence in the aquatic environment, according to the data found in the scientific literature; and (3) their known fate and behaviour in the environment (e.g. water solubility or persistency) and their environmental and toxic relevance (such as their endocrine disrupting potential) [27]. The chemical formulas and main physicochemical properties of the target compounds are shown in Table SD2.

2.2. *Sampling area and sample collection*

A sampling campaign was conducted from 2011 to 2012 in the River Kalamas and Lake Pamvotis, two aquatic ecosystems of great environmental interest, located in the prefecture of Epirus, in the Northwestern part of Greece (Figure 1).

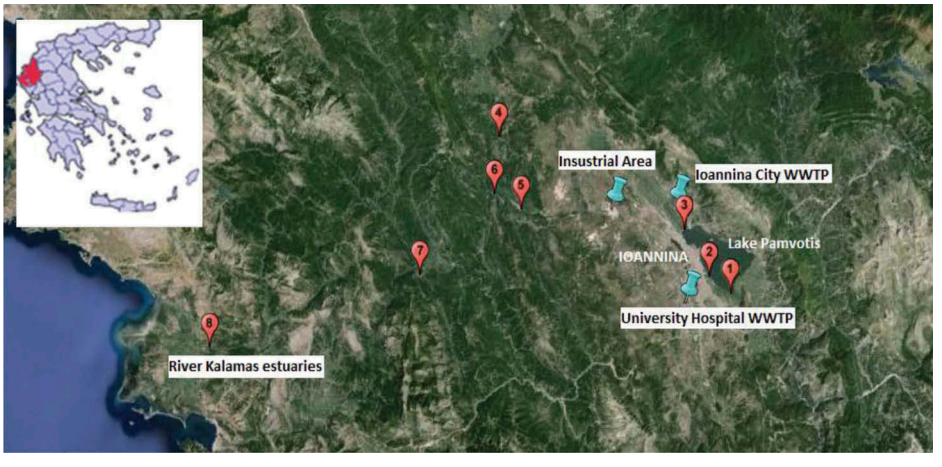

Figure 1. Sampling points along the River Kalamas and Lake Pamvotis (Epirus, Northwestern Greece).

The Kalamas River has its sources in the Northern side of Ioannina prefecture. It flows through several villages (mean annual flow rate 37.5 m^3/s), it has a few tributaries, a catchment area of 1800 km^2 and discharges into the Ionian Sea [28]. The soil types of the river are mainly sandy clay loam with inclusions of fine and coarse sediments. The river sediment is composed mainly of clay minerals, quartz and some feldspar, with an overall organic content less than 10%. The land use is divided between agricultural (75%), aquaculture (10%), urban (5%) and recreation (<5%). Main problems regarding Kalamas basin management focus on agricultural and animal farming polluting activities [29].

Lake Pamvotis is situated in Northwest Greece, and the city of Ioannina lies along its western shoreline. It has been formed from the late Miocene to the Pliocene period; it is a shallow Mediterranean lake and spreads in an area of 22.8 km^2, having a volume of 90×106 m^3, with a mean depth of about 4.3 m and a maximum of 11 m. The catchment has no natural surface outflows and is recharged by karstic springs. It has also a great recreational value (rowing and water skiing) and also supports tourism (island, ferries, lakeside and cafés) and fishing (netting). The trophic status of the lake is eutrophic to hypertrophic [30]. It has a long eutrophication history due to the heavy point and non-point loading of nutrients, with cyanophyte blooms occurring since 1978. An improvement during the 1990s has been observed because of remedial activities such as the removal of pollutant sources from the shoreline and the operation of the municipal and hospital WWTP [29]. In addition, other restoration efforts have been made, such as the reduction of external P-loading, resulting in a major decline in lake nutrient concentrations, but it is still high enough to maintain eutrophic conditions. The lake remains a degraded ecosystem whereas, at the same time, several activities (irrigation, fisheries, and tourism) are still taking place. Drainage from the catchment occurs through a system of sinkholes that drain it to the rivers Arachthos, Louros and Kalamas. Lake Pamvotis is associated with the River Kalamas through a trench, which is recipient of treated urban and industrial wastewater.

The proximity of these ecosystems to Ioannina, a city of approximately 150,000 inhabitants, close to which a medium-range industrial area exists, should be noted. The WWTP of the city discharges the treated sewage directly into the Kalamas River. The WWTP consists of a primary treatment, a secondary biological treatment, removal of phosphorus, denitrification/nitrification and final clarification, and receives urban, industrial as well as the hospital effluent wastewaters. Regarding the hospital plant, it has a capacity of 800 beds and applies a pretreatment, a mix tank and a biological secondary treatment concluding with disinfection. The untreated wastewater is discharged into the urban network which ends up at the municipal WWTP and finally at the aforementioned ecosystems. The existence of few pharmaceutical companies in close proximity should also be mentioned.

For the purposes of the present study, in order to carry out a monitoring programme to cover the four seasons of the year, samplings were conducted seasonally (May, July, November 2011 and February 2012) from eight different sampling points, always close to locations that are potential sources of pharmaceutical contamination. For this reason, the proximity of WWTPs, industrial area, residential areas and urban activities was considered. In total, a number of 32 grab samples were collected. In particular, 12 samples were collected from Lake Pamvotis in shallow areas: Katsika (S1), Lagatsa (S2), Perama (S3). Moreover, 20 samples were obtained from the main flow of the Kalamas River: Theogefyro (S4), Soulopoulo (S5), Paliouri (S6), Vrosina (S7) and Ragio (S8) (Figure 1). In the river, the samples were taken from the middle course, from a depth of about 1 m. In the lake, the samples were taken by a boat, and the sampling stations were located in the mid-depth of the water column. All water samples were collected with a van Dorn sampler (Lab-line, Melrose Park, IL, USA). Weeks with severe weather, such as intense precipitations, were avoided so as to avoid dilution effects. Amber glass

bottles, pre-rinsed with ultrapure water, were used for the collection of water samples (2.5 L). During sampling, physicochemical parameters were measured for each sample. Once the sampling was completed, samples were transported to the laboratory, surrounded with ice.

2.3. *Sample preparation and SPE*

Water samples were vacuum-filtered through 1 μm glass fibre filters GF/B (Whatman, UK) prior to analysis, so that the suspended solid matter can be removed, avoiding thus potential interferences during the analysis. Samples were stored at 4°C and extraction was carried out within 48 hours.

Target analytes were isolated and pre-concentrated from water samples using a standard offline SPE connected to a vacuum pump. With the view to optimise the extraction technique, a few parameters were examined. At first, different extraction disks were tested, to determine which yielded better recoveries, namely SDB-RPS [Poly(styrenedivinylbenzene) sulphonated], SDB-XC [Poly(styrenedivinylbenzene] and C18 (Octadecyl). After that, two different sample volumes were examined (500 and 1000 mL, respectively). Moreover, a range of pH values was also investigated (pH 2, 4, 5, 7 and 8.5). In this step, spiking in real samples without pH adjustment was tested, exhibiting equally high recoveries. Regarding the elution solvent, methanol and ethyl acetate were tested. Finally, the highest recoveries for target analytes were calculated using SDB-RPS disks, loading 1000 mL of sample and being eluted with methanol at neutral pH. Taking into account that the mean pH value of surface waters collected was 7.53 and in order to isolate the analytes in a simpler way, finally, no pH adjustment was determined [19].

Prior to extraction, SDB-RPS disks were preconditioned with 10 mL of acetone, followed by 10 mL of methanol and 10 mL of deionised water. Before the disk became dry, water samples (1000 mL) were passed through the SPE disks, at a flow rate of approximately 10 mL/min, using a vacuum manifold that maintains a constant pressure differential between the inlet and the outlet of the disk. Once the total sample was percolated, disks were rinsed with 2 × 5 mL of deionised water. Afterwards, the disks were dried under vacuum for 10 min to remove residual water, and analytes were eluted with 3 × 5 mL of methanol, drop-by-drop, at flow rate of 1 mL/min. Methanol extracts were evaporated to dryness at approximately 40°C under a gentle stream of nitrogen, reconstituted in a mixture of 1 mL methanol/water 25:75 (v/v) and stored at −20°C until chromatographic analysis.

2.4. *LC-UV/VIS-ESI-MS analysis*

Chromatographic separations were operated using an HPLC system equipped with SIL 20A auto sampler and an LC-20AB pump, both from Shimadzu (Kyoto, Japan). The chromatographic column used for analyte separation was a C18, 150 × 4.6 mm with 5 μm particle size (Restek, USA). An SPD 20A UV-Vis detector coupled in series with the LC-MS 2010 EV mass selective detector equipped with an atmospheric pressure ionisation source electrospray (ESI) interface was used. Injection volume was set at 20 μL. Oven temperature was constant at 40°C, and drying gas during analysis was adapted at 10 mL/min at 200°C while nebulising pressure was 100 psi. Capillary voltage was 4500 V in positive ionisation and −3500 V in negative ionisation, while fragmentation voltage was 5 V in both cases.

The analysis under positive ionisation mode was carried out by gradient elution, which was performed with a binary gradient, where solvent A constituted of water and solvent B of ACN, both with 0.1 % formic acid. Regarding the negative ionisation mode, solvent A was water and solvent B ACN, with no addition of formic acid. For the positive ESI, the elution gradient

started with 90 % A, decreasing to 20 % in 30 min, to 0 % in 5 min and then back to initial conditions within 5 min, while re-equilibration time of 5 min was elapsed in order to restore the column. Regarding to the negative ESI, the analysis was performed according to the following programme: the elution gradient started with 20% of eluent A, increased to 70% in 25 min and raised to 100% in 4 min and reached again the initial conditions in 5 min with a re-equilibration time of 15 min. Flow rate was set to 0.5 mL/min in both cases. As shown above, the total run analysis time was 50 and 40 min for positive and negative modes, respectively.

Cimetidine, paracetamol, atenolol, caffeine, sulfamethazine, phenazone, ciprofloxacin, sulfamethoxazole, carbamazepine, bezafibrate, erythromycin, budesonide, fenofibrate, risperidone were analysed in positive ESI, while oestriol, salicylic acid, ibuprofen, ketoprofen, indomethacin, diclofenac, gemfibrozil, mefenamic acid and triclosan were analysed in negative ESI. For each compound, the precursor molecular ion, $[M + H]+$ or $[M - H]-$ for positive and negative ESI, respectively, and at least one confirming ion were obtained, with the most abundant one to be used for quantification (Table SD3). The only exception was fenofibrate, whose quantification was based only on *m/z* 319, for which the most intense signal was demonstrated. This fact indicates the loss of one methylene and one carbon monoxide group $[M - CH_2 - CO + H]+$ [19].

2.5. *Method performance and validation*

Target pharmaceutical identification and confirmation were conducted with respect to the directions based on the regulations implemented by the European Union (EU) [31]. Therefore, in order to conform to these criteria, three parameters were checked: the retention time of the chromatographic peak from surface water sample compared with that of the pharmaceutical standard, the identification of the target and qualifier ions, and the calculation of the qualifier-to-target ratio. To consider identification as a positive one, the divergence between retention times should not be over 0.50 min. Regarding to qualifier-to-target ratios, the relative abundance percentage should be over 50 % and within 20 % of the standard. Method performance was evaluated in the terms of linearity, extraction recoveries, repeatability and intermediate precision and sensitivity (instrumental limits of detection – IDLs, limits of detection – LODs and limits of quantification – LOQs).

With the view to apply the internal quality control (QC) in every single sequence of the samples, a matrix-matched calibration curve (with at least five points), a reagent blank, a control standard matrix and a spiked sample blank (0.5 μg/L) were analysed.

Calibration curves were generated using linear regression analysis ($r^2 > 0.99$). Matrix-matched calibration curves from surface water SPE extracts were used in order to quantify the target compounds. A five-point calibration curve at concentrations between LOQ and 10 LOQ was generated at the beginning and at the end of each sequence of samples to check system stability. QC samples were also loaded in each set and this is referred to as blank surface water extracts, spiked both at LOQ and 10 times the LOQ level. With the view to calculate the concentrations, recoveries after subtracting the blank samples were applied.

IDLs were determined by injecting a standard solution that has been successively diluted until a concentration equal to a signal-to-noise ratio of 3. LODs and LOQs of the applied method were calculated based on spiked samples of the matrix ($n = 3$) and were considered as the minimum detectable amount of analyte with signal-to-noise ratios of 3 and 10, respectively (Table SD4).

Intra-day and inter-day precision of the proposed chromatographic method was expressed as relative standard deviation (RSD) and was calculated from five repeated injections ($n = 5$) of a spiked extract in the same day (repeatability) and in five successive days (intermediate precision) (Table SD4).

In order to determine the method recoveries ($n = 3$), distilled, river and lake water samples were spiked in triplicate with a mixture of the target compounds at low and high concentration levels of 0.2 and 2 μg/L, respectively. (Table SD5). Blank samples were used, because unspiked samples may contain some of the analytes. Thus, the concentration of the unspiked samples was subtracted from the spiked ones and then divided by the spiking level.

2.6. *Matrix effect*

A major problem that arose during the application of mass spectrometry detectors is ion suppression or enhancement, and it was due to the presence of potentially co-extracted components of the matrix. It takes place in the early stages of the ionisation process in the LC-MS interface, when a component eluted from the chromatography column influences the ionisation of a co-eluted analyte [32]. The significance of matrix effect evaluation tends to be of great importance, since the absence of result correction may lead to inaccurate quantification, either in terms of underestimation or overestimation [33]. An effective way to surpass this problem is to improve sample preparation and the chromatographic selectivity.

The problem is more common when electrospray interfaces are involved, as it may affect a method's linearity, accuracy, precision and sensitivity. Nevertheless, matrices such as surface waters that are studied in this work are considered as uncomplicated ones to cause significant ion suppression or enhancement; however, the use of electrospray ionisations imposes the study of matrix effect in order to validate the results. It is notable that when dealing with waters, a factor that may render the fresh water as a rather complex matrix is the salt content, expressed as salinity or conductivity [33].

With the view to measure the potential interferences due to the matrix effect, spiked water extracts with the mixture of the target analytes were compared to samples spiked in the solvent. The comparison of the chromatographic peaks took place through the Equation (1).

$$\text{Signal suppression } (\%) = 100 - (((\text{Area matrix} - \text{Area Blank}) \times 100) / \text{Area Solvent}) \tag{1}$$

The percentage of suppression is depicted in Figure SD1. In both cases, carbamazepine, gemfibrozil and paracetamol demonstrate severe signal suppression.

2.7. *Ecotoxicological risk assessment*

Ecotoxicological risks were estimated on the basis of RQ method for the selected pharmaceuticals. This method is valuable in order to conduct a screening-level risk assessment. According to literature, the following equation is proposed (Equation (2)) [15,19,34].

$$RQ = \frac{MEC}{PNEC} \tag{2}$$

where *MEC* is the measured environmental concentration, which corresponds to the highest detected concentration, and *PNEC* is the predicted non-effect concentration for every single contaminant. *PNEC* values used are based on acute and chronic toxicity data reported for several aquatic organisms (fish, invertebrates and algae), as shown in Tables SD6 and SD7 (lowest values of EC_{50}, LC_{50} or no observed concentration (NOEC), indicated in bold, were used). *PNEC* values for acute toxicity were estimated by dividing the lowest EC_{50} or LC_{50} with

an assessment factor (AF) of 1000, while *PNEC* values for chronic toxicity were estimated by dividing the lowest NOECs with assessment factors chosen according to the Technical Guidance Document on Risk Assessment of the European Union [35]. Risks are commonly classified into three levels. Low risk is implied when $RQ \leq 0.1$, medium risk when $0.1 < RQ < 1$ and high risk when $RQ \geq 1$ [15,16].

3. Results and discussion

3.1. *Occurrence of PhACs in surface waters*

The method described in this manuscript was applied to verify the occurrence of the 23 target pharmaceuticals in Greek aquatic environment. Table 1 demonstrates the positive findings (PF), which correspond to percentage of each pharmaceutical found in the samples analysed (in total 32), the range and the mean concentrations as well. The results of this first attempt to study the occurrence of such emerging contaminants in the region of Epirus (Northwestern Greece) showed an occurrence of these pharmaceuticals in a range of 0–100%, depending on the compound, the sampling station and the month of collection. It is significant that 21 from the 23 selected pharmaceuticals were detected at least in one sample. The concentrations ranged from very few to some thousands of ng/L; thus, all the results are expressed in ng/L units. According to Nödler *et al*. [36], micro-contaminants are suggested to be source-specific, providing the potential to be employed as indicators for source delineation in monitoring ground and surface water quality.

It is noteworthy that salicylic acid and caffeine were the most abundant compounds, detected in 100% of the water samples, demonstrating also higher concentrations compared to the other compounds. The analgesic paracetamol and the analgesics–antinflammatories ibuprofen and diclofenac were quantified in 81%, 78% and 75% of the analysed samples, respectively. Budesonide was detected in 53% of the samples, at concentrations only below quantification limit (bql), while gemfibrozil occurred in 50% of the samples. Mefenamic acid, bezafibrate, risperidone, atenolol and oestriol were detected only at concentrations bql and for less than the 50% of the total samples. The rest of compounds were sparsely detected (positive findings for less than 35% of the total samples) at various concentrations. Triclosan was detected in only one sampling station (S2) in May and July while indomethacin and sulfamethazine were not detected in any sample. Among the results concerning pharmaceuticals detected at quantifiable levels, the highest concentration was observed for caffeine in S1, in July (3506 ng/L), while the lowest for cimetidine in S8, in May (17 ng/L). Taking into account the mean values of the results, salicylic acid exhibited the highest concentration levels during the monitoring period while phenazone the lowest ones.

Salicylic acid was present in most of the samples at an elevated level. Salicylic acid and paracetamol are the two most popular painkillers, mainly sold as ‘over-the-counter’ (OTC) drugs [37]. In the Greek market, they are both available over the counter. Apart from being a drug itself, salicylic acid is also the main metabolite of acetylsalicylic acid (aspirin). Some of the possible reasons for salicylic acid occurrence in the environment are its natural formation in fresh waters, its uses as keratolytic, dermatice, ingredient of shampoos and preservative of food as well [10]. Aspirin, the parent compound of salicylic acid, is susceptible to direct photolysis by sunlight, and both are hydrolysed, with hydrolysis half-lives ranging from 1.2 h to 12.5 days [21]. Although many studies indicate that removal efficiencies of salicylic acid in WWTPs are exceeding 80% [19,38,39], its presence is very steady and persistent at high levels in aquatic environment. In the present study, the highest concentrations of salicylic acid were detected in May and July for all sampling stations and its maximum presence was in S3, in May

Table 1. Positive findings, ranges of concentrations (ng/L) and corresponding mean values (in brackets) of pharmaceuticals detected in the eight sampling points.

	PF (%)	S1	S2	S3	S4	S5	S6	S7	S8
PAR	78	87–146 (118)	66–156 (123)	bql–107 (75)	n.d.–55 (bql)	64–87 (74)	n.d.–80 (bql)	n.d.–60 (bql)	n.d.–21 (bql)
PNZ	13	n.d.–71 (43)	n.d.	n.d.	n.d.	n.d.–bql	n.d.	n.d.–95 (25)	n.d.
SA	100	312–1990 (1197)	671–1844 (1234)	221–3001 (1473)	193–1560 (710)	606–1232 (880)	332–905 (688)	401–2005 (1117)	bql–1010 (508)
DCF	75	bql–110 (80)	bql–457 (146)	bql–175 (113)	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql
IBU	81	bql–1351 (548)	bql–900 (397)	n.d.–373 (bql)	n.d.–bql	n.d.–bql	n.d.–bql	bql–365 (bql)	n.d.–bql
KPF	34	bql–91 (bql)	n.d.–bql	n.d.–bql	n.d.	n.d.–bql	n.d.	n.d.	n.d.
IND	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MA	19	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql
CAF	100	454–3506 (1524)	560–2891 (1254)	499–1020 (767)	257–1342 (722)	481–2132 (1281)	401–1312 (889)	358–703 (473)	125–401 (239)
BZF	34	n.d.– bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql
FNB	28	bql–91 (43)	bql–78 (37)	n.d.–78 (bql)	n.d.	n.d.	n.d.	n.d.	n.d.
GMF	50	n.d.–602 (333)	n.d.–453 (177)	n.d.–303 (146)	n.d.–200 (bql)	n.d.–bql	n.d.–bql	n.d.–129 (bql)	n.d.–bql
SMT	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SMX	31	n.d.–171 (85)	n.d.–190 (73)	n.d.	n.d.–68 (23)	n.d.	n.d.–18 (bql)	n.d.–18 (bql)	n.d.
CFX	13	n.d.–115 (bql)	n.d.–87 (bql)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ERY	13	n.d.–51 (bql)	n.d.–137 (53)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CBZ	34	n.d.–325 (113)	n.d.–211 (55)	n.d.–406 (125)	n.d.–98 (32)	n.d.–85 (29)	n.d.–41 (bql)	n.d.–57 (bql)	n.d.
RIS	22	n.d.–bql	n.d.–bql	n.d.	n.d.–bql	n.d.–bql	n.d.	n.d.	n.d.
BUD	53	bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql	n.d.–bql
TCS	6	n.d.	n.d.–150 (bql)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ATE	16	n.d.–bql	n.d.–bql	n.d.	n.d.–bql	n.d.–bql	n.d.	n.d.–bql	n.d.
CMT	16	n.d.	n.d.–bql	n.d.	n.d.–20 (bql)	n.d.–18 (bql)	n.d.	n.d.	n.d.–17 (bql)
E3	13	n.d.–bql	n.d.–bql	n.d.–bql	n.d.	n.d.	n.d.	n.d.	n.d.

Notes: PAR, Paracetamol; PNZ, Phenazone; SA, Salicylic acid; DCF, Diclofenac; IBU, Ibuprofen; KPF, Ketoprofen; IND, Indomethacin; MA, Mefenemic acid; CAF, Caffeine; BZF, Bezafibrate; FNB, Fenofibrate; GMF, Gemfibrozil; SMT, Sulfamethazine; SMX, Sulfamethoxazole; CFX, Ciprofloxacin; ERY, Erythromycin; CBZ, Carbamazepine; RIS, Risperidone; BUD, Budesonide; TCS, Triclosan; ATE, Atenolol; CMT, Cimetidine; E3, Oestriol.

(3001 ng/L). These results can be attributed to its frequent and quite high consumption in Greece, due to the aspirin OTC availability, which renders this drug as one of the most popular first-line analgesic [26].

Paracetamol, the other OTC drug included in the present study, was detected in a range of concentrations between n.d. and 156 ng/L. Although the percentage of positive findings was high enough, the concentrations may be lower than expected. This can be grounded on its rapid biodegradation during WWTPs. Furthermore, in WWTPs, it readily reacts with free chlorine, as referred to in previous studies [38], and due to these effects the concentration levels of this drug in effluents are drastically reduced. Elimination efficiencies in WWTPs have been reported to be up to 99% [39] and often reached 100%. Nevertheless, in some cases, high concentrations of paracetamol in surface water and groundwater could be explained by the fact that cities where the samples were taken from do not have WWTPs [40]. In fact, a very low concentration of untreated sewage may cause the occurrence of paracetamol in surface waters, a fact that can be avoided when WWTPs exist [41]. In the present study, paracetamol was detected in 78% of all the analysed samples, and its mean concentration in the sampling period was 61 ng/L.

Ibuprofen-positive findings were 81%. It is reported that ibuprofen is one of the most frequently prescribed drug in Europe and also one of the most common drug residues in surface waters [42]. Ibuprofen has a high metabolic rate in humans and exhibits half-life of less than 1–2 days [18]. Although ibuprofen is easily removed from the WWTPs, it is often detectable in effluents, in very high concentrations, reaching even 28,000 ng/L [42]. In the present study, concentration levels of ibuprofen ranged between bql and 1351 ng/L. Its maximum concentration was detected in S1 in November (1351 ng/L).

Diclofenac is another non-steroidal/anti-inflammatory drug (NSAID) that demonstrated a relatively high occurrence (75% of the analysed samples), while its concentrations ranged from bql to 457 ng/L. Its highest average concentration was observed in autumn, maybe due to the fact that it is used widely for rheumatic diseases, which increase in cold and humid weather [37]. It is used either as a tablet or as a body cream; thus, it can be released in the environment via human excretion or via body cleaning or washing of clothes. Removal rates of diclofenac in WWTPs vary and several studies by various authors have shown contradictory results (0–90%). Diclofenac is one of the so-called emerging contaminants that are yet to be regulated and may enter the dynamic list of priority substances in future updates. It was suggested along with 17-α-ethynylestradiol and β-oestradiol, but none of them was finally included in the Directive 2013/39/EU as priority substances. However, it is included in the first watch list, in order to gather monitoring data for the purpose of facilitating the determination of appropriate measures to address the risk posed [43].

Regarding to the rest of the NSAIDs, they were detected in less than 50% of the real samples, at mean concentrations bql. Average concentrations for ketoprofen and phenazone were bql, while maximum values were 95 and 91 ng/L, respectively. Phenazone is very stable ($\log K_{ow} = 0.38$) and it is not easily eliminated from WWTPs [18]. Ketoprofen demonstrates a limited ability to bioaccumulate and low mobility to the terrestrial compartment if released in water, based upon its $\log K_{ow} = 3.12$. Mefenamic acid did not exceed the quantification levels in any sample, while indomethacin was absent in all of the samples analysed. This result is attributed mainly to the decreased prescription of these NSAIDs compared to other ones, which not only are prescribed but also are consumed widely over the counter. Except for this anthropogenic factor, it is worth mentioning that such acidic pharmaceuticals are frequently detected at higher concentrations in the dry season when the water level is very low and some sections of the river are even dried due to the high water demand in the catchment. This is confirmed in our study, although none of the samples exceeded the LOQ [44].

The psychomotor stimulant caffeine was ubiquitous, demonstrating yet the highest detected concentration (3507 ng/L). More common uses of caffeine, hence likely significant sources of environmental contamination, include it being a key ingredient in coffee, tea, soft drinks and food products (chocolate, dairy desserts, mint and candies). Medicinally, it is a component of hundreds of prescription and OTC drugs, ranging from analgesics to cold medicines; it is used as a cardiac, cerebral and respiratory stimulant, and it also functions as a diuretic. It has been estimated that from coffee alone, an average human consumes 131 mg of caffeine per day and since this compound is metabolised to a great extent in humans, approximately 3.9 mg of it is excreted unchanged in urine [45]. In addition, there is much more amount of caffeine that enters into the sewage system by disposal of unconsumed beverages and rinsing of coffee cups. WWTPs eliminate some of the caffeine, but the elimination efficiency can be quite variable. Removal efficiencies can vary depending on the treatment processes employed, age of the activated sludge, hydraulic retention time, environmental conditions such as temperature and light intensity and physical properties, including the adsorption capacity of compounds on the sludge [46]. Since caffeine is generally readily biodegradable in WWTPs, it has been used as an indicator for the input of raw sewage into surface waters [47]. Furthermore, due to its high occurrence in natural waters and wastewaters, caffeine has been successfully used as an anthropogenic marker for wastewater-associated contaminants [18].

As far as the lipid regulators are concerned, gemfibrozil was the most frequently detected, followed by bezafibrate and fenofibrate. Due to the fact that gemfibrozil has a high log K_{ow} and presents adsorption to soil, it would be expected not to be present in waters. Nevertheless, gemfibrozil was detected in 50% of the analysed samples in concentrations ranging from bql to 602 ng/L. This could be attributed to the fact that gemfibrozil belongs to the class of antihyperlipidaemics, which comprises the highest sales in the worldwide pharmaceutical market [48]. Regarding fenofibrate, although it is a widely consumed drug, it was detected only in 28% of the total samples. This may be attributed to the fact that instead of this non-polar pharmaceutical, its polar metabolite, fenofibric acid, may be present in surface waters. Bezafibrate presented 34% positive findings, only at concentrations bql. This is in good agreement with several studies conducted (Table SD1).

Triclosan was detected in only two samples in S2, in May and July (131 ng/L and 150 ng/L respectively). Triclosan is used as an antimicrobial agent in the manufacture of toothpastes, footwear, shampoos, toilet and hospital hand soaps and medical cosmetics, while recently it is being incorporated into plastic products from children's toys or kitchen utensils such as cutting boards. Triclosan is well removed during the secondary treatment, with values of around 70% [18] This compound is labile to biodegradation and adsorption in the suspended matter in sludge in WWTP suspended matter (relatively high partition coefficient, log K_{ow} = 4.8). Moreover, it is reported that besides being extremely resistant to high and low pH, triclosan is readily degraded in the environment via photodegradation [49].

Antibiotics are one of the most significant groups of pollutants among the vast array of emerging contaminants of anthropogenic and veterinary concern [10]. Among the antibiotics investigated in the present study, sulphamethoxazole appeared to be more persistent than the others, since it is one of the most broad-spectrum antibiotics consumed worldwide. Its concentration showed discrepancies, reaching a maximum concentration of 190 ng/L while the mean concentration was 25 ng/L. The other sulphonamide included in the present work, sulfamethazine, was not detected in any sample. It may be attributed to the fact that this compound can be often found as *N*-acetyl sulfamethazine, its *N*-acetylated metabolite [50]. It is not easy to compare extensively this finding, because of the lack of previous studies on its presence. Ciprofloxacin and erythromycin, which are susceptible to chemical degradation, were occasionally detected at mean concentrations bql, while their maximum concentrations were 115 and 137 ng/L, respectively. This is in good agreement with previous studies [33,36] conducted.

Carbamazepine is one of the most frequently detected drugs in natural waters, at high concentrations, although it is extensively metabolised in humans [40]. In the present study, carbamazepine was present at 34% of the samples analysed, a percentage that was probably expected to be higher, taking into consideration the fact that this drug is considered to be recalcitrant in WWTPs. Its average concentration was 48 ng/L, while its maximum was 406 ng/L (S3, in May). Carbamazepine is reported to be very stable in aquatic environment, and the main reasons of its occurrence in natural waters are both its very low removal rate in WWTPs, less than 10%, and the excretion of glucuronides, which may act as a reservoir from which a later yield of the parent substance can occur [18]. It is considered as a potential tracer in surface water, since it is insufficiently eliminated during wastewater treatment [42]. Moreover, a major issue is that epilepsy ailment lasts for a lifetime; thus, the consumption of antiepileptic drugs is indispensable [40].

It is remarkable that the main metabolites of carbamazepine, namely 10,11-epoxycarbamazepine, 2-hydroxycarbamazepine and 3-hydroxycarbamazepine, have been detected in the environment in more considerable concentrations than carbamazepine itself, which proves that they may be more prominent than the parent compound [33]. This is an explanation for the almost unexpected moderate concentrations at which carbamazepine was detected in this study, taking into consideration the fact that it is one of the most prescribed psychiatric drugs consumed in Greece.

Another drug belonging in the wide therapeutic class of psychiatrics, risperidone, a second-generation atypical antipsychotic, was also investigated and detected sparsely but always in concentrations bql. There is limited information about its occurrence in surface water, but the fact that it is readily metabolised to 9-hydroxy risperidone may be an explanation to the fact that it is not found in the expected concentrations, since it is the highest consumed pharmaceutical treatment of schizophrenia in the Greek market.

Budesonide, a corticosteroid widely consumed worldwide, gave 53% positive findings, but always at levels below the LOQ. Unexpectedly, until now, there are limited literature data to compare its occurrence, which is expected to be rather high, taking into consideration the fact that it is the main ingredient of many formulas used in considerable quantities for large periods within the year, to treat both seasonal and chronic diseases like asthma, chronic obstructive pulmonary disease (COPD) and non-infectious rhinitis.

Atenolol, cimetidine and oestriol were sparsely detected, not exceeding 16% of positive findings each, with a mean concentration bql, while among them, only cimetidine was detected at levels over the quantification limit in only two sampling stations (S5, S6), at concentrations of 18 and 20 ng/L, respectively.

In general, at the sampling stations located along Lake Pamvotis (S1, S2, S3) and thus closer to Ioannina city, the highest total concentrations were observed. Occurrence data for PPCPs in lakes are relatively scarce compared to river data. Lakes impacted by human activity are more vulnerable to PPCP contamination [51]. Lake Pamvotis seems to be a typical example. Sampling stations S5 and S6 followed, maybe due to their proximity to urban areas. The lowest total concentrations were observed for all months at Ragio, the sampling point which is the closest one to the sea. Therefore, dilution could be proposed to have an impact on decrease in the concentration.

For the River Kalamas, the highest mean concentrations were observed in S5, reaching 3580 ng/L in May. The high levels of salicylic acid and caffeine contributed to this slightly differentiated concentrations compared to the other stations, probably because of the urban activities close to it. Similar results were obtained at S7 (2913 ng/L), which is also located in an urban estate.

The presence of pharmaceuticals in this aquatic ecosystem mostly owes to the connection of the river with the WWTP of the Ioannina city, through a ditch. The effluent of this ditch is located very close to sampling station S6 and this is why a gradual decrease in concentrations of pharmaceuticals is observed in consecutive stations. This is because of the dispersion of pharmaceuticals, their dilution in tributaries and the seasonal variation in the volume of water-bodies as well. Furthermore, it is worth mentioning that sampling points' selection was based on their proximity to urban areas, apart from Ioannina city (sparsely populated villages and rural settlements), and, in general, areas affected by various agricultural activities. Hence, a discharge of raw waters is possible.

3.2. *Seasonal variation*

The results obtained from this work enabled the study of the seasonal variation of the 23 selected pharmaceuticals. (Table 2, Figures 2 and 3). Pharmaceuticals detected bql were omitted from the figure. In general, most of the compounds studied showed higher concentrations in the dry season. In particular, caffeine and salicylic acid were the two compounds presenting two-fold concentrations in spring and summer compared to autumn and winter. The seasonal variation in the levels of pharmaceuticals may be associated with the flow conditions in the river system. It is interesting to take into account the fact that the monthly rainfall in Ioannina is approximately 129 mm during the wet season (October to March), which is three times more (51 mm) than that in the dry season(April to September). Therefore, a dilution of the river contents takes place. What is more, the average water temperature is 22°C in the dry season, which is almost twice as high as that in the wet season (12.8°C), and is advantageous to the biodegradation of pharmaceuticals in water [52].

Caffeine presented its highest concentrations in spring (1002 ng/L) and summer (1517 ng/L) and the lowest in winter, concluding that its concentrations might depend on the dilution due to high rainfall in the winter period. In addition, high concentrations in spring and summer might be attributed to the fact that in spring, coffee, tea and various beverages are consumed even cold or hot.

Salicylic acid also showed higher concentrations in spring and summer than in autumn and winter, concluding that dilution due to high rainfall in the winter period played a significant role [20]. The extensive usage of the parent compound, acetylsalicylic acid, in this period, also explains these levels given its degradation to salicylic acid. This observation could be assigned to the lower water flow of the river during spring and the increased degradation of acetylsalicylic acid after an increased consumption and release period during winter [21].

Carbamazepine presents poor elimination in WWTPs and thus is expected in relatively high concentrations in surface waters all over the year. Nevertheless, carbamazepine presented its highest concentrations in spring and lowest in winter, suggesting the effect of dilution, as carbamazepine is very stable against other natural processes such as photodegradation and biodegradation [20].

Ibuprofen showed higher mean concentrations in autumn, when analgesics are consumed more irrationally to encounter and protect against viral diseases, while gemfibrozil presented higher mean concentrations in summer.

Finally, concentrations of triclosan were higher in spring and summer, as the use of shampoos, soaps, deodorants, etc., is more in this period [20]. At this point, it is also remarkable that triclosan is liable to biodegradation and adsorption onto suspended matter (relatively high partition coefficient, log K_{ow} = 4.8); therefore, it is rarely determined in waters. As it was reported in the literature, lower contribution of rainfall and in addition urban run-off to dilution

Table 2. Seasonal occurrence (mean values in brackets) and % positive findings (PFs) per season of pharmaceuticals in surface water samples (ng/L) from the Kalamas River and Lake Pamvotis.

		min–max (mean)							
Therapeutic group	Compound	Spring	(%) PFs	Summer	(%) PFs	Autumn	(%) PFs	Winter	(%) PFs
Analgesics/Antipyretics/ NSDAIDs	Paracetamol	n.d.–146 (64)	62.5	9–156 (59)	100	34–142 (80)	100	n.d.–91 (bql)	50
	Phenazone	n.d.–bql (bql)	12.5	n.d.–95 (12)	12.5	n.d.–56 (bql)	12.5	n.d.–71 (bql)	12.5
	Salicylic acid	905–3001 (1567)	100	674–2005 (1356)	100	99–991 (569)	100	105–842(419)	100
	Diclofenac	n.d.–175 (bql)	87.5	n.d.–98.0 (bql)	87.5	n.d.–457 (89)	37.5	n.d.–132 (bql)	37.5
	Ibuprofen	bql	100	32–502(bql)	100	n.d.–1351 (374)	75	n.d.–bql (bql)	50
	Ketoprofen	n.d.–bql (bql)	37.5	n.d.–91 (bql)	37.5	n.d.–bql (bql)	37.5	n.d.–bql (bql)	37.5
	Indomethacin	n.d.	0	n.d.	0	n.d.	0	n.d.	0
	Mefenamic acid	n.d.–bql (bql)	25	n.d.–bql (bql)	25	n.d.–bql (bql)	12.5	n.d.–bql (bql)	12.5
Lipid regulators	Gemfibrozil	n.d.–303 (bql)	87.5	n.d.–602 (163)	62.5	n.d.–200 (bql)	37.5	n.d.–541 (bql)	87.5
	Fenofibrate	n.d.–34 (bql)	25	n.d.–78 (bql)	37.5	n.d.–46 (bql)	25	n.d.–91(bql)	25
	Bezafibrate	n.d.–bql (bql)	62.5	n.d.–bql (bql)	75	n.d.	0	n.d.	0
Antibiotics	Sulfamethazine	n.d.	0	n.d.	0	n.d.	0	n.d.	0
	Sulfamethoxazole	n.d.–190 (45)	25	n.d.–68 (bql)	62.5	n.d.–68 (bql)	25	n.d.–120 (bql)	12.5
	Ciprofloxacin	n.d.	0	n.d.	0	n.d.–115 (bql)	25	n.d.–bql (bql)	25
	Erythromycin	n.d.	0	n.d.	0	n.d.–137 (bql)	25	n.d.–65 (bql)	25
Antiepileptic	Carbamazepine	n.d.–406 (141)	62.5	n.d.–57 (bql)	37.5	n.d.–122(bql)	12.5	n.d.–89 (bql)	25
Antipsychotic	Risperidone	n.d.–bql (bql)	37.5	n.d.–bql (bql)	37.5	n.d.–bql (bql)	12	n.d.	50
Psychomotor stimulant	Caffeine	198–1730 (1002)	100	124.9–3508 (1516)	100	257–1189 (618)	100	230–560 (438)	100
Glucocorticoid steroid	Budesonide	n.d.–bql (bql)	37.5	n.d.–bql (bql)	87.5	n.d.–bql (bql)	12.5	n.d.–bql (bql)	75
Disinfectant	Triclosan	n.d.–131 (bql)	12.5	n.d.–150 (bql)	12.5	n.d.	0	n.d	0
Oestrogen	Oestriol	n.d.–bql (bql)	25	n.d.–bql (bql)	12.5	n.d.–bql (bql)	12.5	n.d.	0
Beta-blocker	Atenolol	n.d.–bql (bql)	25	n.d.	0	n.d.–bql (bql)	37.5	n.d.	0
H2 receptor antagonist	Cimetidine	n.d.–bql (bql)	50	n.d.	0	n.d.–20 (bql)	12.5	n.d.	0

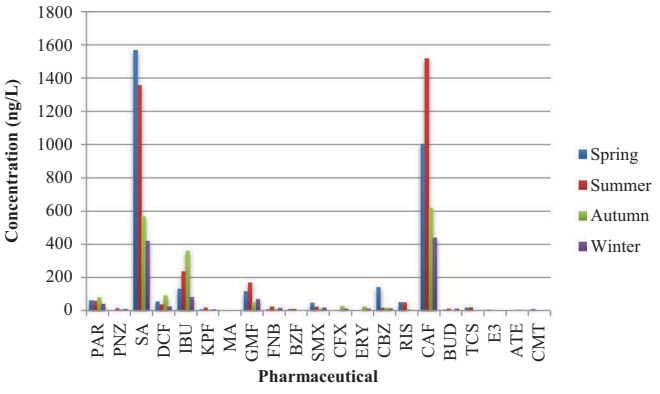

Figure 2. Average concentrations of PPCPs in the River Kalamas and Lake Pamvotis for each sampling month.

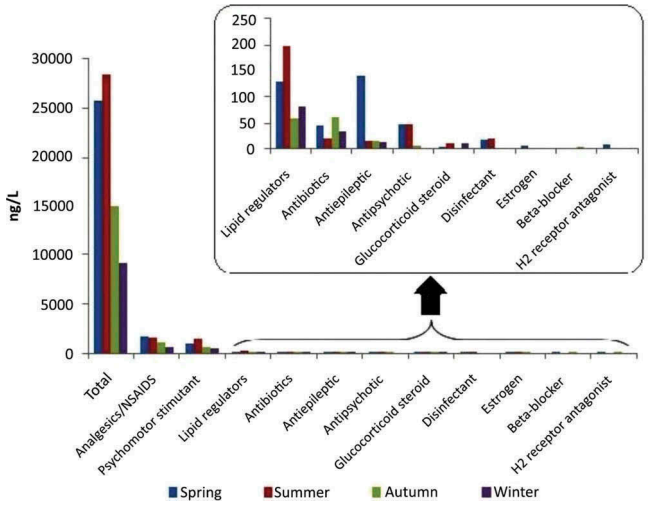

Figure 3. Seasonal total variation for all drugs analysed and for each therapeutic group.

of the influents in the WWTP of the city, might lead to higher concentrations in the summer [20].

Antibiotics showed an evidently higher concentration during autumn and winter, since they are mainly used during this period to treat various seasonal diseases, except for sulfamethoxazole, which showed slightly higher concentrations in spring.

In brief, seasonal variation could be attributed to various factors, both anthropogenic and environmental. In the first case, different needs for pharmaceuticals according to the seasonal illnesses are included, i.e. the decreased need for antibiotics and NSAIDs during summer. In the second case, the fate of pharmaceuticals is directly related to factors such as temperature, radiation, precipitations and degradation processes, namely hydrolysis, photodegradation and biodegradation. Sorption on sediments and suspended matter and wastewater treatment removal efficiency are also of crucial importance for the fate of pharmaceuticals. Thus, it is important to consider which processes are favoured at high temperature, e.g. the photodegradation and

biodegradation increase depending on the UV radiation, while hydrolysis is rather more efficient in lower temperatures [53].

The aforementioned results of this extensive study on the determination of pharmaceuticals in the River Kalamas and Lake Pamvotis illustrate that these closely connected ecosystems are affected to a great extent by anthropogenic activities in the surrounding urban and industrial areas.

3.3. *Ecological risk assessment*

Both acute and chronic toxicity data for algae, invertebrates and fish were employed in the present study so that RQs can be calculated and constitute a tool for ecotoxicological risk assessment. These literature data are shown in Tables SD6 and SD7.

The reason why there is a lack of chronic toxicity data for many of these compounds is due to the fact that pharmaceuticals have been only recently considered as emerging contaminants. Thus, this is a crucial disadvantage for the determination of the risk assessment, since chronic effects are much more likely to be induced rather than acute ones [19]. Hence, some of the pharmaceuticals for which the literature data are deficient have been omitted from Figures 4 and 5.

According to the results obtained for the three levels of aquatic life, five of the selected pharmaceutical compounds seem to pose high risk. To be more specific, salicylic acid, triclosan, erythromycin and sulfamethoxazole pose high acute risk, demonstrating $RQ > 1$, and algae seem to be the most vulnerable species. In terms of chronic toxicity, salicylic acid, caffeine and erythromycin are predominant while the highest RQ values were obtained for fish. The effects that the above-mentioned pharmaceuticals exert in aquatic organisms are various. For instance, regarding to acute risk, it is reported that triclosan disrupts bacterial fatty acid synthesis that can be found in both bacteria and plants. Sulfamethoxazole and sulphonamides in general, have been proved to inhibit folate synthesis pathways in both plants and bacteria. Concerning the chronic risk, erythromycin, as a macrolide antibiotic, inhibits protein synthesis. Salicylic acid and other analgesics have been investigated to identify potential long-term toxic effects in aquatic organisms. The mechanism of action for vertebrates is carried out via the inhibition of cyclooxygenase (COX-1 or COX-2), which is effected by inhibiting prostaglandin synthesis. Finally, chronic exposure to caffeine in many aquatic species has not resulted in any observed effects at concentrations below 1 mg/L [54]. According to a recent study concerning the Greek waters also affected by WWTPs, triclosan and caffeine also presented high RQ levels (in algae), with caffeine presenting the higher one [55].

It is remarkable that although caffeine is found at high levels in environmental matrices, its effect on freshwater organisms is not well documented. According to a study [56] reporting environmental concentrations half-lives and caffeine's lethal and sublethal effects on the freshwater species *Ceriodaphnia dubia, Pimephales promelas*, and *Chironomus dilutus*, caffeine may pose rather negligible risk for most aquatic vertebrates and invertebrates. Consequently, it does not seem to be a threat for freshwater organisms given its current presence in the aquatic environment. Given that its mean half-life is approximately 1.5 days, caffeine levels in streams may not persist such that they will have potential for long-term exposure effects. However, even a quickly degradable drug can act as a persistent chemical. If caffeine is profusely discharged from anthropogenic sources into an environment, it could constantly replenish levels regardless of the amount of caffeine degraded, creating a dynamic equilibrium. Hence, future ecotoxicological research might include potential synergistic, additive or antagonistic effects of caffeine with other compounds.

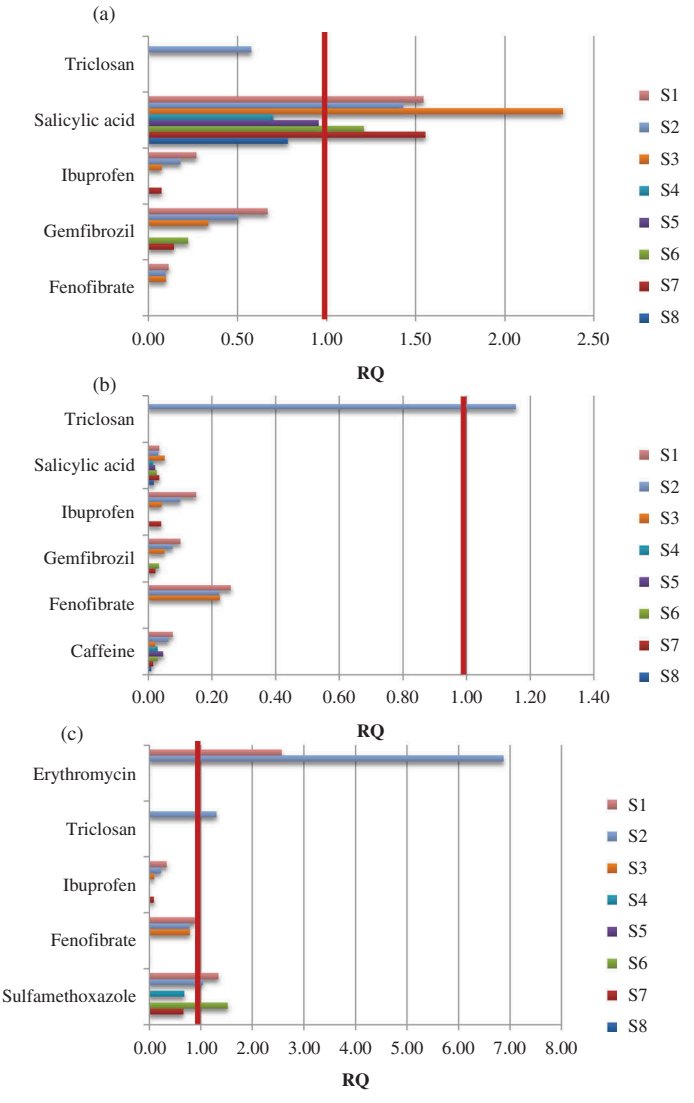

Figure 4. RQs for pharmaceuticals posing significant environmental risk in surface waters estimated for fish (a), invertebrates (b) and algae (c), for acute toxicity.

From the results mentioned above, salicylic acid seems to be of crucial importance since it contributes both to acute and chronic toxicities. The compounds presenting high risk potentials indicate that further research about impact on surface waters, regulatory monitoring and prioritisation on the basis of realistic PNECs should be implemented [57].

However, the estimations made in this study are based on the RQ of a single pharmaceutical. According to Pomati *et al*. [58], the assessment of the risk that the mixture of various pharmaceuticals poses is an issue of concern. The joint ecotoxicity of such chemical cocktails is typically higher than the toxicity of each individual compound [14]. Furthermore, it is claimed that the RQ of the mixture is often more than a factor of 1000 higher than that of a randomly selected pharmaceutical [12]. Since pharmaceuticals do not occur as individual

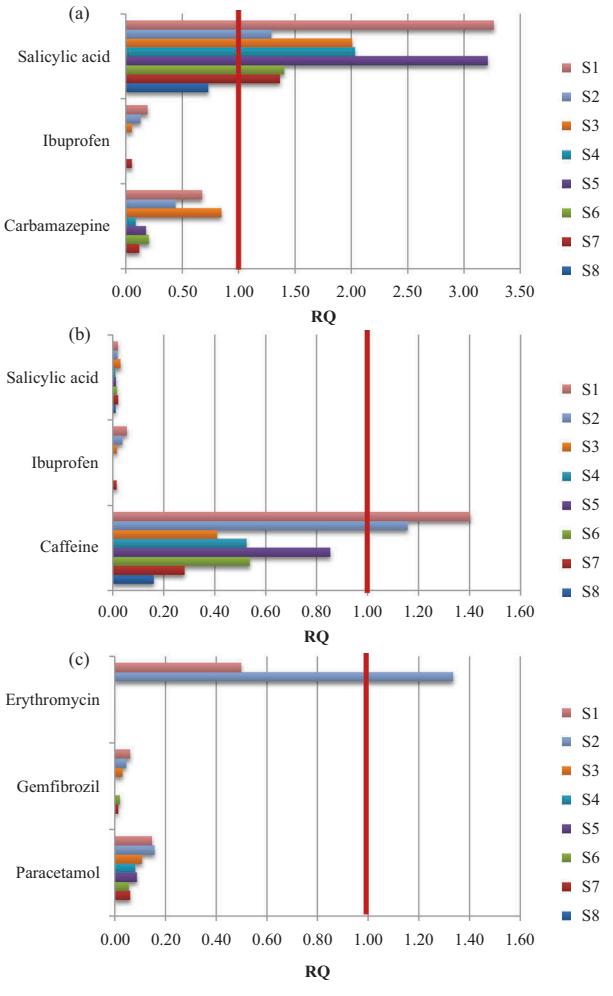

Figure 5. RQs for pharmaceuticals posing significant environmental risk in surface waters estimated for fish (a), invertebrates (b) and algae (c), for chronic toxicity.

substances in an environmental compartment, but a variety of human and veterinary substances are simultaneously used in a given area, there is an obvious need to estimate the overall risk posed by all of the pharmaceuticals present [42]. To the best of our knowledge, this work was the first attempt to investigate the occurrence of pharmaceuticals in these ecosystems as well as to provide preliminary information about environmental risks posed by PPCPs.

4. Conclusions

The present work elucidates the occurrence and environmental risk assessment of 23 pharmaceuticals belonging to various therapeutic classes. The monitoring study was carried out in eight different sampling points along the River Kalamas and Lake Pamvotis, in the region of Epirus (Northwestern Greece), on a seasonal basis. According to the results, the majority of the target compounds were detected, indicating in this way the widespread occurrence of pharmaceuticals in such matrices.

All the selected compounds were present in the samples, except for sulfamethazine and indomethacin. Concentrations ranged from levels bql to 3506 ng/L, with caffeine and salicylic acid being the ubiquitous compounds. The highest concentration corresponded to the psychomotor stimulant caffeine during the summer sampling period. As for the rest of the compounds, it seems that paracetamol, ibuprofen and diclofenac were also frequently detected. With respect to seasonal variation, generally, a discrepancy was observed between dry and wet seasons, probably because of the rainfall during the winter period, among other factors. Higher concentrations were also observed for antibiotics during autumn and winter, as was mentioned before. Although the environmental concentrations of drugs seem to be low, reaching trace levels, the presence of these compounds in mixtures, in combination with their potent pharmacological activities, render them as toxic contaminants for the aquatic organisms.

Results obtained from the environmental risk assessment section showed high acute and chronic risk for some of the investigated compounds such as salicylic acid, caffeine, triclosan, erythromycin and sulfamethoxazole, proving in this way that aquatic ecosystems may be affected to a great extent by such contaminants. Regarding to acute toxicity, algae were the most critical species, while for chronic toxicity, fish were the most important ones. Therefore, the applied method enables the prediction of pharmaceutical residue impacts on the environment, providing in this way a helpful tool to manage properly the surrounding area and take appropriate measures so that the impact posed by pharmaceutical residues can be minimised. Further research should be carried out in order to investigate the contamination caused by metabolites or TP of the active compounds which sometimes may be more toxic than the parent ones [59].

To sum up, taking into consideration the number of the positive findings for the selected compounds, their concentration levels and the potential threat they may pose both to aquatic life and humans in our first attempt to investigate various pharmaceuticals in surface waters in Northwestern Greece, more extensive studies on the occurrence, toxicological impact of pharmaceuticals and their metabolites in surface waters and their effects on public health are required. In future work, more substances should be included so that a prioritisation of contaminants can be feasible. To gain a better understanding of the distribution of the selected pharmaceuticals in fresh waters, the relative importance of different sources of these pharmaceuticals, their seasonal behaviour and the processes that may affect their transport once they enter the aquatic environment should be further researched.

Disclosure statement

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Supplemental data

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