



Pharmaceutically active compounds (PhACs) in surface water: Occurrence, trends and risk assessment in the Tagus River Basin (Spain)

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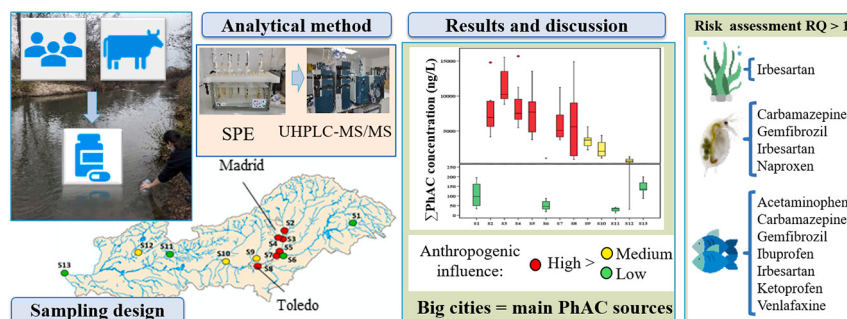
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HIGHLIGHTS

- 22 PhACs and one metabolite were analyzed in surface water from a Tagus river.
- Water contamination by PhACs was strongly related to urban activities.
- Antihypertensives and antidepressants showed the highest concentration levels.
- Valsartan and naproxen levels were lower in the drier (spring and summer) seasons.
- 7 PhACs showed high risk for fish in >45 % of investigated locations.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Yolanda Picó

Keywords:

Pharmaceuticals
Emerging pollutants
Tagus River Basin
River water
UHPLC-MS/MS
Environmental risk

ABSTRACT

In this study, the presence of 23 pharmaceutically active compounds (PhACs) including antibiotics, analgesics, anti-inflammatories, psychiatric and cardiovascular drugs, antifungals and metabolites was investigated in surface waters. A total of 89 samples were collected during 3 years (2020, 2021 and 2022) from a European representative river basin (Tagus, Spain). To elucidate PhAC potential sources, sampling points located in areas with low, median and high anthropogenic influence were selected. The analytical method based on solid phase extraction (SPE) followed by UHPLC-MS/MS analysis was validated meeting SANTE/2020/12830 and SANTE/12682/2019 performance criteria. PhACs were quantified above limits of quantification (LOQs) in 96 % of water samples, being the antihypertensives valsartan (648 ng/L, 87 % quantification frequency) and irbesartan (390 ng/L, 75 %) and the antidepressant *o*-desmethylvenlafaxine (495 ng/L, 76 %) the predominant pollutants. The rest of the target PhACs showed median concentrations between 4 and 172 ng/L with quantification frequencies ranging from 35 to 75 %. Σ PhAC concentrations did not show temporal or seasonal trends. However, valsartan and naproxen presented lower levels in drier (spring and summer) compared to the wetter. Source identification revealed a clear anthropogenic origin since concentrations obtained in highly populated areas were statistically higher ($p < 0.01$) than those quantified in sparsely populated ones. This finding was also confirmed by calculating PhACs mass flow rates, which ranged between 1.4 and 235 kg/y. Finally, data generated were used to estimate the potential risk to the aquatic ecosystem for three trophic levels (phototrophic, invertebrate and vertebrate organisms). Risk quotient ratios (RQs) were calculated for all PhACs at the median (P50) and worst-

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case (max) scenarios. Up to 7 PhACs (acetaminophen, carbamazepine, gemfibrozil, ibuprofen, irbesartan, ketoprofen and venlafaxine) showed high risk for the highest trophic level (fish) in >45 % of investigated locations.

1. Introduction

Pharmaceutical active compounds (PhACs) have contributed to improvements in life expectancy and quality of life of many patients. For this reason, their consumption in medical and veterinary practices has been increasing for decades, driven by a growing need for drugs to cure, relieve symptoms, delay the onset of diseases and prevent chronic and age-related complications (OECD, 2021; Jelic et al., 2009). However, due to discharge into the environment, through pharmaceutical manufacturing, consumption and excretion, and improper disposal of unused or expired products, medicines can reach non-target organisms and unintentional harmful impacts may occur (OECD, 2019). Nonetheless, of all potentially impacted ecosystems, the freshwater compartment would be the most affected by these practices (Sadutto and Picó, 2020). Urban, industrial and hospital effluents will reach the wastewater treatment plants (WWTPs) contaminated with PhACs and there, the lack of appropriate treatments for efficient removal, causes these facilities to discharge their polluted effluents into the rivers (Sadutto and Picó, 2020; Rico et al., 2019; De Barros et al., 2018; Valcárcel et al., 2013; Rúa-Gómez and Püttmann, 2012). For this reason, the scientific community identifies with a broad consensus the WWTP effluents as the main PhAC pollutants source for waterbodies (Lopez et al., 2022; De Barros et al., 2018; Silva et al., 2011).

At least 2000 active pharmaceutical ingredients are being administered worldwide in prescription medicines, over-the-counter therapeutic drugs and medicines for veterinary use (Burns et al., 2018). Nevertheless, from all of them, four categories of medicines stand out that address illnesses whose prevalence has increased notably in OECD countries in recent decades (OECD, 2021). Consumption of antihypertension drugs such as atenolol, irbesartan, metoprolol or valsartan increased by 65 % on average between 2000 and 2019 in OECD countries (OECD, 2021). Probably due to the improved recognition of health mental problems, consumption of psychiatric drugs such as anti-epileptics (carbamazepine) and anti-depressants (venlafaxine or *o*-desmethylvenlafaxine) have doubled in the last two decades. The use of lipid-modifying agents, such as cholesterol-lowering medicines (atorvastatin or gemfibrozil), was even greater with consumption increased by a factor of nearly four. Other drugs of great concern are antibiotics (azithromycin, clarithromycin, erythromycin, sulfamethoxazole and trimethoprim) because of their contribution to antimicrobial resistance through overuse or misuse (OECD, 2019). In some cases, substances with antifungal properties like thiabendazole, are also used as plant protection products (PPP), expanding the possible sources of emissions to the ecosystem (European Commission, 2009). Finally, analgesics and non-steroidal anti-inflammatory drugs are also important since their consumption has grown significantly in the last decade (Ahmed et al., 2023; OECD, 2023).

PhAC degradation times range from days to months (Table S1). However, aquatic organisms may be chronically exposed to them because they are continuously being discharged to surface waters causing unintended, undesired adverse effects, including mortality, as well antibiotic resistant bacteria (Arenas-Sánchez et al., 2019), endocrine disruption (Lopez et al., 2022; McEneff et al., 2014; Gros et al., 2012) and changes in physiology (Chabchoubi et al., 2023) and behavior (Brodin et al., 2014). In addition, the presence of chemicals in rivers has been reported as an important pathway for the transport and mobilization of pollutants to seas and oceans (Navarro et al., 2020) but before this happens treated water use in agricultural activities or even for human consumption may arouse high preoccupation. In line with the latter scenario, the recast Drinking Water Directive (EU) 2020/2184

recognized a growing public concern about the effects of emerging compounds including pharmaceuticals on human health through the use of water intended for human consumption and adopted on 19 January 2022, the first watch list that includes 17-beta-estradiol, a medication and naturally occurring steroid hormone (European Commission, 2022a). Similarly, the Water Framework Directive (WFD) has protected EU surface waters from chemical pollution since 2000, establishing a watch list mechanism to improve the available information on identifying the substances of greatest concern, and forcing Member States to monitor the substances on the list at least once per year for up to four years. The watch lists (2015–2022) have included PhACs and some of them (azithromycin, carbamazepine, clarithromycin, erythromycin and ibuprofen) have been then moved to the list of priority substances in the proposal for a Directive amending the Water Framework Directive, the Groundwater Directive and the Environmental Quality Standards Directive published in October 2022 (European Commission, 2022b). Currently, the proposal for a Directive amending the WFD and the watch list includes 9 and 11 PhACs, respectively. However, to the best of the author's knowledge, there are no maximum levels in the European Union for the presence of these chemicals in WWTP effluents. Therefore, aging populations, advances in healthcare, and intensification of meat and fish production spurring the demand for medicines worldwide (OECD, 2019) allows us to predict an increase in the release of pharmaceuticals into the environment, both in areas heavily burdened by these anthropogenic activities and in more remote areas such as Antarctica (Postigo et al., 2023). The presence of these chemicals, even in low quantities, may pose a risk to the environment and therefore it is mandatory to address their occurrence in all environmental compartments. For this reason, the present study aimed to assess the presence of 22 PhACs and one metabolite in surface water from a highly anthropogenic watershed. Potential sources, influence of land uses, WWTP discharges and/or presence of livestock farms in quantified concentrations were investigated. Besides, the effect of flow rates and the impact on temporal and seasonal variations were evaluated. Finally, data generated were used to evaluate the risk associated with their presence in the freshwater ecosystem.

2. Material and methods

2.1. Reagents and chemicals

Native and deuterated standards were purchased from LabStandard (Barcelona, Spain) (acetaminophen, atenolol, atorvastatin, azithromycin, gemfibrozil, ibuprofen, ketoprofen, metoprolol, miconazole, venlafaxine, acetaminophen-d3 and atenolol-d7), LGC Dr. Ehrenstorfer (Augsburg, Germany) (carbamazepine, clarithromycin, clotrimazole, *o*-desmethylvenlafaxine, erythromycin, fluconazole, irbesartan, naproxen, sulfamethoxazole, thiabendazole, trimethoprim, valsartan, ibuprofen-d3 and sulfamethoxazole-d4), Trc Canada (North York, ON, Canadá) (gemfibrozil-d6) and LGC LoGiCal (Luckenwalde, Germany) (venlafaxine-d6). Methanol (HPLC grade) was purchased from J.T. Baker (Phillipsburg, NJ, USA). Acetonitrile (HPLC grade), formic acid (98 %) and ammonium acetate were purchased from Scharlab SL (Barcelona, Spain). Oasis HLB (6 mL, 500 mg) cartridges were obtained from Waters (Milford, MA, USA).

Individual stock solutions were in acetonitrile, except gemfibrozil which was in hexane and miconazole, metoprolol, thiabendazole, venlafaxine, acetaminophen-d3, atenolol-d7 and venlafaxine-d6 which were in methanol, all of them at a concentration of 100 mg/L. These solutions were used for preparing two working standard solutions,

native and deuterated mixtures separately, both at a concentration of 1 mg/L in acetonitrile. All standard solutions were stored at -20°C .

2.2. Study area and sample collection

The selected watershed was the Spanish Tagus Basin, which is the third largest in the Iberian Peninsula, occupying an area of 55,800 km² in Spain. It includes cities with high (Madrid, 3.3 million inhabitants, [Instituto Nacional de Estadística \(INE\), 2022](#)), medium (Toledo, 0.08 million inhabitants) and low (Aranjuez, 59,762 inhabitants) population and areas under industrial pressure, or with agriculture and livestock production importance. Anthropogenic discharges threaten the quality of the basin's ecosystems and several pollutants, including perfluoroalkylated substances ([Navarro et al., 2020](#)) and neonicotinoids ([Casillas et al., 2022](#)) have been identified in its waters. Tagus Basin is located in an area characterized by a Mediterranean climate strongly continental, with hot dry summers and cold winters, and is experiencing serious climate change suffering a decrease in rainfall and making droughts a recurring phenomenon. The river flows through ecosystems with rich flora and fauna including natural and conservation reserves, such as the Alto Tajo Natural Park, the Monfragüe National Park, the International Tagus Natural Park and the Tagus Estuary Natural Reserve ([Navarro et al., 2020](#)). For these reasons, the selection of this study area is appropriate to evaluate PhAC potential risk on the aquatic ecosystem.

A total of 89 surface water samples were collected between 2020 and 2022 in thirteen locations (S1–S13) along the Tagus River Basin ([Fig. 1](#), and supplementary material (SM) Table S2). Sampling points were selected to represent areas with low (S1, S6, S11 and S13), medium (S9, S10 and S12) and high anthropogenic influence (S2 to S5, S7 and S8). This classification was based on land uses (urban, crops, forest...), the presence of WWTPs (from <2000 to >150,000 equivalent inhabitants) and livestock farms (from <10 to >50) of the watershed ([Geo Portal, 2023](#)). Complete details are described in the SM and [Fig. S2](#). Annual sampling campaigns were carried out during the autumn of 2020, 2021

and 2022. In addition, during 2022 seasonal monitoring (winter, spring, summer and autumn) was conducted to investigate seasonal changes in PhAC levels. Water samples were collected with polypropylene bottles and, once in the laboratory, were frozen at -20°C until analysis. Additionally, nine field blanks were also monitored in a sampling point with high anthropogenic influence (S3) and a remote location (S12).

2.3. Chemical analysis

The selection of target analytes was conducted with a strengths, weaknesses, opportunities, and threats (SWOT) analysis with data collected from an extensive bibliographic review and results of interviews with experts (scientists, companies, legislators, government technicians, worker associations and environmental-ecological groups) ([CEMEF, 2023](#)). The following method was validated for the determination of target analytes. Water samples (1 L) were filtered through glass fiber filters under negative pressure before extraction and spiked with 50 ng of deuterated internal standards (acetaminophen-d3, atenolol-d7, gemfibrozil-d6, ibuprofen-d3, sulfamethoxazole-d4 and velafaxine-d6). Extraction and purification were conducted on an Oasis HLB SPE cartridge (6 mL, 500 mg; Waters, Milford, MA, USA) preconditioned with 10 mL of methanol and 10 mL of Milli-Q water. Then, the sample was loaded onto a cartridge and let dry for 1 h. Finally, analytes were eluted using 10 mL of methanol and concentrated to 300 μL under a gentle nitrogen stream. Details related to SPE elution volume optimization are shown in SM.

2.4. Instrumental determination

PhAC chromatographic separation was carried out by an ultra high performance liquid chromatography (UHPLC) ExionLC system (SCIEX, MA) using a Luna® Omega 1.6 μm C18 100 Å column (100 $\times$ 2.1 mm i.d, Phenomenex). UHPLC system was coupled to a Triple Quad™ 3500 MS/MS System (SCIEX, MA) equipped with a Turbo V™ ion source (SCIEX,

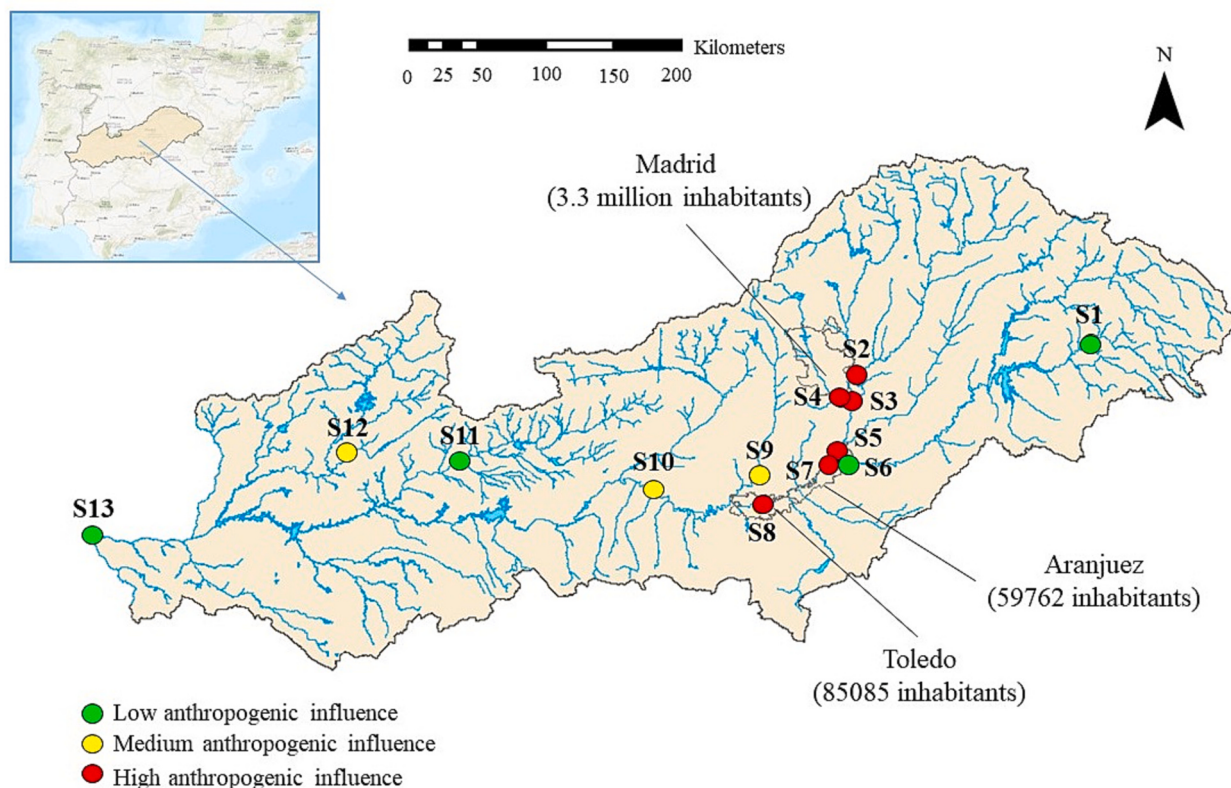


Fig. 1. Geographical sample distribution in the studied area (Tagus River Basin).

MA). Several mobile phases were tested (Table S4) but formic acid 0.02 % (A) and methanol (B) were the solvents that gave the sharpest and more symmetrical peaks at a flow rate of 0.350 mL/min. The optimized gradient program was: 5 % of B for 1.0 min, a linear gradient up to 95 % of B in 8.5 min (kept 5.5 min) and finally back to initial conditions (5 % of B). The target compounds were determined in multiple reaction monitoring (MRM) mode. Two transitions were acquired for each target analyte and mass spectrometry (MS) parameters including declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) were also optimized.

2.5. QA/QC and statistical analysis

The validated method meets performance criteria as indicated in SANTE/2020/12830 and SANTE/12682/2019. Limits of quantification (LOQs), defined as the lowest validated concentration fulfilling criteria for recovery (70–120 %), precision ($RSD \leq 20$ %), and identification (retention time within ± 0.1 min of standards and MS/MS ion ratio within 30 %) were 5 ng/L for all PhACs except ibuprofen (25 ng/L) (Table S8). Limits of detection (LODs), as the concentration giving a signal to noise ratio of 3:1 were calculated from the lowest validated level (LOQ). All LODs were well below the maximum acceptable limits in the detection method defined for PhACs in the watch list for Union-wide monitoring in the field of water policy (European Commission, 2022c). Linearity was assessed by calibration curves which were generated using linear regression analysis over the established concentration range (0.2–400 ng/L). The complete results of validation experiments are shown in SM. Field and procedural blanks were processed and analyzed with every batch of samples under the same conditions. In addition, instrumental blanks consisting of methanol injections were run before sample injection to check memory effects and contamination from the chromatograph system. Concentrations in the field, procedural and instrumental blanks were below LODs for all analytes.

Statistical analyses were carried out with SPSS 14.0 for Windows. Analyte concentrations were not normally distributed (Shapiro-Wilk W and Kolmogorov-Smirnov tests), hence Spearman rank correlation coefficient was derived to investigate bivariate relationships. For exploring bivariate associations (Spearman test) values <LOQs were removed. However, for the descriptive statistical analysis, PhACs with quantification frequencies (Qf) above 30 %, values <LOQs were replaced by LOQs divided by the square root of 2. Principal component analysis (PCA) and Kruskal-Wallis H-test were run to evaluate temporal, seasonal and geographic distributions.

2.6. Calculation of risk assessment parameters

In order to assess the potential risk of PhACs for aquatic organisms, risk quotients (RQs) were calculated for three trophic levels (phototrophs, invertebrates and vertebrates). RQs were calculated at worst (RQ_{max}) and general (RQ_{50}) scenario as the ratio between the measured environmental concentration at maximum (MEC_{max}) or median (MEC_{50}) concentrations and the predicted no effect concentration (PNEC) (Eq. (1)) according to Technical Guidance Document on Risk Assessment of the European Chemicals Bureau (European Commission, 2003). PNECs were calculated by dividing toxicological data by an assessment factor (AF) as reported by Molnar et al., 2021 (Eq. (2)). Complete details are shown in SM.

$$RQ_{max} = \frac{MEC_{max}}{PNEC} \text{ or } RQ_{50} = \frac{MEC_{50}}{PNEC} \quad (1)$$

$$PNEC = \frac{(NOEC \text{ or } LOEC \text{ or } E(L)C50)}{AF} \quad (2)$$

3. Results and discussion

3.1. PhAC distribution in river water

The main statistics descriptives of PhAC concentrations are shown in Table 1. In addition, PhAC levels at each location are depicted in Fig. 2 and detailed for all sampling campaigns in Table S10. PhACs were quantified above LOQs in 96 % of water samples. Most of the PhACs were quantified in >50 % of the samples, but valsartan, *o*-desmethylvenlafaxine and irbesartan presented the highest concentration levels (648, 390 and 495 ng/L, respectively; median) and Qf above 75 %. Quantification frequencies decreased for the antibiotic erythromycin (37 %) and its metabolite anhydroerythromycin (49 %), and the anti-inflammatories ibuprofen and ketoprofen that resulted on Qfs of 47 % and 35 %, respectively. The lipid regulator atorvastatin and the fungicides miconazole and clotrimazole were not detected in any sample, which could be associated with their low water solubility (<0.05 mg/L; Table S1).

Of all investigated substances, antidepressants and antihypertensives stand out. The antidepressant venlafaxine was quantified in 69 % of the samples with a median concentration of 127 ng/L but also *o*-desmethylvenlafaxine presented high quantification frequency (76 %) and a four times higher median concentration (495 ng/L) than venlafaxine. The presence of *o*-desmethylvenlafaxine in surface water could be attributed to two origins. Firstly, *o*-desmethylvenlafaxine is used as the active ingredient in some antidepressants but it could be attributed to a metabolic origin since around 30 % of a venlafaxine dose is excreted in the urine as *o*-desmethylvenlafaxine (Rúa-Gómez and Püttmann, 2012), and therefore venlafaxine consumption could indirectly contribute to increase levels of *o*-desmethylvenlafaxine in surface water. It is worth to mention that similar ($p > 0.05$) *o*-desmethylvenlafaxine/venlafaxine ratios (3.8 ± 1.8 ; mean $\pm$ sd) were obtained among sampling points. The use of antidepressants has increased in recent years in most developed regions such as Europe, Canada, Australia and Chile (OECD, 2023). In Spain, >23 million packs were sold in 2022, 16 % more than in 2020, confirming this growing trend in antidepressant consumption (Table S11). However, even though both antidepressants are included in the WFD's watch list since 2020, studies reporting concentrations of *o*-desmethylvenlafaxine are scarce (Table S12).

Elevated blood pressure levels are highly prevalent and the major reason for cardiovascular events worldwide (Mills et al., 2020). For this reason, it is not surprising that the antihypertensive agents valsartan and irbesartan presented high quantification frequencies (>75 %) and median concentrations of 648 and 390 ng/L, respectively. However, results of special interest the fact that beta-blockers atenolol and metoprolol also used to treat hypertension were quantified in fewer samples (66 % and 58 %) and showed lower ($p < 0.01$) concentration levels (97 ng/L for atenolol and 14 ng/L for metoprolol) compared to angiotensin receptor blockers (ARBs; valsartan and irbesartan) even though beta-blockers investigated in the present study are thousand times more soluble in water (Table S1). This result may reflect a higher use of ARBs compared to beta-blockers. As happened for hypertension, high blood cholesterol levels are one of the causes that increase the risk of cardiovascular disease, which is the leading cause of death globally (Jung et al., 2022; WHO, 2021). Two lipid regulators were investigated in the present study. Gemfibrozil was quantified in 74 % of the samples with a median concentration of 133 ng/L. In contrast, atorvastatin, which had the highest consumption data (>60 million packs per year, Table S11), was below LODs in all samples. In this case, its low water solubility (Table S1) and rapid photodegradation under light conditions (Klementová et al., 2021) could play an important role in this result.

According to the World Health Organization, antibiotic resistance is one of the biggest threats to global health, food security, and development today (OECD, 2021). Therefore, macrolide (azithromycin, clarithromycin and erythromycin), sulfonamides (sulfamethoxazole) and diaminopyrimidines (trimethoprim) antibiotics were investigated in

Table 1Concentration (mean $\pm$ sd, median, minimum-maximum; ng/L) and quantification frequency (samples % > LOQ) of PhACs analyzed.

Group	PhACs	Mean $\pm$ sd (ng/L)	Median (ng/L)	Min – max (ng/L)	QF (%)	LOQ (ng/L)
Antidepressants	O-Desmethylvenlafaxine	593 $\pm$ 663	495	<0.1–2907	76	5
	Venlafaxine	168 $\pm$ 190	127	<0.3–1079	69	5
Antiepileptics	Carbamazepine	65 $\pm$ 62	59	<0.1–262	75	5
Antibiotics	Azithromycin	16 $\pm$ 20	5	<0.7–115	52	5
	Clarithromycin	18 $\pm$ 22	6	<0.1–115	56	5
	Erythromycin	24 $\pm$ 81	4	<0.2–609	37	5
	Anhydroerythromycin ^a	276 $\pm$ 1056	4	<0.2–7996	49	–
	Sulfamethoxazole	101 $\pm$ 162	62	<0.1–1274	73	5
	Trimethoprim	44 $\pm$ 58	14	<0.1–242	61	5
Antihypertensives	Atenolol	244 $\pm$ 422	97	<0.4–3167	66	5
	Irbesartan	404 $\pm$ 452	390	<0.1–2100	75	5
	Metoprolol	25 $\pm$ 30	14	<0.1–123	58	5
	Valsartan	1147 $\pm$ 1428	648	<0.2–6543	87	5
	Atorvastatin	n.d.	n.d.	n.d.	–	5
Lipid regulators	Gemfibrozil	228 $\pm$ 285	133	<0.3–1473	74	5
Analgesics	Acetaminophen	56 $\pm$ 180	9	<0.8–1168	58	5
Anti-inflammatories	Ibuprofen	150 $\pm$ 300	18	<5.2–1800	47	25
	Ketoprofen	67 $\pm$ 136	4	<1.6–607	35	5
	Naproxen	80 $\pm$ 102	47	<1.2–554	72	5
Antifungal	Clotrimazole	n.d.	n.d.	n.d.	–	5
	Fluconazole	530 $\pm$ 1345	172	<0.1–9745	75	5
	Miconazole	n.d.	n.d.	n.d.	–	5
	Thiabendazole	17 $\pm$ 21	8	<0.1–107	58	5
	Σ PhACs	213 $\pm$ 613	21	4–9745	96	

n.d.: not detected.

^a Anhydroerythromycin did not fulfill all validation requirements (SANTE/2020/12830 and SANTE/12682/2019) and therefore reported concentrations should be considered semiquantitative.

surface waters. Sulfamethoxazole and trimethoprim combinations, also known as co-trimoxazole are antimicrobials used to treat many bacterial infections. Both active substances present similar water solubility (Table S1) and therefore it is not surprising that the two were frequently quantified in water samples (73–61 %, 62–14 ng/L; sulfamethoxazole - trimethoprim). On the contrary, even though macrolides antibiotics are more widely consumed (Table S11), their lower water solubility may trigger lower concentration levels in surface water (5, 6, 4 ng/L; median values for clarithromycin, azithromycin and erythromycin). Nevertheless, degradation of PhACs could also affect antibiotic patterns in water, especially for macrolides antibiotics. Erythromycin degrades at acidic pH and in aquatic environments exists principally in the degraded form as anhydroerythromycin (Wang et al., 2012). Erythromycin analytical degradation was considered negligible (<2 %) during validation experiments and therefore anhydroerythromycin/erythromycin ratios obtained (12 $\pm$ 6.4; mean $\pm$ sd) suggested that anhydroerythromycin levels found in the present study (276 ng/L; 49 % Qf) could be attributed to environmental degradation of its precursor. This metabolite, in contrast to o-desmethylvenlafaxine, has no pharmacological activity and is not used for the manufacture of medicines.

According to data reported by the Ministry of Health, non-opioid analgesics and non-steroidal anti-inflammatory drugs derived from propionic acid are at the top of the list of packages billed to the National Health System in Spain (Table S11). Among the studied anti-inflammatory drugs, the most abundant and concentrated is naproxen (47 ng/L, 72 %), followed by ibuprofen (18 ng/L, 47 %) and ketoprofen (4 ng/L, 35 %). As for analgesics, the median concentration of acetaminophen was 9 ng/L with a Qf of 58 %. Both Qf and concentration were lower than would be expected since this is a group of drugs widely consumed in Spain and very soluble in water. Biotransformation has been reported to be the predominant pathway for acetaminophen elimination under dark incubation conditions (Liang et al., 2016) and removal efficiencies in municipal WWTPs are around 95 % (Gracia-Lor et al., 2012; Wu et al., 2023). Therefore, it is to be hoped low acetaminophen levels in surface waters even in areas with high anthropogenic influence.

PhACs used in the treatment of fungal infections such as clotrimazole, fluconazole, miconazole and thiabendazole are less consumed than

some of the medicines described above. As mentioned before clotrimazole and miconazole present very low water solubility and it is not surprising that were below LOD in all cases. On the contrary, fluconazole is highly soluble in water (Table S1) and was quantified in 75 % of the samples, reaching the fourth-highest median concentration (172 ng/L). In some cases, drugs with biocidal or antifungal capabilities are used to address human and veterinarian diseases as well as to treat or control plagues in agricultural activities. This is the case of thiabendazole, which is used to treat gastrointestinal roundworm in animals and humans, but also to control a variety of fruit and vegetable diseases such as mold, blight, rot and stains caused by various fungi (US EPA, 2002). In Spain, up to 17 authorized pesticide formulations contain thiabendazole in accordance with the register of phytosanitary products of the Ministry of Agriculture, Fisheries and Food (Ministry of Agriculture, Fisheries and Food, 2023). Nevertheless, the influence of this origin seems to be low considering that maximum levels were obtained in sampling points located in heavily populated areas highlighting that thiabendazole presence in water is mostly related to human consumption.

In general, PhAC levels in surface water range between tens to hundreds of ng/L and even could reach values of μ g/L (Table S12). Nevertheless, direct comparison between data reported in other studies even at the same river basin must be performed with caution, since the selection of sampling points, especially in rivers with high anthropogenic influence, greatly conditions the results obtained. To compare PhAC concentration reported in other studies, it is mandatory to know sampling point characteristics and daily flows, because otherwise, temporal trends sensed by comparing data reported in the literature may lead to wrong conclusions. For this reason, only studies reporting data for the same river basin were considered.

To the best of the author's knowledge, this is the first time that fluconazole, irbesartan, o-desmethylvenlafaxine and metoprolol, have been quantified in the Tagus watershed. Moreover, fluconazole concentrations were the first reported in Europe. Most of the studies conducted in the Tagus river focused their research on the upper part of the river (Rico et al., 2019; Valcárcel et al., 2011, 2013) covering the most anthropogenic area of the watershed. Therefore, it is not surprising that carbamazepine, clarithromycin, erythromycin and naproxen concentrations reported in the present study, which included also remote areas,

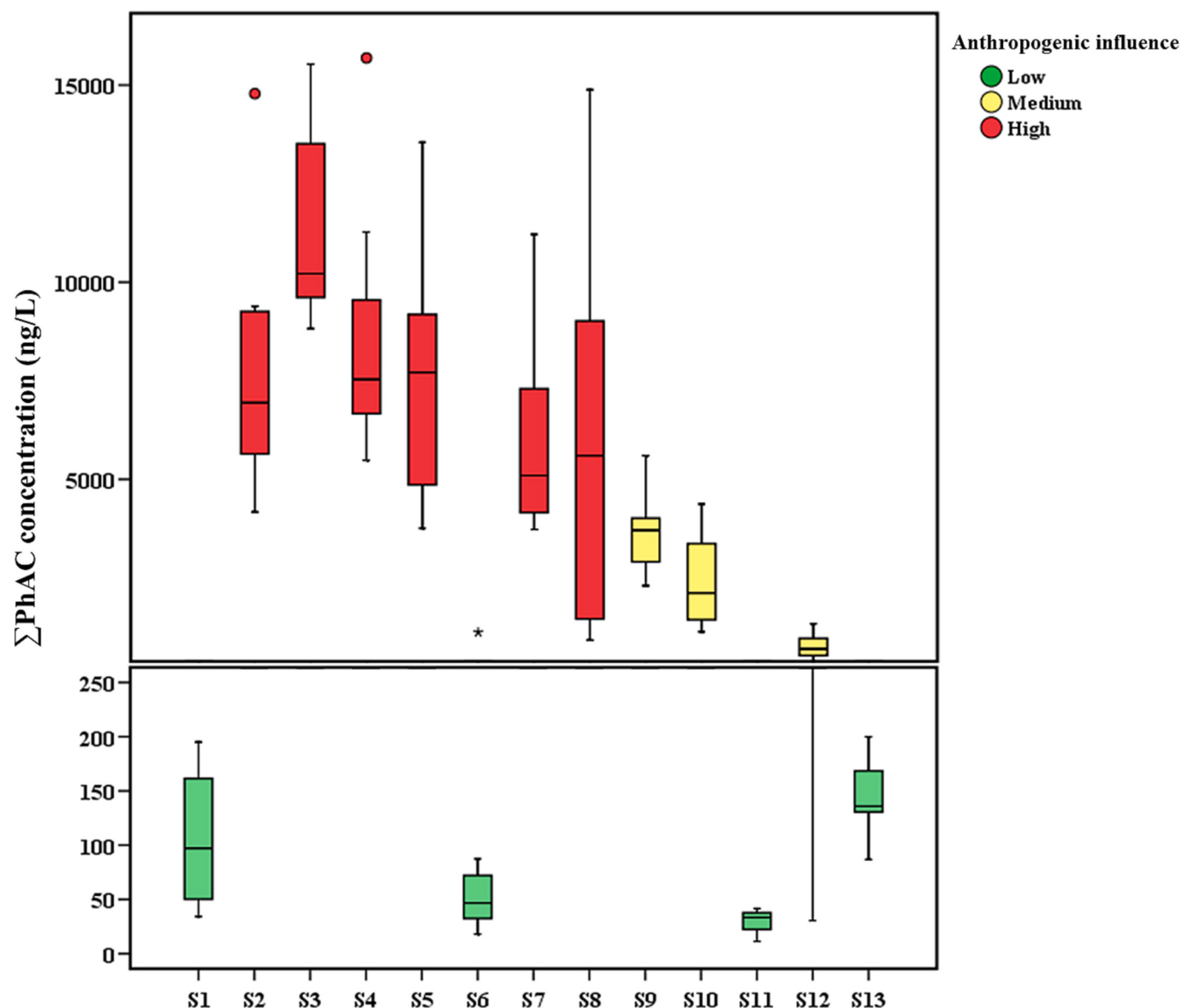


Fig. 2. Box and whisker plot of the concentration (ng/L) for the sum of PhACs at each sampling location. Green, yellow and red boxes corresponded to low, medium and high anthropogenic influenced areas, respectively.

are lower compared to those previously published (Rico et al., 2019, Valcárcel et al., 2011, 2013). However, venlafaxine concentrations reported here ($<LOQ$ –1079 ng/L; min-max) were in the same range (100–1003 ng/L) as those described by Valcárcel et al. (2013) in samples collected downstream (100 m) WWTP emission points thirteen years before, revealing an increase in the presence of this antidepressant in surface waters.

3.2. PhACs spatial and temporal trends

In light of the high mean standard deviations obtained for calculated concentrations (Table 1), the selection of the sampling point seems to greatly influence the levels of drugs found even in small areas. To address this issue data generated around Aranjuez city were investigated (Fig. S7). S5, S6 and S7 were collected in an area of 10 km², but S6 and S7 were located in the Tagus River, upstream and downstream of the city of Aranjuez, while S5, corresponded to an affluent (Jarama River) that flows between S6 and S7. As can be observed, quantified concentrations in S7 result from the dilution of those found in S5 with waters coming from S6 evidencing that the selection of a sampling point can determine

the PhAC content to a greater extent.

As could be expected, sampling points subjected to high anthropogenic influence in their watershed presented higher ($p < 0.05$) PhAC concentrations than medium and low influenced locations (Fig. 2). Interestingly, among these former sampling points, those located in urban areas and subjected to WWTP discharges (S2, S3 and S4) revealed maximum pollutant levels. On the other hand, livestock farms (S5, S7, S10 and S12) did not seem to play an important role in the presence of PhACs in surface waters. From these results, it could be inferred that urban areas and WWTP discharges could represent the main sources of PhAC. To delve into this origin, principal component analysis (PCA) was performed with log-transformed concentrations, and scores and loading plots are shown in Fig. 3. Models depicted in three principal components (PC) 86 % of the variance. The first component included most PhACs (Table S13) and explained 73 % of the variance. The second component depicted 7 % of the variance and is mainly influenced by erythromycin and anhydroerythromycin levels and finally acetaminophen influenced (6 % variance) the third component. The scores plotted in Fig. 3 (right) grouped sampling points with low population influence on the negative side of PC1, while samples subjected to medium and high influence are

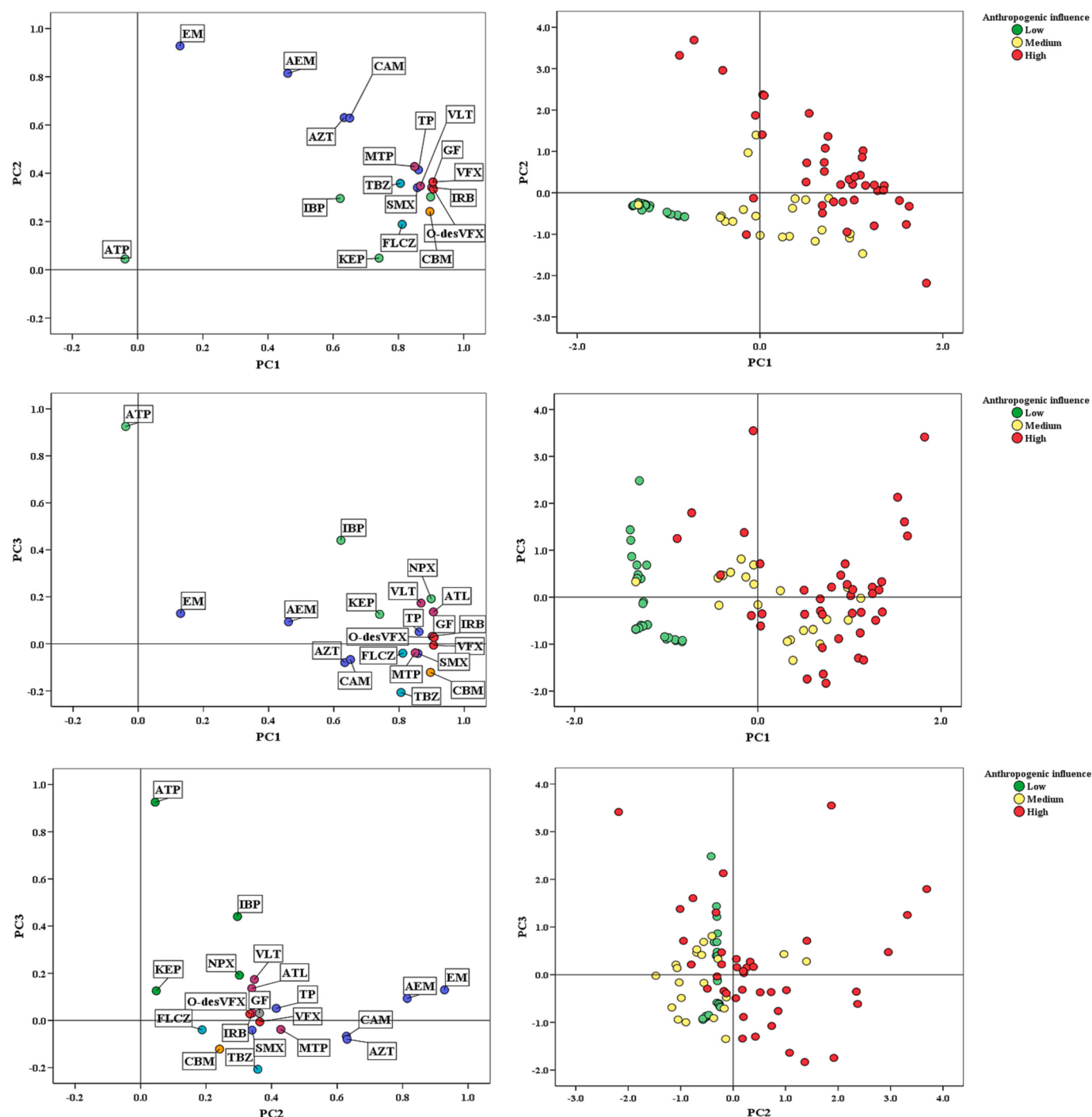


Fig. 3. Diagrams of dispersion related to the three components resulting PCA. Loading plots (left) contribution of each variable to each component. Scores plots (right), markers set by population influence: low (green), medium (yellow) and high (red). ATP (acetaminophen), ATL (atenolol), AZT (azithromycin), CBM (carbamazepine), CAM (clarithromycin), EM (erythromycin), AEM (anhydroerythromycin), FLCZ (fluconazole), GF (gemfibrozil), IBP (ibuprofen), IRB (irbesartan), KEP (ketoprofen), MTP (metoprolol), NPX (naproxen), O-desVFX (o-demethylvenlafaxine), SMX (sulfamethoxazole), TBZ (thiabendazole), TP (trimethoprim), VLT (valsartan), VFX (venlafaxine).

distributed mostly on the right side. Spearman correlation coefficients for drugs included in PC1 revealed a statistically significant positive association among most of them (Table S14) suggesting a similar origin that according to PCA results, may be related to population influence. This hypothesis was investigated by Kruskal-Wallis H-test and samples obtained at highly populated sites presented statistically significant ($p < 0.01$) higher PC1-PhAC surface water values than samples collected from areas with low population density (Fig. 3). This behavior was also found for PhACs governing PC2. Interestingly, erythromycin and

anhydroerythromycin were positively correlated ($r_s = 0.877$, $p < 0.01$) but none of them were associated with the other drugs analyzed. A similar result was obtained for acetaminophen in the third component but in this case, population density did not influence concentrations in surface water.

Seasonal and temporal trends of PhAC concentrations were evaluated using non-parametric statistical techniques (Kruskal-Wallis H test). At first, it might be expected that higher consumption of PhACs should trigger higher environmental levels. However, this tendency could not

be observed ($p > 0.05$) for $\sum$ PhAC concentrations obtained in 2020, 2021 and 2022 (Fig. S5). Nevertheless, these findings must be viewed with caution since maybe a longer sampling period could provide another result. In the same manner, no seasonal differences ($p > 0.05$) were found for $\sum$ PhAC levels in surface waters but in this case, this result may be influenced by river flow rates.

The effect of climate change in Mediterranean river basins (increases in the length of meteorological dry spells and droughts; Kovats et al., 2014; Noto et al., 2023; Schleussner et al., 2016) would likely lead to a decrease in flow rates. For this reason, to maintain a minimum ecological flow and ensure water supply to the population and irrigation in a climate change scenario, flows are regulated, storing water in rainy periods and releasing it from reservoirs when the flow decreases to excess. This is a common practice in Mediterranean rivers, which are heavily dammed and could explain the lack of flow rate seasonal variations (Table S3) in some sampling points (S4, S6, S9 and S12). This fact could buffer seasonal variations when data are analyzed together (Fig. S5). However, evaluating the concentration of each analyte in areas with greater anthropogenic influence (red), significant differences ($p < 0.02$) for valsartan and naproxen between drier (spring and summer) and wetter (autumn and winter) seasons were obtained (Fig. S6). This result could be influenced by higher WWTP degradation rates (Alfonso-Muniozguren et al., 2021; Kosma et al., 2020) during the hottest months and the movement of inhabitants in holiday periods (2020–2021; Instituto Nacional de Estadística (INE), 2022) considering that that Madrid loses up to a third of its population during these months.

3.3. Mass flow rates of PhACs in Tagus River Basin

As mentioned before, pollutant levels in surface water could be diluted or concentrated depending on the river flow rates. To evaluate this potential behavior, quantified concentrations (ng/L) were combined with mean daily flows (m^3/s) to obtain mass flow rates (kg/y) at each location and sampling period. After this data flow normalization (Table S15), the mass flow pattern maintained the result obtained for surface water concentrations. The median mass flow rates calculated for target PhACs ranged as follows: 1.4–7.9 kg/y (erythromycin, azithromycin, ketoprofen, clarithromycin, thiabendazole, anhydroerythromycin, acetaminophen, metoprolol and trimethoprim), 15 kg/y (ibuprofen and naproxen), 23 kg/y (carbamazepine), 32 kg/y (sulfamethoxazole), 49–53 kg/y (atenolol and venlafaxine), 65–77 kg/y (gemfibrozil and fluconazole) and $> 100 \text{ kg}/\text{y}$ (irbesartan, *o*-desmethylvenlafaxine and valsartan). As expected, samples collected in sparsely populated areas, with low flow rates (S1, S6, S11) did not represent a remarkable source of PhACs. In contrast, more populated zones (S2 to S5, S7 and S8) showed significantly higher PhAC mass flow rates (Kruskal-Wallis H test - $p < 0.01$). S3 presented the highest mass flow rates for the sum of PhACs (6557 kg/y) followed by S5 (5452 kg/y), S8 (4678 kg/y) and S7 (4602 kg/y). It should be underlined that within the zones with high anthropogenic influence, S2 and S4, which had large amounts of PhACs, became those with the lowest mass flow rate due to they presented the smallest flows. It is also noteworthy that after mass flow rates calculation, 11 (atenolol, azithromycin, clarithromycin, anhydroerythromycin, gemfibrozil, ibuprofen, ketoprofen, naproxen, trimethoprim, valsartan and venlafaxine) of 23 studied analytes showed lower mass flow rates in drier seasons (spring and summer) than in wetter seasons (autumn and winter).

3.4. Risk assessment

The risk associated with the presence of the studied PhACs in the aquatic ecosystem was assessed through the RQ ratios calculated for three representative aquatic organisms: phototrophic organisms (algae; Table S16), invertebrates (crustaceans, mainly *Daphnia magna*; Table S17) and vertebrates (fish; Table S18) at the median (RQ_{50}) and maximum (RQ_{max}) scenarios. In general, $\text{RQ} < 0.01$ denotes a negligible

risk, $\text{RQ} < 0.1$ reveals a low risk, $0.1 < \text{RQ} < 1$ represents a medium risk, and $\text{RQ} > 1$ indicates a high ecological risk to aquatic organisms (Molnar et al., 2021). According to this classification, irbesartan was the only PhAC that presented a high risk for phototrophic organisms, in sampling points located in highly populated areas. RQ_{max} for acetaminophen took values above 1 in S5, S6 and S7 but this may reflect an occasional discharge, since considering the general situation ($\text{RQ}_{50} < 0.1$) acetaminophen poses a low risk in these zones. Up to five PhACs presented a moderate risk (atenolol, azithromycin, ibuprofen, valsartan and venlafaxine) for algae since both RQ_{max} and RQ_{50} offered values between 0.01 and 0.1. Again, as in the case of irbesartan, the most densely populated zones presented medium risk while the areas with lower population loads presented low risk for phototrophic organisms. In the case of invertebrate organisms (*Daphnia magna*, *Thamnocephalus platyurus* or *Ceriodaphnia dubia*; Table S17), 4 PhACs (carbamazepine, gemfibrozil, irbesartan and naproxen) aroused RQs (RQ_{max} and RQ_{50}) above 1. While irbesartan did not change its behavior, carbamazepine and naproxen increased their risk from negligible or low for algae to moderate or high for crustaceans in most areas studied. But this effect was more noticeable for gemfibrozil, which moved from a negligible risk for phototrophic organisms (Table S16) to a high risk for invertebrate organisms in most sampled areas (Table S17). A similar effect was observed for ibuprofen in vertebrate organisms (fish; table S18). This drug showed negligible, low or moderate risk at lower trophic levels, but for vertebrate organisms calculated RQ ratios reached values well above 1 even at the median scenario, highlighting a significant risk to high trophic aquatic organisms. In addition to ibuprofen, other 7 PhACs (acetaminophen, carbamazepine, fluconazole, gemfibrozil, irbesartan, ketoprofen, naproxen and venlafaxine) presented a moderate or high risk for fish. It should be mentioned here that carbamazepine and ibuprofen are proposed to be included in the list of priority substances in the proposal for a Directive amending the WFD. This inclusion will result in the establishment of environmental quality standards (EQS) for these drugs.

The effects of PhACs on aquatic organisms can be additive but also synergistic or antagonistic (Gao et al., 2023; Galus et al., 2013). Nevertheless, according to Omar et al. (2019), total RQ_{mix} can be calculated as the sum of each RQ for PhACs with the same mechanism of action, such as the macrolide antibiotics, β -blockers, angiotensin receptor blockers, anti-inflammatories and antidepressants evaluated in the present study (Tables S16, S17 and 18). With this approach, ARBs presented a high risk for the three assessed organisms (algae, crustaceans and fish) assessed in locations classified as high and medium anthropogenic influenced. Antidepressants presented a high risk for vertebrate organisms in these areas but interestingly, anti-inflammatories did also at locations with low anthropogenic influence.

When evaluating the risk posed by the presence of these PhACs in the aquatic ecosystem, it is important to consider not only the calculated concentrations but also their frequency of quantification. Compounds with low Qf but high-risk levels, such as ketoprofen, could represent a sporadic high risk. On the other hand, frequently quantified chemicals like valsartan with low or moderate risk levels for the 3 trophic levels could pose a long-term hazard to the aquatic ecosystem that must be investigated. Results obtained reinforce that efforts should be made to study the presence of these pollutants in surface waters and obtain reliable data that are essential to evaluate their risk to the aquatic ecosystem.

4. Conclusions

The presence of PhACs in surface water was evaluated in one of the major European watersheds. The drugs with $\text{QF}\% > 70\%$ and the highest concentrations were valsartan (antihypertensive), *o*-desmethylvenlafaxine (antidepressant), irbesartan (antihypertensive), fluconazole (fungicide), gemfibrozil (lipid modifier) and venlafaxine (antidepressant). On the other hand, atorvastatin, clotrimazole, and

miconazole were not quantified in any of the samples. Concentration levels and estimated mass flow rates, in urban areas were statistically higher than those at non-urban locations, pointing out the big cities as the main PhAC pollution sources, that could be associated to their manufacture, distribution, consumption and/or inappropriate disposal. Results did not reveal an increasing trend in the levels of PhACs in surface waters during the period studied (2020, 2021 and 2022), but lower valsartan and naproxen surface water concentrations were obtained in the drier season (spring and summer) compared to the wetter (autumn and winter). The potential risk for three trophic levels (phototrophic, invertebrate and vertebrate organisms) was assessed by calculating the risk quotient for all PhACs at median (P_{50}) and worst case (max) scenarios. The presence of 7 of the PhACs studied may pose a high risk to vertebrate organisms in the aquatic ecosystem. In addition, irbesartan and gemfibrozil, two of the most concentrated PhACs, also presented a risk to invertebrate organisms and algae. Some of the target analytes (azithromycin, carbamazepine, clarithromycin, erythromycin and ibuprofen) have been recently included in the list of priority substances of the proposal for a Directive amending the WFD. Therefore, data reported in the present study may help decision makers to evaluate the sources and risks associated with the presence of these chemicals in the aquatic environment.

CRedit authorship contribution statement

Silvia Royano: Data curation, Investigation, Formal analysis, Methodology, Validation, Writing – original draft. **Adrián de la Torre:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft. **Irene Navarro:** Investigation, Methodology, Validation, Writing – review & editing. **María Ángeles Martínez:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This research is part of the project PID2019-105990RB-I00 funded by the MCIN/AEI/10.13039/501100011033. The authors thank Confederación Hidrográfica del Tajo for providing the surface water samples and information related to daily flows.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.167422>.

References

- Ahmed, F., Tschärke, B., O'Brien, J.W., Hall, W.D., Cabot, P.T., Sowa, P.M., Samanipour, S., Thomas, K.V., 2023. National wastewater reconnaissance of analgesic consumption in Australia. *Environ. Sci. Technol.* 57, 1712–1720. <https://doi.org/10.1021/acs.est.2c06691>.
- Alfonso-Muniozguren, P., Serna-Galvis, E.A., Bussermaker, M., Torres-Palma, R.A., 2021. A review on pharmaceuticals removal from waters by single and combined biological, membrane filtration and ultrasound system. *Ultrason. Sonochem.* 76, 105656. <https://doi.org/10.1016/j.ultsonch.2021.105656>.
- Arenas-Sánchez, A., Rico, A., Rivas-Tabares, D., Blanco, A., García-Doncel, P., Romero-Salas, A., Nozal, L., Vighi, M., 2019. Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 2: spatio-temporal analysis and ecological risk assessment. *Sci. Total Environ.* 667, 222–233. <https://doi.org/10.1016/j.scitotenv.2019.02.286>.
- Brodin, T., Piovano, S., Fick, J., Klaminder, J., Heynen, M., Jonsson, M., 2014. Ecological effects of pharmaceuticals in aquatic systems—impacts through behavioural alterations. *Philos. Trans. R. Soc. B* 369, 20130580. <https://doi.org/10.1098/rstb.2013.0580>.
- Burns, E.E., Carter, L.J., Snape, J., Thomas-Oates, J., Boxall, A.B.A., 2018. Application of prioritization approaches to optimize environmental monitoring and testing of pharmaceuticals. *J. Toxicol. Environ. Health Part B* 21 (3), 115–141. <https://doi.org/10.1080/10937404.2018.1465873>.
- Casillas, A., De la Torre, A., Navarro, I., Sanz, P., Martínez, M.A., 2022. Environmental risk assessment of neonicotinoids in surface water. *Sci. Total Environ.* 809, 151161. <https://doi.org/10.1016/j.scitotenv.2021.151161>.
- CEMEF, 2023. Emerging Pollutants in River Ecosystems. Environmental Transfer and Associated Risk (CEMEF 2020–2023). PID2019-105990RB-I00. <http://projects.ciema.es/web/cecef/inicio-titulo>.
- Chabchoubi, I.B., Lam, S.S., Pane, S.E., Ksibi, M., Guerriero, G., Hentati, O., 2023. Hazard and health risk assessment of exposure to pharmaceutical active compounds via toxicological evaluation by zebrafish. *Environ. Pollut.* 324, 120698. <https://doi.org/10.1016/j.envpol.2022.120698>.
- De Barros, A.L.C., Schmidt, F.F., de Aquino, S.F., Afonso, R.J.C.F., 2018. Determination of nine pharmaceutical active compounds in surface waters from Paraopeba River Basin in Brazil by LTPE-HPLC-ESI-MS/MS. *Environ. Sci. Pollut. Res.* 25, 19962–19974. <https://doi.org/10.1007/s11356-018-2123-y>.
- European Commission, 2003. Technical Guidance Document on Risk Assessment. Part II. Available at: <https://op.europa.eu/en/publication-detail/-/publication/212940b8-3e55-43f8-8448-ba258d0374bb>.
- European Commission, 2009. Regulation (EC) no 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. *Off. J. Eur. Union* L 30, 1–50.
- European Commission, 2022a. Commission implementing decision (EU) 2022/679 of 19 January 2022. *Off. J. Eur. Union* L 3, 1–3.
- European Commission, 2022b. Proposal for a Directive of the European Parliament and of the Council Amending Directive 2000/60/EC Establishing a Framework for Community Action in the field of Water Policy, Directive 2006/118/EC on the Protection of Groundwater Against Pollution and Deterioration and Directive 2008/105/EC on Environmental Quality Standards in the Field of Water Policy, pp. 1–81.
- European Commission, 2022c. Commission implementing decision (EU) 2022/1307 of 22 July 2022. *Off. J. Eur. Union* L 5, 1–5.
- Galus, M., Jeyarajana, J., Smith, E., Li, H., Metcalfe, C., Wilson, J.Y., 2013. Chronic effects of exposure to a pharmaceutical mixture and municipal wastewater in zebrafish. *Aquat. Toxicol.* 132–133, 212–222. <https://doi.org/10.1016/j.aquatox.2012.12.016>.
- Gao, F., Wen, H., Feng, S., Li, M., Zhu, L., Zhang, Y., Xi, Y., Xiang, X., 2023. The elevated toxicity of the biodegradation product (guanyurea) from metformin and the antagonistic pattern recognition of combined toxicity: insight from the pharmaceutical risk assessment and the simulated wastewater treatment. *Sci. Total Environ.* 892, 164747. <https://doi.org/10.1016/j.scitotenv.2023.164747>.
- Geo Portal, 2023. Ministry for Ecological Transition and Demographic Challenge. <https://sig.mapama.gob.es/geoportal/> (accessed 10 July 2023).
- Gracia-Lor, E., Sancho, J.V., Serrano, R., Hernández, F., 2012. Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. *Chemosphere* 87, 453–462. <https://doi.org/10.1016/j.chemosphere.2011.12.025>.
- Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. *J. Chromatogr. A* 1248, 104–121. <https://doi.org/10.1016/j.chroma.2012.05.084>.
- Instituto Nacional de Estadística (INE), 2022. Official population figures of Spanish municipalities in application of the Ley de Bases del Régimen Local (Art. 17). Madrid: Population by municipality and sex. <https://www.ine.es/jaxiT3/Datos.htm?t=2881> (accessed 20 July 2023).
- Jelić, A., Petrović, M., Barceló, D., 2009. Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry. *Talanta* 80, 363–371. <https://doi.org/10.1016/j.talanta.2009.06.077>.
- Jung, E., Kong, S.Y., Ryu, H.H., Shin, S.D., 2022. Serum cholesterol levels and risk of cardiovascular death: a systematic review and a dose-response meta-analysis of prospective cohort studies. *Int. J. Environ. Res. Public Health* 19 (14), 8272. <https://doi.org/10.3390/ijerph19148272>.
- Klementová, S., Petráňová, P., Fojtíková, P., 2021. Photodegradation of atorvastatin under light conditions relevant to natural waters and photoproducts toxicity assessment. *Open J. Appl. Sci.* 11, 489–499. <https://doi.org/10.4236/ojapps.2021.114035>.
- Kosma, C.I., Kapsi, M.G., Konstas, P.S.G., Trantopoulos, E.P., Boti, V.I., Konstantinou, I. K., Albanis, T.A., 2020. *Environ. Res.* 191, 110152. <https://doi.org/10.1016/j.envres.2020.110152>.
- Kovats, R.S., Valentini, R., Bouwer, L., Georgopoulou, E., Jacob, D., Martin, E., Rounsevell, M., Soussana, J., 2014. Climate change 2014: impacts, adaptation, and vulnerability. Part B Reg. Asp. Eur. 1267–1326.

- Liang, C., Lan, Z., Zhang, X., Liu, Y., 2016. Mechanism for the primary transformation of acetaminophen in a soil/water system. *Water Res.* 98, 215–224. <https://doi.org/10.1016/j.watres.2016.04.027>.
- Lopez, F.J., Pitarch, E., Botero-Coy, A.M., Fabregat-Safont, D., Ibáñez, M., Marín, J.M., Peruga, A., Ontañón, N., Martínez-Morcillo, S., Olalla, A., Valcárcel, Y., Varó, I., Hernández, F., 2022. *Sci. Total Environ.* 812, 152567. <https://doi.org/10.1016/j.scitotenv.2021.152567>.
- McEneff, G., Barron, L., Kelleher, B., Paull, B., Quinn, B., 2014. A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent, receiving marine waters and marine bivalves. *Sci. Total Environ.* 476–477, 317–326. <https://doi.org/10.1016/j.scitotenv.2013.12.123>.
- Mills, K.T., Stefanescu, A., He, J., 2020. The global epidemiology of hypertension. *Nat. Rev. Nephrol.* 16 (4), 223–237. <https://doi.org/10.1038/s41581-019-0244-2>.
- Ministry of Agriculture, Fisheries and Food, 2023. Registration of Phytosanitary Products. <https://servicio.mapa.gob.es/regfiweb> (accessed 21 April 2023).
- Molnar, E., Maasz, G., Pirger, Z., 2021. Environmental risk assessment of pharmaceuticals at a seasonal holiday destination in the largest freshwater shallow lake in Central Europe. *Environ. Sci. Pollut. Res.* 28, 59233–59243. <https://doi.org/10.1007/s11356-020-09747-4>.
- Navarro, I., De la Torre, A., Sanz, P., Martínez, M.A., 2020. Perfluoroalkyl acids (PFAAs): distribution, trends and aquatic ecological risk assessment in surface water from Tagus River basin (Spain). *Environ. Pollut.* 256, 113511. <https://doi.org/10.1016/j.envpol.2019.113511>.
- Noto, L.V., Cipolla, G., Pumo, D., Francipane, A., 2023. Climate change in the Mediterranean Basin (part II): a review of challenges and uncertainties in climate change modeling and impact analyses. *Water Resour. Manag.* 37, 2307–2323.
- OECD, 2019. Pharmaceutical Residues in Freshwater: Hazards and Policy Responses, OECD Studies on Water. OECD Publishing, Paris. <https://doi.org/10.1787/c936f42d-en>.
- OECD, 2021. Health at a Glance 2021: OECD Indicators. OECD Publishing, Paris. <https://doi.org/10.1787/ae3016b9-en>.
- OECD, 2023. Stat. Pharmaceutical Market. <https://stats.oecd.org/Index.aspx?ThemeT=releid=9> (accessed 15 February 2023).
- Omar, T.F.T., Aris, A.Z., Yusoff, F.M., Mustafa, S., 2019. Risk assessment of pharmaceutically active compounds (PhACs) in the Klang River estuary, Malaysia. *Environ. Geochem. Health* 41, 211–223. <https://doi.org/10.1007/s10653-018-0157-1>.
- Postigo, C., Moreno-Merino, L., López-García, E., López-Martínez, J., López de Alba, M., 2023. Human footprint on the water quality from the northern Antarctic Peninsula región. *J. Hazard. Mater.* 453, 131394. <https://doi.org/10.1016/j.jhazmat.2023.131394>.
- Rico, A., Arenas-Sánchez, A., Alonso-Alonso, C., López-Heras, I., Nozal, L., Rivas-Tabares, D., Vighi, M., 2019. Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 1: screening, quantitative analysis and comparison of sampling methods. *Sci. Total Environ.* 666, 1058–1070. <https://doi.org/10.1016/j.scitotenv.2019.02.250>.
- Rúa-Gómez, P.C., Püttmann, W., 2012. Occurrence and removal of lidocaine, tramadol, venlafaxine, and their metabolites in German wastewater treatment plants. *Environ. Sci. Pollut. Res.* 19, 689–699. <https://doi.org/10.1007/s11356-011-0614-1>.
- Sadutto, D., Picó, Y., 2020. Sample preparation to determine pharmaceutical and personal care products in an all-water matrix: solid phase extraction. *Molecules* 25 (21), 5204. <https://doi.org/10.3390/molecules25215204>.
- Schleussner, C.-F., Lissner, T.K., Fischer, E.M., Wohland, J., Perrette, M., Golly, A., Rogelj, J., Childers, K., Schewe, J., Frieler, K., 2016. Differential climate impacts for policy-relevant limits to global warming: the case of 1.5 C and 2 C. *Earth Syst. Dynam.* 7 (2), 327–351.
- Silva, B.F., Jelčić, A., López-Serna, R., Mozeto, A.A., Petrović, M., Barceló, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. *Chemosphere* 85, 1331–1339. <https://doi.org/10.1016/j.chemosphere.2011.07.051>.
- US EPA, 2002. Prevention, Pesticides and Toxic Substances (7508C) EPA-738-F-02-002 May 2002. R.E.D. FACTS Thiabendazole and Salts. https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-060101_1-May-02.pdf.
- Valcárcel, Y., González Alonso, S., Rodríguez-Gil, J.L., Gil, A., Catalá, M., 2011. Detection of pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and potential ecotoxicological risk. *Chemosphere* 84, 1336–1348. <https://doi.org/10.1016/j.chemosphere.2011.05.014>.
- Valcárcel, Y., González Alonso, S., Rodríguez-Gil, J.L., Castaño, A., Montero, J.C., Criado-Alvarez, J.J., Mirón, I.J., Catalá, M., 2013. Seasonal variation of pharmaceutically active compounds in surface (Tagus River) and tap water (Central Spain). *Environ. Sci. Pollut. Res.* 20, 1396–1412. <https://doi.org/10.1007/s11356-012-1099-2>.
- Wang, J., MacNeil, J.D., Kay, J.F., 2012. Chemical Analysis of Antibiotic Residues in Food, p. 27, 132 and 194.
- WHO, 2021. Cardiovascular diseases (CVDs). <https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-cvds> (accessed 17 July 2023).
- Wu, J., Liu, Z., Ma, Q., Dai, L., Dang, Z., 2023. Occurrence, removal and risk evaluation of ibuprofen and acetaminophen in municipal wastewater treatment plants: a critical review. *Sci. Total Environ.* 891, 164600. <https://doi.org/10.1016/j.scitotenv.2023.164600>.