Biodiversity restoration and conservation of inland water ecosystems for environmental and human well-being

BioReset

BiodivRestore-406

2020 - 2021 Joint Call

Joint COFUND Call on "Conservation and restoration of degraded ecosystems and their biodiversity, including a focus on aquatic systems"

Deliverable 1.1.1

Data from monitoring of pharmaceuticals in surface waters and WWTP effluents and influents

Lead Beneficiary	Work package	Delivery month
REQUIMTE	**1**	**18**

1. Executive Summary

BioReset proposes to advance treatment processes (chemical, physical, biological and their combination) to promote ecosystem recovery and conservation and to develop assessment strategies. Diatoms will be used to model ecosystem conservation and restoration since their communities show high levels of biodiversity. The diatoms will provide an expeditious method to compare different recovery strategies and water treatment processes, allowing to address timescale and key conservation/restoration questions. The full environmental, economic, and social viability of the upgraded and innovative treatment technologies will be assessed. Based on this knowledge, scale-up studies in geographically different sites (Portugal and Spain) will be performed to ascertain the technical and economic feasibility at a larger scale and recommended action guidelines will be issued.

BioReset also envisages the creation of a representative space-time picture of the presence of emerging contaminants in inland waters and its correlation to effects on diatom communities. For this, powerful analytical techniques, such as gas- and liquid chromatography, will be used. Besides these methods, and to obtain real-time information, miniaturized analytical platforms that can perform fast, and on-site monitoring will also be employed.

Deliverable 1.1.1 provides details about the monitoring of pharmaceuticals in river water and wastewater treatment plant (WWTP) effluents and influents, developed in Work Package (WP) 1.

2. Task description

WP1 regards analytical methods to analyse emerging contaminants (EC, pharmaceuticals and microplastics) in inland waters using established and novel methods. Task 1.1 focuses on monitoring pharmaceuticals with ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) and microplastics with GC-Pyr-MS/MS. The methodology for pharmaceutical analysis, including extraction and analysis, is described in the team members' previous works [1-3]. It involved solid-phase extraction (SPE) followed by UHPLC-MS/MS. The target compounds include pharmaceuticals from various therapeutic groups, as well as their corresponding metabolites and one degradation product.

3. WP1 - Task 1.1 team members

The Team members in WP1, Task 1.1, regarding pharmaceutical analysis, are:

Name	Organization	Role	Name	Organization	Role
Cristina Delerue-Matos	REQUIMTE	Task coordinator	Magda Almeida	AdCL	Researcher
Manuela Correia	REQUIMTE	Researcher	Roberto Barbosa	AdCL	Researcher
Paula Paíga	REQUIMTE	Researcher	Ana Soares	AdCL	Researcher
Sandra Jorge	AdCL	Researcher			

4. Developed activities

Sampling Campaigns

Sampling campaign 2018-2019

During the previous project ("REWATER – Sustainable and Safe Water Management in Agriculture: Increasing the Efficiency of Water Reuse for Crop Growth While Protecting Ecosystems, Services, and Citizens' Welfare"), two sampling campaigns were conducted in 2018 and 2019. Samples were collected at five points along the Lis River (located in the Leiria region, central Portugal) and from both the influent and effluent of two wastewater treatment plants (WWTPs) discharging into the river. The target compounds included 33 pesticides and 83 pharmaceuticals. Solid-phase extraction (SPE) was used to extract the pollutants in the samples, which were then analyzed by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The results related to pesticides were obtained and published during the REWATER project. However, the pharmaceutical data remained unanalyzed and unpublished at that time.

At the beginning of the BioReset project, the chromatograms of the 83 pharmaceuticals from multiple therapeutic classes (nonsteroidal anti-inflammatory drugs, analgesic, antibiotics, psychiatric drugs, β-blockers, lipid regulator and cholesterol lowering statin drugs, calcium channel blocker, fibrate lipid lowering agent, proton pump inhibitor, antipsychotic, antidiabetic drug, stimulant,

anorexics, anxiolytics, and laxatives) were integrated and analyzed. Notably, the sampling campaigns were conducted at the same sites where monitoring data had been collected by our team since 2013 [1,3]. It was observed that 33 compounds were common across all sampling campaigns (2013, 2014, 2017, 2018, and 2019). This allowed for a temporal analysis, correlating data across years. A total of 111 samples were collected from 2013 to 2019, including 65 from river sites, 23 from WWTP effluents, and 23 from WWTP influents. These findings provide valuable insights into pharmaceutical contamination trends in the river ecosystem and WWTP discharges over time, enhancing understanding of environmental and public health impacts.

Sampling Campaigns 2022-2023

In 2022, a sampling campaign was performed at the Ribeira de Frades wastewater treatment plant (WWTP) in the municipality of Coimbra, central Portugal, with samples collected from river water, WWTP effluents and influents.

In 2023, monitoring expanded to three WWTP locations. Surface water samples were collected from Cacia (municipality of Aveiro, central-northern Portugal) and Ribeira de Frades (upstream and downstream), and effluent samples were gathered from São Jacinto (municipality of Aveiro, central-northern Portugal), Ribeira de Frades, and Cacia WWTPs. A total of 9 samples were collected, and 97 compounds which include pharmaceuticals, metabolites, and degradation products (nonsteroidal anti-inflammatory drugs, analgesic, antibiotics, psychiatric drugs, β-blockers, lipid regulator and cholesterol lowering statin drugs, calcium channel blocker, fibrate lipid lowering agent, proton pump inhibitor, antipsychotic, antidiabetic drug, stimulant, anorexics, anxiolytics, laxatives, and pharmaceuticals administered to Alzheimer's and Parkinson's diseases) were monitored. Surface water and wastewater samples were extracted using SPE and analyzed with UHPLC-MS/MS.

A summary of the types and the number of samples collected in each sampling campaign is presented in Table 1.

Table 1. Types and numbers of samples collected in each sampling campaign.

Sample	Sampling campaign				
	2018	2019	Temporal analysis (2013 to 2019)	2022	2023
River	5	5	65	1	3
WWTP Effluent	2	2	23	1	3
WWTP Influent	2	2	23	1	
Total	**9**	**9**	**111**	**3**	**6**

Sample treatment

Surface water and wastewater samples were collected by the team members of AdCL, and once received in the laboratory, samples were vacuum filtered through a 0.45 µm nylon membrane filter.

Pharmaceuticals under study

The analytes under study (pharmaceuticals, metabolites, and degradation product) in each sampling campaign are listed in Table 2.

Table 2. Pharmaceuticals, metabolites, and degradation products analyzed in each sampling campaign (X - compound analysed).

Compound	Sampling campaign						
	Temporal analysis					2022	2023
	2013	2014	2017	2018	2019		
Acetaminophen	X	X	X	X	X	X	X
Acetylsalicylic acid	X	X	X	X	X	X	X
Alprazolam			X	X	X	X	X
Amoxicillin			X	X	X	X	X
Ampicillin			X	X	X	X	X
Anfepramone			X	X	X	X	X
Atorvastatin			X	X	X	X	X
Atenolol			X	X	X	X	X
Azithromycin	X	X	X	X	X	X	X
Bupropion			X	X	X	X	X
Caffeine			X	X	X	X	X
Carbamazepine	X	X	X	X	X	X	X
Carboxyibuprofen	X	X	X	X	X	X	X
Cathine			X	X	X	X	X
Chlorocycline			X	X	X	X	X
Chlorpromazine			X	X	X	X	X
Ciprofloxacin	X	X	X	X	X	X	X
Citalopram	X	X	X	X	X	X	X
Citalopram N-oxide			X	X	X	X	X
Citalopram propionic acid			X	X	X	X	X
Clarithromycin	X	X	X	X	X	X	X
Clobenzorex			X	X	X	X	X
Desmethylcitalopram			X	X	X	X	X
O-Desmethylvenlafaxine			X	X	X	X	X
Diazepam	X	X	X	X	X	X	X
Diclofenac	X	X	X	X	X	X	X
Didemethylcitalopram			X	X	X	X	X
Diltiazem			X	X	X	X	X
Doxycycline			X	X	X	X	X
Enrofloxacin	X	X	X	X	X	X	X
Ephedrine			X	X	X	X	X
10, 11-epoxycarbamazepine	X	X	X	X	X	X	X
Erythromycin			X	X	X	X	X
Fenfluramine			X	X	X	X	X
Fenofibrate			X	X	X	X	X
Fentermine			X	X	X	X	X
Fluoxetine	X	X	X	X	X	X	X
Gemfibrozil			X	X	X	X	X
Hydroxyibuprofen	X	X	X	X	X	X	X
Ibuprofen	X	X	X	X	X	X	X
Ketoprofen	X	X	X	X	X	X	X
Lansoprazole			X	X	X	X	X
Lomefloxacin			X	X	X	X	X
Lorazepam			X	X	X	X	X
Mazindol			X	X	X	X	X
Metformin			X	X	X	X	X
DI-Methamphetamine			X	X	X	X	X
Moxifloxacin			X	X	X	X	X
Naproxen	X	X	X	X	X	X	X
Nimesulide	X	X	X	X	X	X	X
DI-Norephedrine			X	X	X	X	X
Norfloxacin			X	X	X	X	X
Norfluoxetine	X	X	X	X	X	X	X
Norsertraline	X	X	X	X	X	X	X
Ofloxacin	X	X	X	X	X	X	X
Oxytetracycline			X	X	X	X	X
Paroxetine	X	X	X	X	X	X	X
Phenolphthalein			X	X	X	X	X
Potassium clavulanate			X	X	X	X	X
Pravastatin			X	X	X	X	X
Propranolol			X	X	X	X	X
Prulifloxacin			X	X	X	X	X
Rimonabant	X	X	X	X	X	X	X
Salicylic acid			X	X	X	X	X
Sertraline	X	X	X	X	X	X	X
Sibutramine			X	X	X	X	X
Simvastatin			X	X	X	X	X

Table 2. Pharmaceuticals, metabolites, and degradation products analyzed in each sampling campaign (X - compound analysed) (*cont.*).

Compound	Sampling campaign						
	Temporal analysis					2022	2023
	2013	2014	2017	2018	2019		
Sulfadiazine	X	X	X	X	X	X	X
Sulfadimethoxine	X	X	X	X	X	X	X
Sulfamethazine	X	X	X	X	X	X	X
Sulfamethizole			X	X	X	X	X
Sulfamethoxazole	X	X	X	X	X	X	X
Sulfamethoxypyridazine	X	X	X	X	X	X	X
Sulfapyridine	X	X	X	X	X	X	X
Sulfaquinoxaline			X	X	X	X	X
Sulfathiazole			X	X	X	X	X
Synephrine			X	X	X	X	X
Tetracycline			X	X	X	X	X
Topiramate			X	X	X	X	X
Trazodone	X	X	X	X	X	X	X
Trimethoprim	X	X	X	X	X	X	X
Venlafaxine	X	X	X	X	X	X	X
Zonisamide			X	X	X	X	X
Benserazide						X	X
Pramipexole						X	X
Carbidopa						X	X
Galantamine						X	X
Rasagiline						X	X
Amantadine						X	X
Apormorphine						X	X
Ropinirole						X	X
Rivastigmine						X	X
Selegiline						X	X
Safinamide						X	X
Donepezil						X	X
Rotigotine						X	X
Entacapone						X	X

Extraction and analysis

The analytes were extracted using SPE and analyzed by UHPLC-MS/MS on a triple-quadrupole mass spectrometer operating in electrospray ionization (ESI) mode. Lab Solutions LC-MS software (version 5.80, Shimadzu) was used for system control and data processing, with quantification performed by Multiple Reaction Monitoring (MRM). The extraction and chromatographic conditions - including column selection, program settings, elution mode, mobile phases, flow rate, oven temperature, source parameters, ionization mode, precursor ions, product ions, mass spectrometry conditions, and ion ratio - were optimized. The optimized SPE procedure developed and chromatographic conditions are detailed in the author's previous publications [1-3] and summarized in the following paragraph.

Target compounds were isolated using Strata-X SPE cartridges (200 mg, 3 mL). To each sample (50 mL of WWTP influent, 100 mL of WWTP effluent, or 250 mL of river water), a suitable volume of 0.1 M ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2EDTA) solution was added to achieve a final concentration of 0.1% (g solute/g solution). The pH of each sample was adjusted to 2 using 37% HCl. Pre-treated samples were passed through SPE cartridges conditioned with 5 mL of methanol and equilibrated with 5 mL of ultrapure water, followed by 5 mL of ultrapure water adjusted to pH 2. For the clean-up step, 5 mL of ultrapure water was passed through each cartridge, after which cartridges were left under maximum vacuum pressure for 1 hour. Analytes were eluted with 10 mL of methanol, evaporated to dryness under nitrogen, and reconstituted in 500 µL of acetonitrile (30:70, v/v). Finally, 5 µL of the isotope labelled internal standard (ILIS) mixture was added to standards and samples.

Several chromatographic programs were used for the analysis of the target pharmaceuticals [1-3]. For the analysis in negative ionization mode, a Kinetex C18 column (2.6 × 150 mm i.d., 1.7 µm particle size) from Phenomenex (USA) was used, and chromatographic separation was achieved using ultrapure water and acetonitrile at a flow rate of 0.22 mL/min. For the analysis in positive ionization mode, the chromatographic separation was carried out in a Cortecs® UPLC C18+column (100 × 2.1mmi.d.; 1.6 µm particle size) from Waters (Milford, MA, USA), using 0.1% formic acid in ultrapure water and acetonitrile, at a flow rate of 0.3 mL/min. For both ionization modes, the injection volume was 5 µL, column oven was set at 30°C, and the auto sampler was operated at 4°C.

Analytical Method Validation: Ensuring Accuracy and Reliability

Linearity, method detection limits (MDLs), method quantification limits (MQLs), precision (intra- and inter-day), recovery, and matrix effects (ME) were included in the validation tests. For calibration curves, the area was plotted against analyte concentration using linear regression analysis. The MDLs and MQLs were determined using the minimum detectable amount of each analyte with signal-to-noise ratios of 3 and 10, respectively. Intra- and inter-day analyses were expressed as the relative standard deviation (RSD (%)). Recovery was calculated by comparing the areas of the quantification ion for samples spiked before solid-phase extraction (pre-spiked sample) with the areas of the quantification ion for samples spiked after solid-phase extraction (post-spiked sample). The matrix effect was assessed by comparing the area of a standard prepared in the matrix with the area of the standard prepared in solvent.

5. Results

Validation of the analytical method

Validation analysis was performed in each sampling campaign. Method linearity was confirmed graphically across a concentration range of 0.5 to 1000 µg/L (12 levels), with correlation coefficients (R) exceeding 0.997, indicating a strong linear relationship between the analyte concentration and the peak area. The method led to good precision values, with RSD (%) of intra- and inter-day analysis lower than 10%. Recovery tests involved three fortification levels per matrix, with two extractions per level. Results were consistent for all levels and in all sampling campaigns, showing RSD<10%. SPE extraction yielded satisfactory recoveries for most compounds in river water and wastewater matrices. For the sampling campaigns conducted in 2018 and 2019, 63.9% of compounds in river water, 57.8% in WWTP effluents, and 54.2% in WWTP influents showed recoveries above 75%. In the 2022 and 2023 campaigns, 36.1% of compounds in surface waters had recoveries higher than 75%, 48.2% had recoveries between 50% and 75%, and 15.7% had recoveries below 50%. Additionally, 74.7% of compounds had recoveries higher than 75%, 4.82% had recoveries between 50% and 75%, and 20.5% had recoveries below 50% in wastewater. Matrix effects (ME) in surface water, WWTP effluent, and WWTP influent were assessed. As expected, in general, higher ME was observed in wastewaters when compared with surface water. The MDL and MQL were determined for all matrices where pharmaceuticals were detected. In the 2018–2019 sampling campaigns, MDL ranged from 0.0200 to 57.7 ng/L in river water, 0.0500 to 168 ng/L in WWTP effluent, and 0.100 to 391 ng/L in WWTP influent. For the 2022–2023 campaigns, MDL ranged from 0.0200 to 57.2 ng/L in surface waters and 0.0500 to 182 ng/L in wastewaters. Higher MDL values were found in WWTP influent matrix compared to WWTP effluents and river water. The lowest MDL values showed the method's high sensitivity and revealed qualities of UHPLC-MS/MS for accurate quantification and confirmation of trace levels of the pharmaceuticals, metabolites, and degradation products in environmental samples.

Occurrence of Pharmaceuticals in Surface Waters and Wastewaters

Sampling campaign 2018/2019

From the sampling campaign performed in 2018 and 2019, of the 83 target analytes, 45 in 2018 and 40 in 2019 were detected in at least one sample (Tables 3 and 4). More pharmaceuticals were found in the wastewater samples when compared with the number of pharmaceuticals detected in river samples. The obtained results indicate an increase in both the number of detected analytes and their total concentrations along the river course, with the highest values generally observed downstream of both WWTPs, highlighting their influence on the river. Among the therapeutic classes investigated, nonsteroidal anti-inflammatory drugs/analgesics, antibiotics, and psychiatric drugs showed a higher number of detected pharmaceuticals. Concentrations ranged from below method detection limit (<MDL) to 3.20 µg/L (caffeine) and <MDL to 639 µg/L (hydroxyibuprofen) in 2018, and from <MDL to 0.848 µg/L (diclofenac) and <MDL to 53.0 µg/L (caffeine) in 2019 for the analyzed water samples.

Figure 1 illustrates several key findings: the total number of detected pharmaceuticals, the sum of their concentrations, the distribution of detected pharmaceuticals across sampling sites, the count of pharmaceuticals with concentrations at the µg/L level, the number of detected metabolites and degradation products, the classification of pharmaceuticals by therapeutic class, and the number of pharmaceuticals with a 100% detection frequency.

Table 3. Pharmaceuticals, metabolites and degradation products detected in each sample and their concentration (ng/L) in the sampling campaign of 2018.

Compound	River sample SP1		River sample SP2		River sample SP3		River sample SP4		River sample SP5		WWTP Effluent-E1		WWTP Effluent-E2		WWTP Influent-I1		WWTP Influent-I2	
	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)
Carbamazepine	8.50	4.0	n.d.		166	5.6	41.7	13	266	8.0	1337	7.6	858	1.7	1048	3.7	652	1.0
10,11-Epoxycarbamazepine	n.d.		n.d.		n.d.		n.d.		n.d.		86.9	12	n.d.		79.9	15	56.1	4.8
Citalopram	n.d.		n.d.		14.4	8.9	n.d.		41.5	13	158	3.2	158	0.60	131	13	144	12
Citalopram propionic acid	n.d.		9.30	1.2	19.4	0.74	11.1	5.3	24.0	2.8	124	2.1	89.6	18	61.2	0.69	52.3	13
Desmethylcitalopram	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		198	10	n.d.	
Didemethylcitalopram	n.d.		n.d.		n.d.		n.d.		n.d.		61.6	0.083	n.d.		n.d.		n.d.	
Diazepam	n.d.		n.d.		n.d.		n.d.		13.9	1.5	n.d.		n.d.		n.d.		73.2	7.8
Fluoxetine	n.d.		<MDL		<MDL		<MDL		<MDL		13.5	37	5.72	25	<MDL		<MDL	
Paroxetine	n.d.		n.d.		n.d.		n.d.		n.d.		17.8	8.3	n.d.		n.d.		n.d.	
Sertraline	n.d.		n.d.		n.d.		n.d.		8.74	10	84.9	1.7	50.8	11	121	1.2	106	2.7
Trazodone	n.d.		n.d.		2.15	11	n.d.		35.2	19	111	2.0	126	9.1	29.1	13	115	8.5
Venlafaxine	6.19	27	3.85	15	45.6	2.1	9.34	6.9	124	6.5	370	4.7	488	7.3	367	3.9	408	4.4
Bupropion	n.d.		n.d.		27.1	1.6	15.7	7.2	60.6	2.2	169	8.5	205	0.064	178	1.1	201	11
Azithromycin	n.d.		n.d.		187	8.4	n.d.		532	7.4	4399	2.8	4370	3.1	652	14	1096	9.1
Clarithromycin	n.d.		n.d.		99.1	9.8	23.0	5.1	187	2.6	2566	2.2	1291	1.2	2214	8.6	1690	5.8
Ciprofloxacin	n.d.		n.d.		n.d.		n.d.		n.d.		580	13	482	15	939	10	1814	1.1
Ofloxacin	n.d.		n.d.		n.d.		n.d.		n.d.		1037	11	382	18	1071	4.0	954	16
Trimethoprim	n.d.		n.d.		n.d.		n.d.		38.1	19	41.9	10	<MDL		<MDL		<MDL	
Sulfamethoxazole	n.d.		n.d.		n.d.		n.d.		n.d.		97.7	9.0	67.5	16	945	2.6	917	14
Sulfapyridine	n.d.		n.d.		11.6	20	n.d.		n.d.		n.d.		n.d.		696	5.9	955	16
Oxytetracycline	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		8685	19	n.d.		n.d.	
Tetracycline	n.d.		n.d.		55.1	18	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
Alprazolam	n.d.		n.d.		n.d.		n.d.		n.d.		78.7	11	104	10	n.d.		n.d.	
Lorazepam	n.d.		n.d.		n.d.		n.d.		n.d.		656	7.5	n.d.		n.d.		n.d.	
Acetaminophen	<MDL		77.6	7.3	20.6	7.9	142	9.7	93.8	18	194	14	182	5.3	96770	2.6	82702	7.2
Acetylsalicylic acid	n.d.		n.d.		n.d.		n.d.		n.d.		84.7	6.8	n.d.		99.5	4.7	115	4.0
Salicylic acid	106	16	348	13	180	17	118	21	205	4.2	215	3.4	271	15	15719	5.8	29012	20
Ibuprofen	1.38	29	4.78	3.8	69.1	0.63	45.5	2.8	94.7	8.1	79.5	14	290	6.3	5862	2.5	12490	15
Carboxyibuprofen	n.d.		n.d.		1277	2.8	n.d.		n.d.		n.d.		n.d.		392986	0.069	639403	12
Hydroxyibuprofen	15.3	0.59	36.2	7.2	1673	1.5	388	0.65	1295	4.9	3035	0.42	2336	18	169633	7.3	283651	8.7
Diclofenac	n.d.		n.d.		285	3.4	13.1	1.9	112	1.6	1606	0.95	1648	18	1939	6.8	3316	11
Ketoprofen	<MDL		<MDL		10.1	0.37	<MDL		42.4	15	140	4.7	222	1.6	66.3	15	564	15
Naproxen	n.d.		n.d.		156	2.0	12.3	12	28.3	6.2	534	2.8	95.4	6.0	2225	7.1	3004	8.5
Gemfibrozil	n.d.		5.72	12	15.2	14	9.46	23	39.9	3.1	80.9	2.5	141	6.0	109	5.4	189	3.1
Simvastatin	n.d.		n.d.		n.d.		n.d.		n.d.		<MDL		n.d.		n.d.		n.d.	
Pravastatin	n.d.		n.d.		n.d.		n.d.		n.d.		235	0.081	n.d.		n.d.		n.d.	
Phenolphthalein	n.d.		n.d.		<MDL		<MDL		<MDL		n.d.		n.d.		n.d.		n.d.	
Ephendrine	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		4786	1.1	5816	4.4
Fentermine	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		193	7.7
Topiramate	n.d.		0.66	6.9	77.4	2.9	23.4	3.6	192	0.58	730	3.7	985	1.7	n.d.		1074	0.40
dl-Methamphetamine	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		8.2	14	n.d.	
Caffeine	57.9	12	482	12	3202	1.8	628	4.6	474	5.8	19312	0.80	844	18	53251	14	67203	4.7
Atenolol	n.d.		n.d.		n.d.		n.d.		n.d.		1835	8.4	n.d.		2432	11	3012	9.2
Diltiazem	n.d.		n.d.		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL	
Fenofibrate	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		<MDL		n.d.		356	4.7

Table 4. Pharmaceuticals, metabolites and degradation products detected in each sample and their concentration (ng/L) in the sampling campaign of 2019.

Compound	River sample SP1		River sample SP2		River sample SP3		River sample SP4		River sample SP5		WWTP Effluent-E1		WWTP Effluent-E2		WWTP Influent-I1		WWTP Influent-I2	
	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)	Conc (ng/L)	RSD (%)
Carbamazepine	0.593	4.0	2.43	16	45.7	5.6	21.8	13	185	8.0	834	7.6	639	1.7	733	3.7	633	1.0
10,11-Epoxycarbamazepine	n.d.		n.d.		n.d.		n.d.		n.d.		57.0	12	n.d.		n.d.		n.d.	
Citalopram	n.d.		n.d.		6.64	8.9	<MDL		46.2	13	15	3.2	153	0.60	71.6	13	81.2	12
Citalopram propionic acid	n.d.		n.d.		n.d.		n.d.		n.d.		134	2.1	28.1	18	n.d.		n.d.	
Desmethylcitalopram	n.d.		n.d.		n.d.		n.d.		n.d.		185	5.1	n.d.		n.d.		n.d.	
Fluoxetine	5.79	13	6.42	2.8	8.53	13	7.19	13	21.1	10	66.8	37	63.2	25	82.1	15	81.8	6.2
Paroxetine	n.d.		n.d.		n.d.		n.d.		n.d.		112	8.3	n.d.				n.d.	
Sertraline	n.d.		n.d.		12.5	12	9.34	3.0	21.4	10	89.7	1.7	87.8	11	224	1.2	171	2.7
Trazodone	n.d.		n.d.		34.0	11	15.8	3.7	148	19	344	2.0	362	9.1	298	13	414	8.5
Venlafaxine	n.d.		n.d.		8.26	2.1	n.d.		n.d.		n.d.		n.d.		397	3.9	n.d.	
Bupropion	n.d.		n.d.		n.d.		<MDL		28.5	2.2	60.6	8.5	77.9	0.064	52.2	1.1	147	11
Azithromycin	n.d.		n.d.		73.0	8.4	6.20	5.7	41.7	7.4	210	2.8	34.3	3.1	139	14	15.1	9.1
Ciprofloxacin	n.d.		n.d.		n.d.		n.d.		n.d.		257	13	n.d.		264	10	378	1.1
Clarithromycin	n.d.		n.d.		69.4	9.8	9.73	5.1	31.6	2.6	166	2.2	16.6	1.2	158	8.6	12.5	5.8
Ofloxacin	n.d.		n.d.		<MDL		<MDL		n.d.		110	11	41.7	18	<MDL		39.9	16
Sulfadiazine	114	9.3	n.d.		n.d.		n.d.		n.d.		n.d.		<MDL		n.d.		20.6	5.7
Sulfamethazine	n.d.		n.d.		n.d.		4.87	13	n.d.		n.d.		n.d.		n.d.		n.d.	
Sulfamethoxazole	n.d.		n.d.		n.d.		6.96	12	22.1	1.8	n.d.		n.d.		179	2.6	291	14
Sulfapyridine	n.d.		n.d.		n.d.		15.2	14	n.d.		n.d.		n.d.		245	5.9	353	16
Trimethoprim	<MDL		n.d.		n.d.		n.d.		80.6	19	215	10	236	17	236	16	349	15
Tetracycline	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		332	3.6	n.d.		n.d.	
Chlorocycline	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		1006	1.6	n.d.		n.d.	
Acetaminophen	35.2	11	44.3	7.3	51.7	7.9	43.7	9.7	148	18	70.8	14	494	5.3	3094	2.6	31965	7.2
Carboxyibuprofen	n.d.		n.d.		43.8	2.8	109	6.3	n.d.		n.d.		n.d.		14282	0.069	21480	12
Diclofenac	n.d.		n.d.		119	3.4	72.5	1.9	848	1.6	2272	0.95	2799	18	2178	6.8	3372	11
Hydroxyibuprofen	49.0	0.59	65.4	7.2	480	1.5	300	0.65	226	4.9	10002	0.42	299	18	11364	7.3	11273	8.7
Ibuprofen	3.41	29	8.88	3.8	57.8	0.63	43.1	2.8	35.9	8.1	2457	13.6	163	6.3	4655	2.5	8229	15
Ketoprofen	17.5	7.0	18.8	3.2	28.6	0.37	71.7	0.71	61.3	15	22	4.7	179	1.6	384	15	552	15
Naproxen	n.d.		n.d.		50.5	2.0	40.9	12	130	6.2	900	2.8	492	6.0	3126	7.1	1696	8.5
Nimesulide	n.d.		6.50	15	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
Salicylic acid	54.6	16	74.9	13	75.4	17	85.0	21	125	4.2	978	3.4	142	15	4612	5.8	32733	20
Gemfibrozil	n.d.		n.d.		n.d.		n.d.		25.8	3.1	82.0	2.5	78.9	6.0	n.d.		n.d.	
Astorvatatin	n.d.		n.d.		68.3	2.7	n.d.		n.d.		686	0.22	298	4.4	507	3.8	n.d.	
Fenfluramine	n.d.		n.d.		n.d.		n.d.		n.d.		20.9	4.2	n.d.		n.d.		n.d.	
dl-Methamphetamine	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		<MDL		n.d.		n.d.	
Phenolphthalein	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		406	4.4	n.d.	
Topiramate	n.d.		n.d.		35.8	2.9	24.5	3.6	237	0.58	894	3.7	858	1.7	n.d.		548	0.40
Caffeine	76.8	12	65.9	12	151	1.8	399	4.6	145	5.8	836	0.80	n.d.		35104	14	52959	4.7
Atenolol	n.d.		n.d.		n.d.		n.d.				1215	8.4	n.d.		3171	11	2368	9.2
Diltiazem	n.d.		n.d.		n.d.		<MDL		25.6	14	24.8	15.0	n.d.		28.4	12	n.d.	

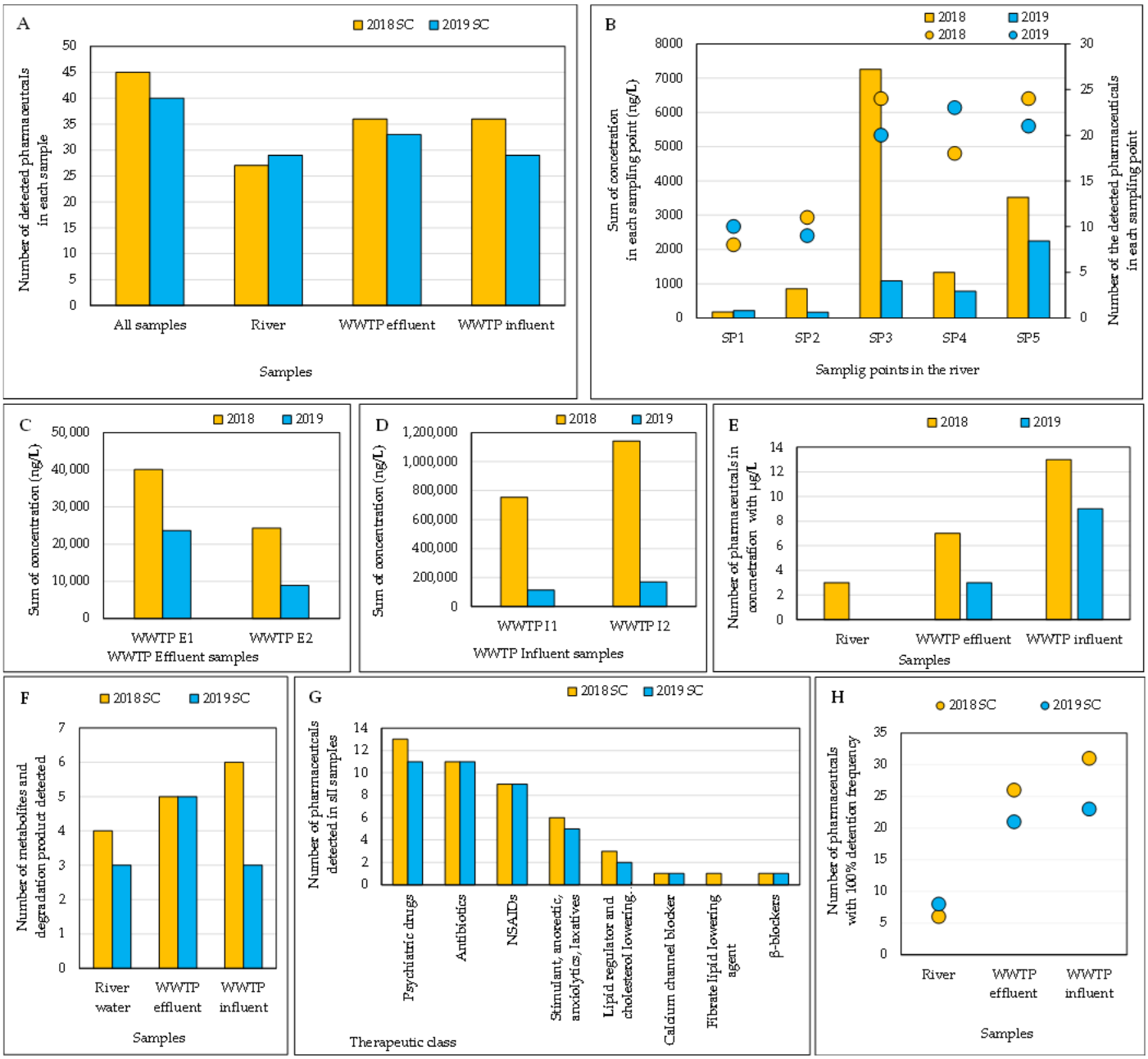

Figure 1. Results for each type of sample (river water and WWTP effluent and influent samples) in the sampling campaigns performed in 2018 and 2019: (A) Number of detected pharmaceuticals; (B) Sum of the concentrations and number of detected pharmaceuticals in each sampling site in surface water samples; (C) Sum of the concentrations of detected pharmaceuticals in WWTP effluent samples; (D) Sum of the concentrations of detected pharmaceuticals in WWTP influent samples; (E) Number of pharmaceuticals with concentration at µg/L level, (F) Number of metabolites and degradation product; (G) Number of pharmaceuticals detected by therapeutic class; and (H) Number of pharmaceuticals with 100% of detection frequency.

Temporal analysis from 2013 to 2019

Sampling campaigns have been conducted at the same sites since 2013 [1, 3], and 33 compounds were common across all campaigns (2013, 2014, 2017, 2018, and 2019). The study focuses on pharmaceuticals, particularly antibiotics and psychiatric drugs, due to their high prescription rates and media attention. Additionally, the widespread use of NSAIDs and analgesics presents challenges for control, as they are prescribed for chronic inflammatory conditions and are available over the counter for various ailments. The results are presented in Figure 2, with concentrations shown on graphs representing the sums within therapeutic families. From 2013 to 2019, comparing the three therapeutic classes, NSAIDs/analgesics consistently showed the highest concentrations, except for samples from WWTP-E1 and -E2 in the 2018 SC, where antibiotics were predominant (Figure 2). These findings are in line with the widespread usage of NSAIDs across all age groups of the population. For most of the analyzed samples, the sum of the concentration is higher between 2017 and 2019 when compared with the results found in 2013 and 2014.

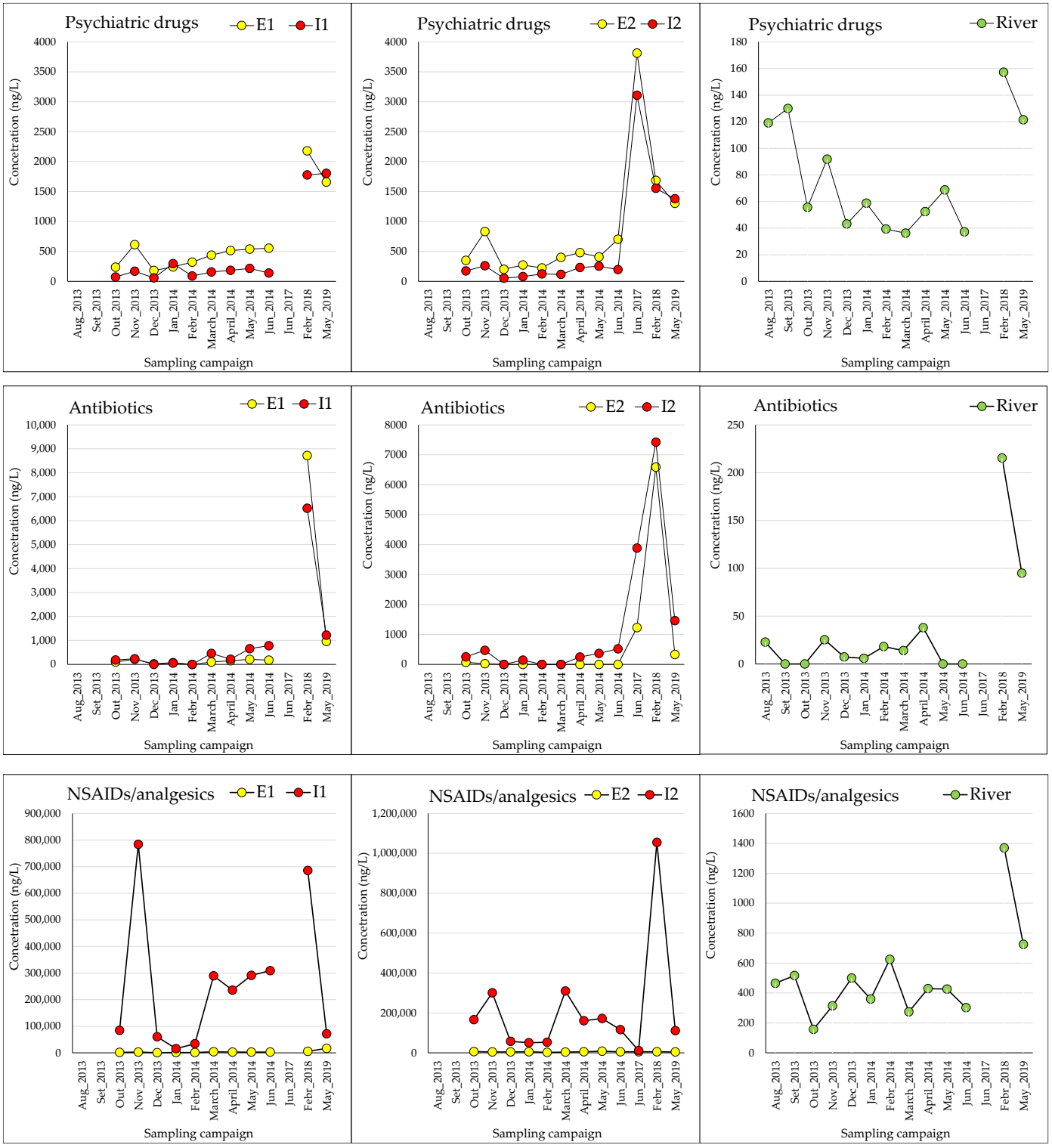

Figure 2. Temporal analysis for NSAIDs/analgesics, antibiotics, and psychiatric drugs from 2013 to 2019.

Sampling campaigns 2022 - 2023

In the sampling campaigns of 2022 and 2023, 97 compounds belonging to different therapeutic classes were analysed. The number of pharmaceuticals and the sum of concentration found in surface waters and wastewaters are presented in Figure 3.

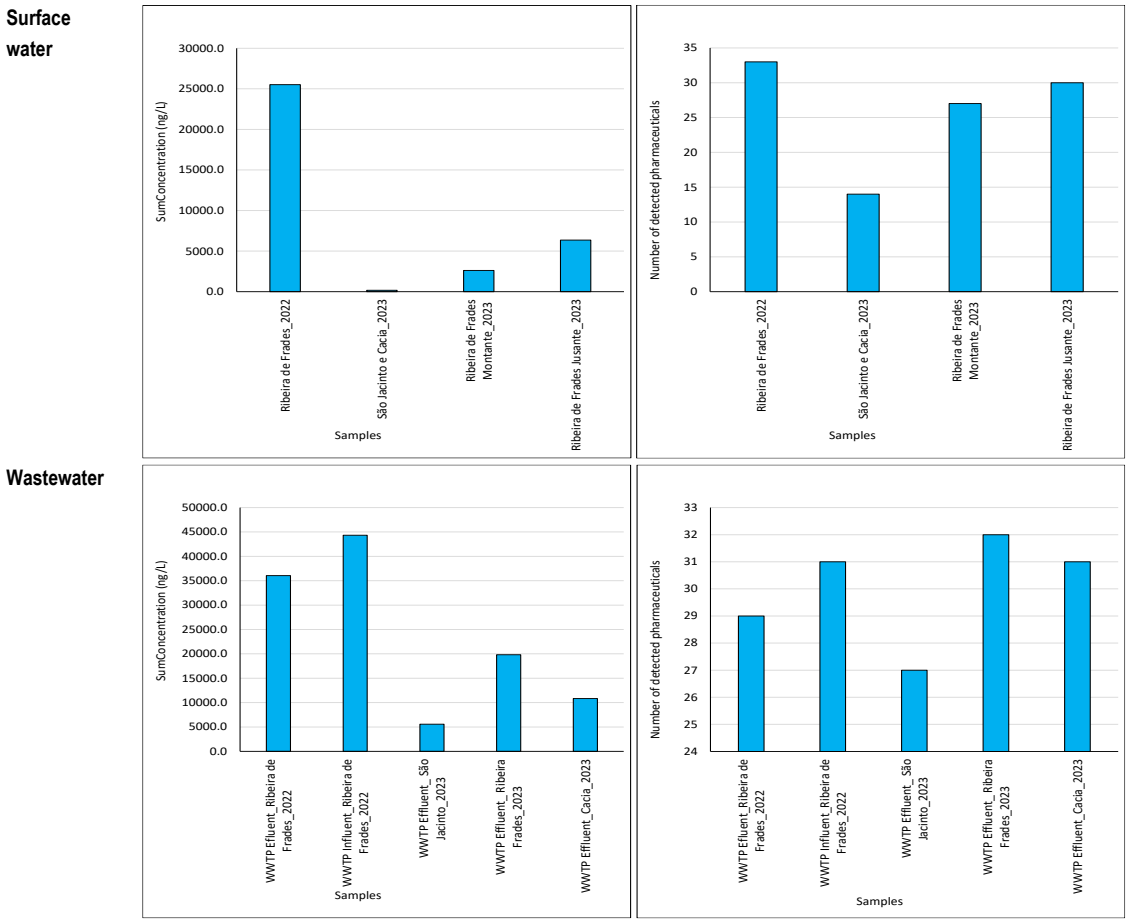

Figure 3. Sum of concentration (ng/L) and the number of pharmaceuticals detected in each sampling point in surface wasters and wastewaters samples.

Analysing both sampling campaigns, several compounds, including both pharmaceuticals and their metabolites, were consistently detected across all sampling sites. 100% detection frequency was found for 12 compounds (acetaminophen, ampicillin, azithromycin, caffeine, fluoxetine, gemfibrozil, 2-hydroxyibuprofen, ibuprofen, ketoprofen, mazindol, naproxen, and salicylic acid) in surface waters, and for 24 compounds (acetaminophen, ampicillin, azithromycin, caffeine, fluoxetine, gemfibrozil, 2-hydroxyibuprofen, ibuprofen, ketoprofen, mazindol, naproxen, salicylic acid, alprazolam, atorvastatin, bupropion, carbamazepine, citalopram, clarithromycin, *O*-desmethylvenlafaxine, diclofenac, ofloxacin, sertraline, venlafaxine, and trazodone) in wastewater. It must be highlighted that either in surface water and wastewater, the metabolites carboxyibuprofen, 2-hydroxyibuprofen, citalopram propionic acid, *O*-desmethylvenlafaxine, and 10,11-epoxy carbamazepine, as well as the degradation product salicylic acid, were detected.

Concentrations at the µg/L level were observed for atorvastatin (1620 ng/L), caffeine (3214 ng/L), *O*-desmethylvenlafaxine (2304 ng/L), diclofenac (4367 ng/L), 2-hydroxyibuprofen (5623 ng/L), ibuprofen (2267 ng/L), and ketoprofen (1338 ng/L) in surface waters, and for atorvastatin (1760 ng/L), caffeine (12664 ng/L), *O*-desmethylvenlafaxine (2364 ng/L), diclofenac (4882 ng/L), 2-hydroxyibuprofen (7767 ng/L), ibuprofen (4442 ng/L), ketoprofen (1247 ng/L), acetaminophen (3638 ng/L), carbamazepine (1359 ng/L), carboxyibuprofen (7068 ng/L), salicylic acid (8353 ng/L), and venlafaxine (1029 ng/L) in wastewater. The lowest concentrations and lower number of pharmaceuticals were detected in the São Jacinto WWTP effluent, as well as in the surface waters of both São Jacinto and Cacia.

The high detection frequency of certain pharmaceuticals (e.g., acetaminophen, caffeine, ibuprofen) in both surface water and wastewater highlights the persistent nature of pharmaceutical contamination in the environment. These findings underscore the need for continued monitoring and evaluation of pharmaceutical pollutants in aquatic environments.

6. Associated indicators

Publications

1. Paula Paíga, Luísa Correia-Sá, Manuela Correia, Sónia Figueiredo, Joana Vieira, Sandra Jorge, Jaime Gabriel Silva, Cristina Delerue-Matos, Temporal Analysis of Pharmaceuticals as Emerging Contaminants in Surface Water and Wastewater Samples: A Case Study, *in preparation*.
2. Paula Paíga, Sónia Figueiredo, Manuela Correia, Magda André, Roberto Barbosa, Sandra Jorge, Cristina Delerue-Matos, Occurrence of 97 Pharmaceuticals in Wastewater and Receiving Waters: Analytical Validation and Treatment Influence, *in preparation*.

References

[1] P. Paíga, L.H.M.L.M. Santos, S. Ramos, S. Jorge, J.G. Silva, C. Delerue-Matos. Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and seasonal variation. Science of the Total Environment 573 (2016) 164-177.

[2] P. Paíga, L.H.M.L.M. Santos, C. Delerue-Matos. Development of a multi-residue method for the analysis of human and veterinary pharmaceuticals and some of their metabolites in aqueous environmental matrices by SPE-UHPLC-MS/MS. Journal of Pharmaceutical and Biomedical Analysis 135 (2017) 75-86.

[3] P. Paíga, M. Correia, M.J. Fernandes, A. Silva, M. Carvalho, J. Vieira, S. Jorge, J.G. Silva, C. Freire, C. Delerue-Matos. Assessment of 83 pharmaceuticals in WWTP influent and effluent samples by UHPLC-MS/MS: Hourly variation. Science of the Total Environment 648 (2019) 582-600.