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Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices

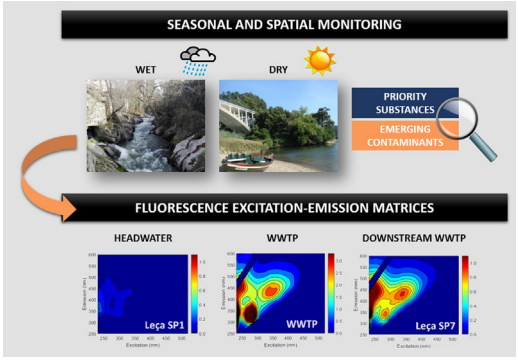
Marta O. Barbosa [a], Ana R. Ribeiro [a,*], Nuno Ratola [b], Ethan Hain [c], Vera Homem [b], M. Fernando R. Pereira [a], Lee Blaney [c], Adrián M.T. Silva [a]

[a] Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal
[b] LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, Porto, Portugal
[c] University of Maryland Baltimore County, Department of Chemical, Biochemical, and Environmental Engineering, 1000 Hilltop Circle, Baltimore, MD 21250, USA

HIGHLIGHTS

- Spatial and seasonal variations of multi-class micropollutants were studied.
- Four stressed rivers in Portugal were monitored in dry and wet seasons.
- Ketoprofen, tramadol, enrofloxacin and thiacloprid were measured at highest levels.
- The fluorescence EEMs of surface water matched the distribution of micropollutants.
- Correlation of fluorescence signatures found for azithromycin, carbamazepine, EHMC.

GRAPHICAL ABSTRACT



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ABSTRACT

The European Union (EU) has recommended the monitoring of specific priority substances (PSs, Directive 2013/39) and some contaminants of emerging concern (CECs, Decision 2015/495) in surface waterbodies. The present study provides spatial distributions and temporal variations of a wide range of multi-class PSs and CECs in four stressed rivers in Portugal (Ave, Leça, Antuã, and Cértima). Thirteen micropollutants were found in all four rivers, including the priority pesticide isoproturon (up to 92 ng L^{-1}), various pharmaceuticals (up to 396 ng L^{-1}), and the UV-filter 2-ethyl-hexyl-4-methoxycinnamate (EHMC, up to 562 ng L^{-1}) identified in Decision 2015/495. The industrial priority compound perfluorooctanesulfonic acid (PFOS) was found in three rivers (Antuã, Cértima, and Leça) below the method quantification limit, together with four pharmaceuticals not included in these EU guidelines. The already banned priority pesticide atrazine was detected in Ave, Antuã, and Leça (up to 41 ng L^{-1}) and simazine in Cértima and Leça (up to 26 ng L^{-1}). Acetamiprid and imidacloprid (included in Decision 2015/495) were only detected during the dry season in the Ave. Leça river was selected as a waterbody case study for assessment of fluorescence excitation-emission matrices (EEMs). These results matched the spatial distribution trend of micropollutants along the river, with stronger fluorescence response and higher concentrations being found downstream of industrial areas and urban wastewater treatment plants (WWTPs). Moreover, the fluorescence signature of surface water collected downstream of an urban WWTP aligned very well with that obtained for the respective WWTP effluent. Thus, actions are needed to preserve a good environmental status of these stressed European waterbodies.

* Corresponding author.
E-mail address: ritalado@fe.up.pt (A.R. Ribeiro).

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1. Introduction

Environmental contamination of aquatic compartments by organic micropollutants is a subject of major concern in the last two decades (Cho et al., 2014; Tousova et al., 2017). Surface waters are constantly exposed to such contaminants, which mainly originate from agricultural runoff and discharge of effluents from industrial and municipal wastewater treatment plants (WWTPs), with the latter considered the major source of some classes of micropollutants found in river waters (Cho et al., 2014; Barbosa et al., 2016). The occurrence of organic micropollutants in rivers at residual concentrations (Yan et al., 2013; Robles-Molina et al., 2014; Tsui et al., 2014; Campanha et al., 2015; Dai et al., 2015; Ccanccapa et al., 2016; González-Alonso et al., 2017; Wilkinson et al., 2017; Sousa et al., 2018) can lead to adverse effects for aquatic wildlife and human health, limiting the use of water for recreation, irrigation, and consumption (Vasconcelos Ferreira et al., 2010; Gorito et al., 2017).

To tackle these problems, current European Union (EU) recommendations suggest the regular monitoring of an extensive range of chemical and biological parameters in surface waters (Directive, 2000), including a list of 45 substances for priority action (priority substances, PSs) with environmental quality standards (EQS) set up for some compounds (Directive, 2008; Directive, 2013) and a Watch List of contaminants of emerging concern (CECs) (Decision_495, 2015). Moreover, Directive, 2013/39/EU set the Maximum Allowable Concentration-EQS (MAC-EQS), corresponding to the concentration that should not be exceeded at any representative monitoring point for any given surface water body. In this context, the monitoring of PSs and CECs in surface waters is a useful tool not only to assess pollution sources, but also to ensure efficient management of water resources and the protection of aquatic flora and fauna (Tousova et al., 2017).

The occurrence of particular PSs and CECs in Portuguese rivers has been reported in Ave river (Ribeiro et al., 2016a; Rocha et al., 2013), Leça river (Rocha et al., 2012), Douro river (Ribeiro et al., 2016b), Ria de Aveiro (Rocha et al., 2016), and Guadiana river (Palma et al., 2014). However, an integrated study comprising the spatial distributions and temporal variations of a wide range of PSs and CECs belonging to different classes has not been concurrently conducted in multiple rivers. Therefore, the purpose of the present study was to perform two seasonal monitoring campaigns of 39 organic micropollutants in four stressed Portuguese rivers: i) Ave; ii) Leça; iii) Antuã; and, iv) Cértima. Contamination levels of the target compounds in these rivers were investigated during dry and wet seasons, and the water samples, collected at different points on each river, were analyzed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), after sample preparation by solid phase extraction (SPE).

Quantitative PSs/CECs analysis require significant costs, which often limit the scope of the sampling plan for specific projects. For this reason, quick, inexpensive screening tools that provide insights into PSs/CECs occurrence and concentration would not only allow optimization of sampling strategies, but also elicit new research questions on the occurrence and fate of PSs/CECs in surface water systems. Excitation-emission matrix (EEM) analyses are increasingly being used to describe the fluorescence properties of dissolved organic matter (DOM) for characterization (Yamashita et al., 2008), source-tracking (Cuss et al., 2016), and fate/transformation (Mangalgiri et al., 2017) purposes. We posit that EEM analysis may serve as a useful screening tool for representative PSs/CECs given their chemical similarity with selected molecules in the DOM matrix. This concept has been previously explored with respect to CEC occurrence (Yang et al., 2013) and transformation (Yan et al., 2017). For example, Yang et al. (2013) found significant correlations for caffeine, sulfamethoxazole, acetaminophen, and ciprofloxacin concentrations with the summed volume from regions I, II, and IV of the EEMs of water samples collected from the Pearl river (China). Nevertheless, the correlation of other PSs/CECs with specific EEM regional volumes needs to be explored to develop location-based screening tools for other watersheds, especially as the DOM matrix and PSs/CECs use vary by watershed. In this study, potential correlations between EEMs and PSs/CECs occurrence and concentration were investigated in the Leça river. This report is the first to evaluate the spatiotemporal distribution of PSs/CECs and fluorescence EEMs, as well as the correlations between these water quality parameters, in a Portuguese river, and the results have important implications for other water systems around the world.

2. Materials and methods

2.1. Chemicals and materials

All reference standards (i.e., acetamiprid, alachlor, atenolol, atorvastatin, atrazine, azithromycin dihydrate, bezafibrate, carbamazepine, ceftiofur, chlorfenvinphos, citalopram hydrobromide, clarithromycin, clindamycin, clofibric acid, clopidogrel hydrogen sulfate, clothianidin, diclofenac sodium, diphenhydramine, 2-ethylhexyl-4-methoxycinnamate (EHMC), enrofloxacin, erythromycin, fluoxetine hydrochloride, hydrochlorothiazide, imidacloprid, isoproturon, ketoprofen, methiocarb, metoprolol tartrate, norfluoxetine oxalate, ofloxacin, perfluorooctanesulfonic acid (PFOS), propranolol, simazine, thiacloprid, thiamethoxam, tramadol hydrochloride, trimethoprim, venlafaxine hydrochloride, and warfarin; >98% purity) and surrogate standards (i.e., acetamiprid-d3, azithromycin-d3, atrazine–d5, diclofenac-d4, fluoxetine–d5, ketoprofen–d3, methiocarb-d3, and ofloxacin-d3) were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol (MS grade) and ethanol (HPLC grade) were acquired from VWR International (Fontenay-sous-Bois, France) and Fisher Scientific (Leicestershire, UK), respectively. Formic and sulfuric acid were obtained from Merck (Darmstadt, Germany), and ultrapure water was supplied by a Milli-Q water system (resistivity of 18.2 MΩ cm at 25 °C). Oasis® HLB (Hydrophilic-Lipophilic Balanced) cartridges (150 mg, 6 mL), used for sample preparation, were purchased from Waters (Milford, MA, USA).

2.2. Sampling area

Two sampling campaigns were performed in the dry (September 2016) and wet seasons (February 2017). During the sampling period, the weather was characterized by a mean atmospheric temperature of 23 °C in September 2016 and 11 °C in February 2017. The average precipitation was 24.3 and 113.5 mm in September and February, respectively (www.ipma.pt accessed on May 2018). The selection of Ave, Leça, Antuã, and Cértima rivers was based on the following: recognized contamination due to adjacent land-use patterns (i.e., residential, agricultural, and industrial areas); the existence of tributaries and WWTPs that can have a negative impact on the quality of these water courses; and, the presence of drinking water treatment plants (DWTPs) that may be affected by surface water pollution. Sample collection was performed along the whole course of the four target rivers (8 sampling points (SPs) for Leça and Cértima rivers and 9 SPs for Ave and Antuã rivers, Fig. 1), comprising locations near the source and mouth, as well as strategic areas subjected to impacts from urban, agricultural, or WWTP activities. The GPS coordinates of the SPs for each river are given in Table S1, Supplementary Material.

2.2.1. Ave river

The Ave river, which is situated in the North of Portugal, has an extension of about 100 km and a drainage basin area of 1340 km^2. The headwaters are located in Cabreira Mountain (1260 m above mean sea level, a.m.s.l.), and the estuary is located in Vila do Conde, along the Atlantic coast. The most important tributaries are the Este and Vizela rivers at the right and left banks, respectively. The average flow in the Ave river was 2.96 $m^3\ s^{-1}$ in the dry season (September 2016) and

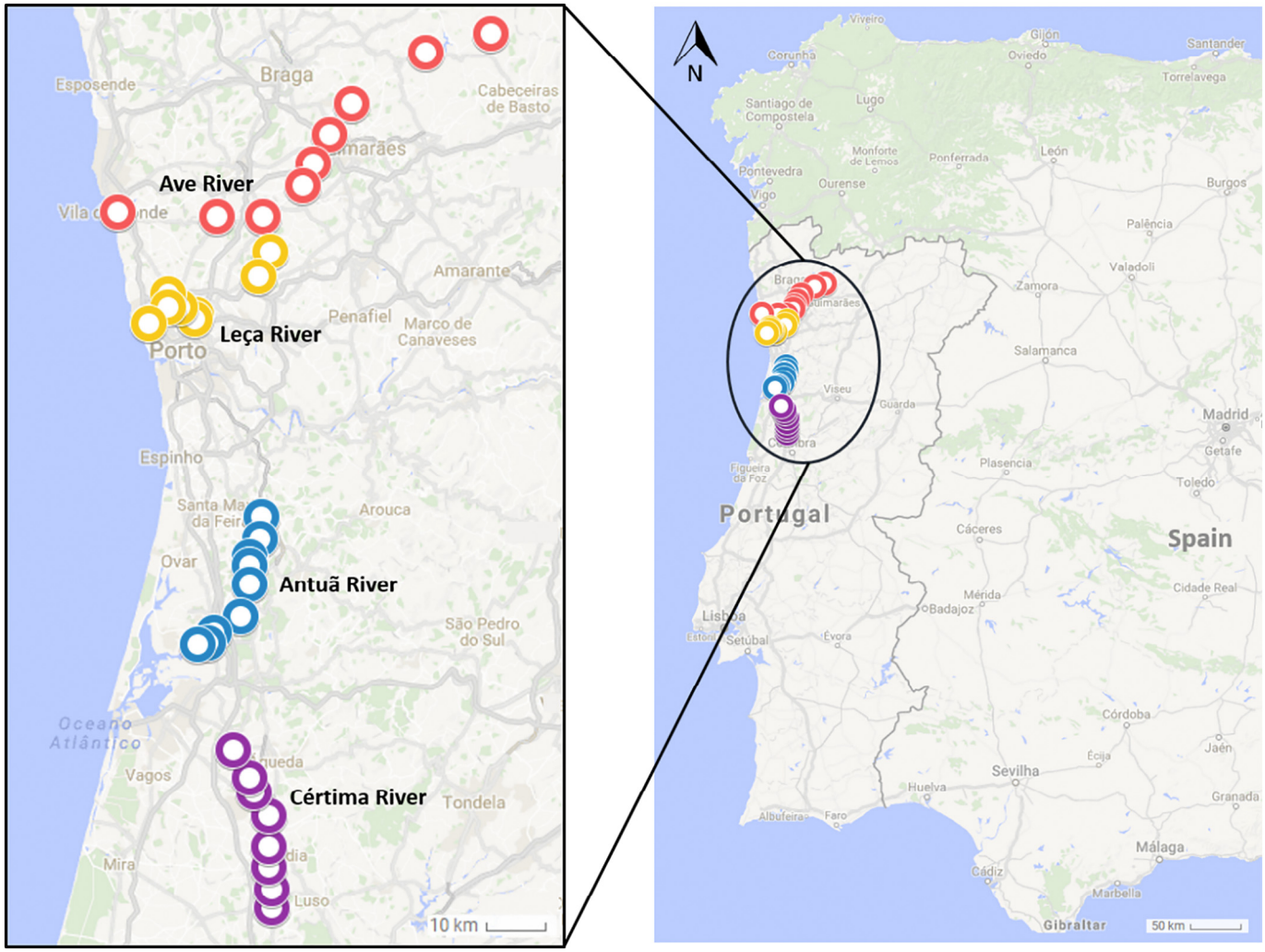


Fig. 1. Ave, Leça, Antuã, and Cértima rivers (Portugal) and the location of each sampling site.

63.08 $m^3\ s^{-1}$ in the wet season (February 2017) (www.snirh.pt accessed on May 2018). The water resources are used for manufacturing and irrigation of rural activities. Most of the river basin area is used for agricultural and livestock activities (Ribeiro et al., 2016a). Water quality problems observed in this area are associated with high industrial density, including the textile sector (largest industry), leather tanning, rubber manufacture, and plastic production. Some industrial effluents are still illegally discharged into the water courses without treatment (Rocha et al., 2013).

2.2.2. Antuã river

Antuã river (extension of 38 km) has its source at Romariz, Santa Maria da Feira (400 m a.m.s.l.) and drains into the Atlantic Ocean through the Ria de Aveiro. Antuã basin is one of the sub-basins of the Vouga river with a total area of approximately 149 km^2. The main tributaries are Pintor stream, Cercal stream, and Ínsua river on the left bank and Arrifana stream on the right bank. Antuã river is characterized by an average flow of 4 $m^3\ s^{-1}$, with the monthly average ranging between 0.6 $m^3\ s^{-1}$ in August and 10 $m^3\ s^{-1}$ in February (www.cesam.ua.pt/files/8_Congresso_Agua.pdf accessed on May 2018). Low water quality in this river stems from industrial and urban discharges, runoff from agricultural fields, and discharges from livestock farms. Agriculture is the principal activity within the borders of the Antuã river basin, which is situated near the city of Estarreja (Cerqueira et al., 2008).

2.2.3. Cértima river

The 43 km long Cértima river is located in North-Central Portugal and serves as a sub-tributary of the Vouga river, which drains into the Atlantic Ocean through the Ria de Aveiro coastal lagoon. The river source is at Buçaco Mountain (380 m a.m.s.l.), and the basin drains an area of 538 km^2. In its lower section, the river valley opens widely to form Pateira de Fermentelos lake, a sensitive wetland classified as a *Ramsar* site (i.e., wetlands of international importance designated under the *Ramsar Convention*). The river narrows again at Requeixo, where the Cértima discharges into the Águeda river (Serpa et al., 2014). The Cértima river has a flow rate of 7.17 $m^3\ s^{-1}$ at SP8 in the wet season and 0.13 $m^3\ s^{-1}$ during the dry season (Sena, 2007). The main tributaries are the Serra and Levira rivers and Ribeira do Pano (Cerqueira et al., 2005). Agriculture, domestic discharges, and industrial activities are the major sources of chemical pollution in the Cértima river basin (Vasconcelos Ferreira et al., 2010).

2.2.4. Leça river

On its flow towards the Atlantic Ocean, Leça river has an extension of 45 km and drains an area of 190 km^2 (Rocha et al., 2012), with an average flow of 3.4 $m^3\ s^{-1}$ (http://maretec.mohid.com accessed on May 2018). The source of Leça river is located at Monte Córdova, Santo Tirso (475 m a.m.s.l.) and its mouth is located at Leixões Harbor basin, an important international harbor having dock facilities for commercial, cruise, and fishing vessels and an oil terminal. The main tributaries are

Ribeira do Arquitecto and Ribeira do Leandro, both on the right bank. The river receives effluents from several industries, some of which are untreated, and urban WWTPs (Gomes et al., 2014). Leça river was selected as a case study to evaluate the correlation of fluorescence EEMs to PSs and CECs concentrations since it is located between highly urbanized and industrialized regions belonging to the Porto metropolitan area, which represents the largest Port in Northern Portugal. One sample collected after the secondary biological treatment stage of an urban WWTP was also analyzed for comparison with surface samples.

2.3. Sample collection and preparation

Surface water samples were collected in the middle of each river, using a bottle sampler. Subsequently, samples were transferred to 1 L amber glass bottles and stored at 4 °C until extraction, which was performed within 24 h. Leça river surface water and wastewater effluent samples were frozen at −20 °C until analysis of the respective fluorescence EEMs. Several parameters, such as pH, conductivity, oxidation-reduction potential, temperature, salinity, dissolved oxygen, and total dissolved solids, were analyzed on site using a HI98194 Multiparameter Meter (HANNA® instruments; Woonsocket, RI, USA). Before SPE, all samples were filtered through 1.2-μm glass-fiber filters (47 mm GF/C, Whatman™; Maidstone, United Kingdom) and the pH was adjusted to 3 using sulfuric acid.

2.4. SPE–UHPLC–MS/MS method

An offline SPE–UHPLC–MS/MS method was applied for quantification of the target organic micropollutants according to previous works (Ribeiro et al. 2015; Barbosa et al., 2016). Briefly, Oasis® HLB cartridges were sequentially conditioned with 4 mL of ethanol and 4 mL of ultrapure water at a flow rate of 1 mL min^{-1}. Sample loading of 500 mL of surface water samples was carried out at a constant flow rate of 10 mL min^{-1}, using a vacuum manifold unit. The washing step was performed with 4 mL of ultrapure water, and the cartridges were then dried under vacuum for 45 min. The elution step was performed at a flow rate of 1 mL min^{-1} with 4 mL of ethanol and the extracts were evaporated to dryness in a Centrivap Concentrator® device (LABCONCO® Corporation, Kansas City, MO, USA). The dried extracts were reconstituted in 250 μL of ethanol, and the resulting solutions were filtered through 0.22 μm polytetrafluoroethylene syringe filters (Membrane Solutions, Kent, WA, USA).

Surface water sample analysis was performed by UHPLC-MS/MS, using a Shimadzu Corporation apparatus (Tokyo, Japan), consisting of an UHPLC (Nexera) with two pumps (LC-30AD), an autosampler (SIL-30AC), an oven (CTO-20AC), a degasser (DGU-20A 5R), and a system controller (CBM-20A) with proper software (LC Solution Version 5.41SP1) coupled to a triple quadrupole mass spectrometer (Ultra Fast Mass Spectrometry series LCMS-8040). Analytical separation occurred along a Kinetex™ XB-C18 100 Å column (100 × 2.1 mm i.d.; 1.7 μm particle diameter) supplied by Phenomenex, Inc. (Torrance, CA, USA). The mobile phase consisted of (A) 0.1% formic acid aqueous solution and (B) methanol operated in gradient mode. Column oven and autosampler temperatures were set at 35 °C and 4 °C, respectively, and the injection volume was 5 μL. Selected reaction monitoring (SRM) transitions between the precursor ion and the two most abundant fragment ions were evaluated to quantify and confirm the identity of each compound. SRM1 was used for quantification purposes and the ratio between SRM1 and SRM2 was used for qualitative confirmation, along with the analyte retention time. Detailed analytical parameters and method selectivity, linear range, and limits of detection and quantification are described in the Supplementary Material (Tables S2 and S3).

2.5. Fluorescence excitation-emission matrices (EEMs)

Fluorescence EEMs of Leça river and wastewater samples collected during the wet season were measured using a Horiba Aqualog fluorescence spectrophotometer (Horiba Scientific; Edison, NJ USA). For all samples, 3-mL aliquots were added to a 1-cm quartz cuvette for analysis. Excitation wavelengths were incrementally increased from 209 to 620 nm using 3-nm steps, and the emission spectrum was recorded at 244–822 nm with 2.33-nm steps. Fluorescence EEMs of environmental samples were blank-corrected using LC-MS grade water. Inner-filter effects were corrected using the controlled dilution approach (Luciani et al., 2009; Kothawala et al., 2013). The 1st and 2nd order Rayleigh scattering lines were removed using the Horiba masking tool. A sealed Raman water fluorescence standard (Agilent Technologies; Santa Clara, CA USA) was used to convert all data to Raman Units (Timko et al., 2015). Corrected EEMs were plotted in Matlab (Mathworks; Natick, MA, USA), and regional volumes were calculated according to Chen et al. (2003). The corrected EEMs were considered in terms of tyrosine (region I), tryptophan (region II), fulvic acid (region III), soluble microbial product (region IV), and humic acid (region V)-like fluorescence. Pearson correlations were conducted to assess relationships between CECs concentrations for the compounds detected at all sampling sites (i.e., azithromycin, carbamazepine, and EHMC) and regional/total volumes from the EEM analysis. The correlations were considered statistically significant at a 95% confidence interval (p-value < 0.05). All statistical analyses were performed in R-studio 3.5.0.

3. Results and discussion

3.1. Physicochemical characterization

To assess the water quality and anthropogenic impacts in the four rivers, physicochemical parameters, namely pH, temperature, dissolved oxygen, conductivity, salinity, total dissolved solids, and turbidity, were measured at all sampling sites in both seasons (Table S4). The pH values ranged between 5.0 and 8.1 in the Ave, between 6.3 and 7.2 in the Leça, between 6.7 and 7.4 in the Antuã, and between 6.9 and 8.0 in the Cértima. This parameter affects the solubility of nutrients, and the values measured in all rivers (between 5 and 8) are optimal for plankton growth and nutrient availability (Ribeiro et al., 2016a, 2016b). The pH was generally higher during the wet season in comparison to the dry season. The pH was constant during each season along the Leça and Cértima rivers. In the Ave, pH increased from SP1 to SP9 (close to the mouth of the river), whereas pH increased slightly from SP1 to SP5 and then decreased from SP5 to SP9 in the Antuã. Dissolved oxygen concentrations varied slightly in each season but were generally higher during the wet season, except in the case of the Ave. In Ave and Leça, a gradient of conductivity, salinity, and total dissolved solids was detected from SP1 until the last SP, where the values were higher by at least one order of magnitude, since these SPs were located in the estuary. The same increasing trend was not observed between SP5 and SP9 of Antuã river or for SP2 to SP3 and SP5 to SP9 of Cértima river. These three physicochemical parameters (i.e., conductivity, salinity, and total dissolved solids) were typically higher during the dry season in all rivers.

3.2. Distribution and seasonal variation of target micropollutants in four Portuguese rivers

Thirteen micropollutants, namely azithromycin, carbamazepine, clarithromycin, clindamycin, diclofenac, diphenhydramine, EHMC, fluoxetine, isoproturon, metoprolol, thiacloprid, tramadol, and venlafaxine, were found in all four rivers (Table S5). Many of these ubiquitously detected compounds are pharmaceuticals, and their occurrence is related to overall consumption and recalcitrance to wastewater treatment with domestic and hospital effluents being the

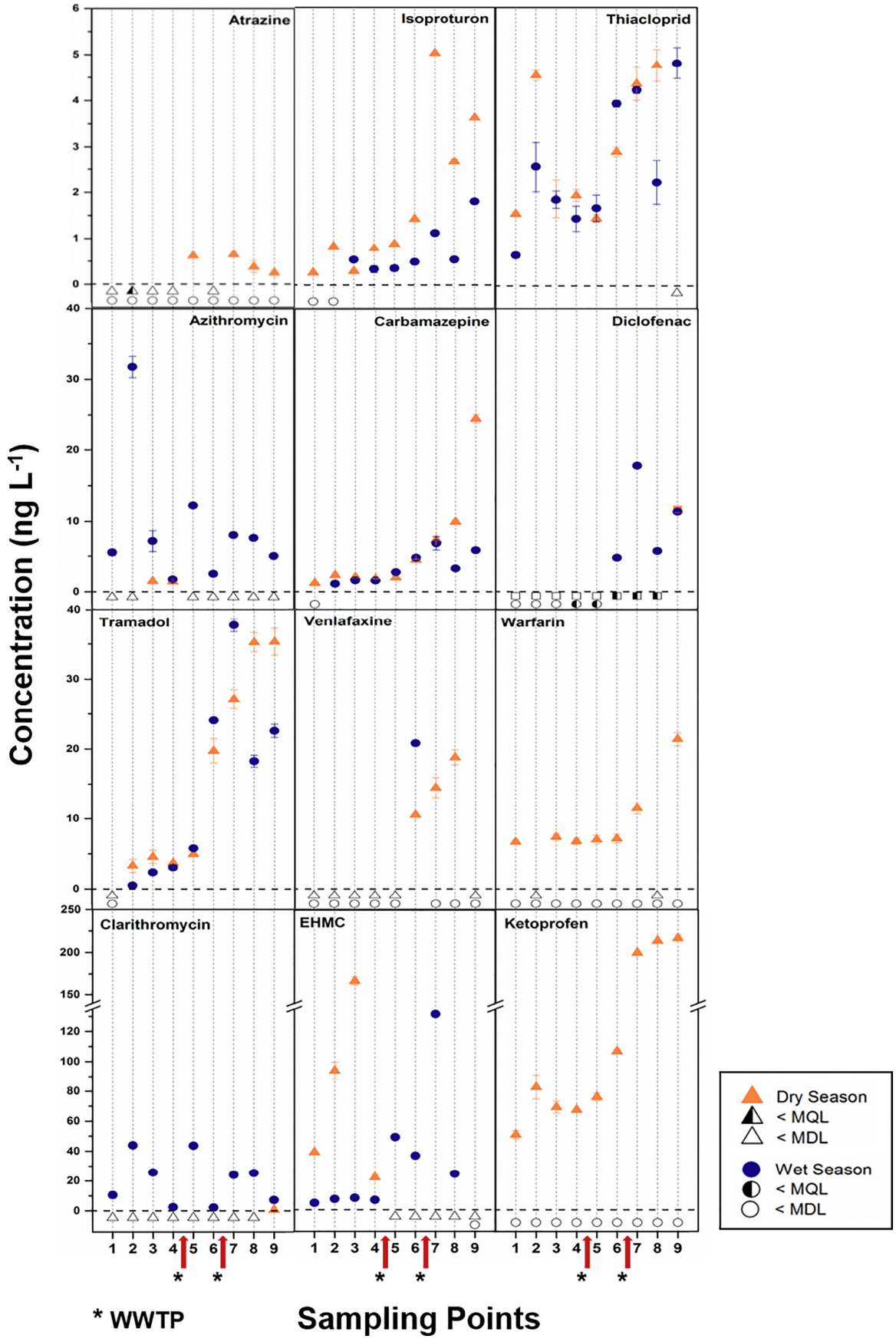


Fig. 2. Spatial distribution and concentrations of micropollutants in Ave river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in Ave river, please see Fig. S1).

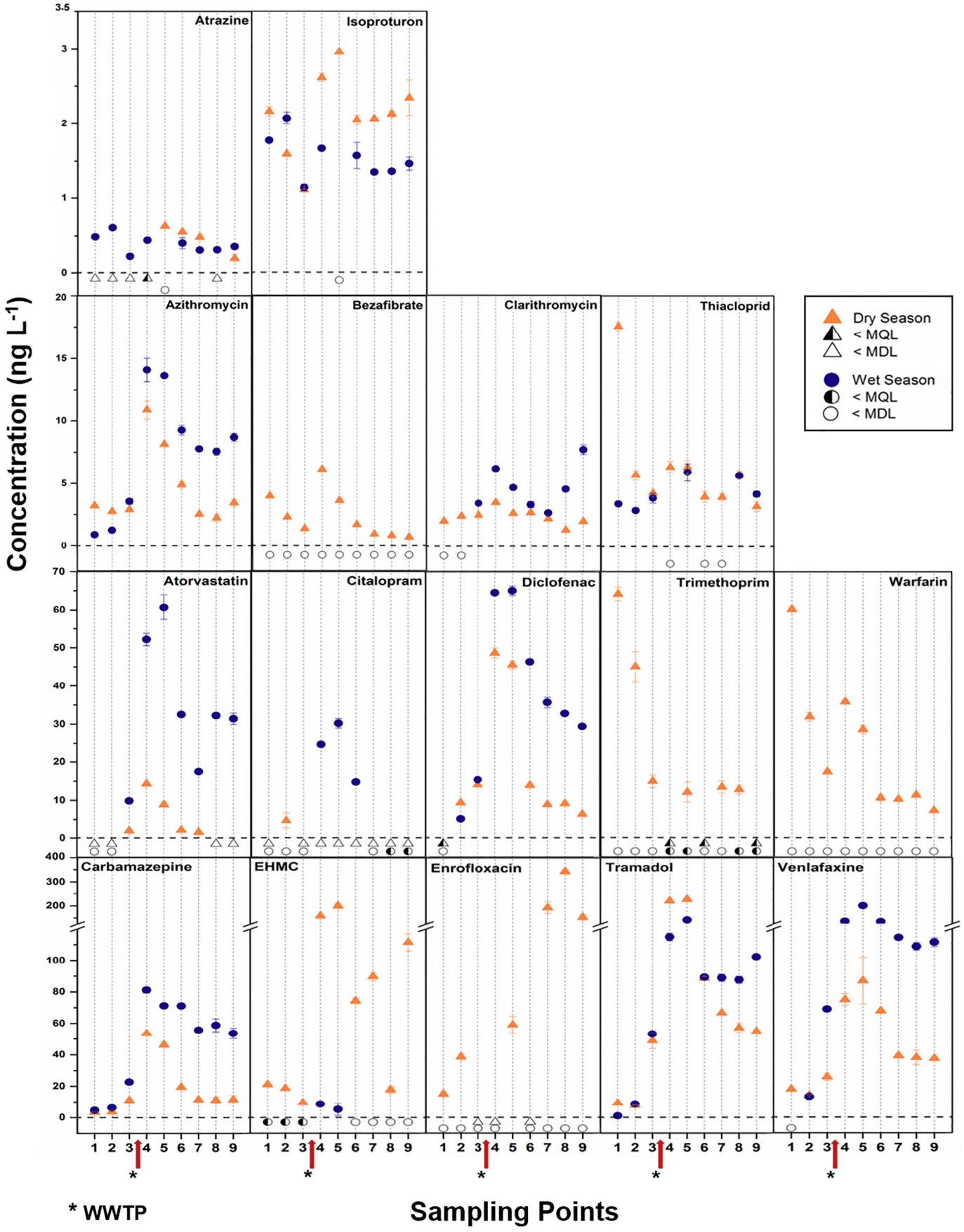


Fig. 3. Spatial distribution and concentrations of micropollutants in Antuã river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in Antuã river, please see Fig. S2).

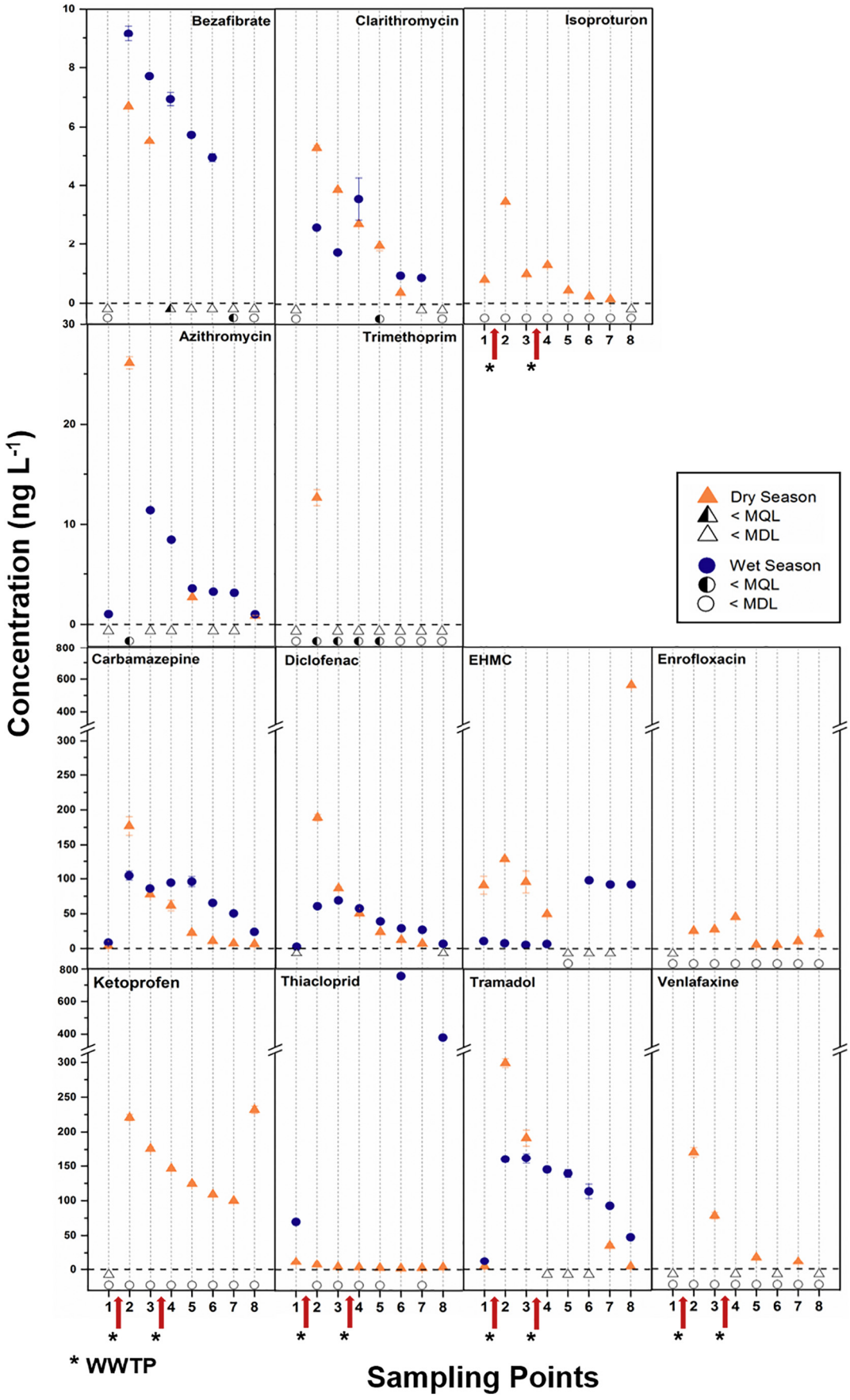


Fig. 4. Spatial distribution and concentrations of micropollutants in Cértima river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in Cértima river please see Fig. S3).

main sources (Chitescu et al., 2015). For pesticides, isoproturon was found below 5.0 ng L^{-1}, except in Leça river, where it was detected at higher concentrations (9.42–92.5 ng L^{-1},), but still below the 1.0 μg L^{-1} MAC-EQS set in Directive, 2013/39/EU and the maximum admissible concentration for pesticides in drinking water, established by Directive 98/83/EC as 0.1 μg L^{-1} for individual pesticides and 0.5 μg L^{-1} for their sum (Directive, 1998). On the contrary, thiacloprid was quantified above the maximum value allowed for individual pesticides in drinking water at two SPs of Cértima river. Bezafibrate, enrofloxacin, PFOS, propranolol, and trimethoprim were also identified in the Antuã, Cértima, and Leça rivers. PFOS was always below the MAC-EQS set in Directive, 2013/39/EU. Acetamiprid and imidacloprid were only detected in the Ave in the dry season, being acetamiprid below the method quantification limit (MQL) at three SPs and imidacloprid quantified at SP6 and below the MQL at SP9. In this river, the pharmaceuticals, ketoprofen (also in Cértima) and warfarin (also in Antuã), were quantified at almost all SPs in the dry season. Atrazine was detected in the Ave, Antuã, and Leça rivers. This banned triazine pesticide was present at levels <1.58 ng L^{-1}, except at SP9 of Leça, where it reached 41 ng L^{-1}. Simazine, which is also a triazine pesticide, was detected in Cértima and Leça rivers, with the highest concentration (26 ng L^{-1}) measured at Leça SP9. Both triazine pesticides were always below their MAC-EQS (2.0 μg L^{-1} for atrazine and 4.0 μg L^{-1} for simazine). The sum of all pesticides was below the maximum admissible concentration (0.5 μg L^{-1}) for pesticides in drinking water, defined in Directive 98/83/EC (Directive, 1998). Atorvastatin was identified in the wet and dry seasons in the Antuã (up to 61 ng L^{-1}) and Leça (up to 24 ng L^{-1}) rivers. The antidepressant drug citalopram was only quantified at 30 ng L^{-1} in the Antuã river. Overall, the concentrations of the micropollutants were generally lower in the wet season, which can be attributed to dilution effects associated with the higher flow rates in all rivers. For some compounds, the opposite trend was observed, namely higher concentrations were determined in the wet season. These findings may be attributed to seasonal differences in consumption (e.g., antibiotics), as reported in other works (Nannou et al., 2015). The lower temperatures and shorter daylight hours of the winter season may also impede biodegradation and phototransformation mechanisms resulting in less environmental transformation (Meierjohann et al,. 2016).

3.2.1. Ave river

Eighteen of the thirty nine target compounds were detected in the Ave. Figs. 2 and S1 show the spatial distribution and concentrations of micropollutants in dry and wet seasons. Some target compounds, namely acetamiprid, atrazine, clindamycin, diphenhydramine, imidacloprid, ketoprofen, metoprolol, and warfarin, were only quantified in the dry season, which may be related to the lower precipitation and flow rates observed. From the target micropollutants, those with frequency of occurrence higher than 50% in the Ave were as follows: clindamycin, diphenhydramine, fluoxetine, ketoprofen, and warfarin in the dry season; the macrolide antibiotics (i.e., azithromycin and clarithromycin) and EHMC in the wet season; and isoproturon, thiacloprid, carbamazepine, and tramadol in both seasons. The highest concentrations determined in this river corresponded to the anti-inflammatory ketoprofen, which was found at all SPs during the dry season at concentrations between 50 and 217 ng L^{-1}. This anti-inflammatory compound was also determined at high concentrations in the Llobregat river (Spain) (Osorio et al., 2012), due to its broad use in human medicine. EHMC was detected during the dry season at four SPs (SP1–4) at concentrations up to 168 ng L^{-1}. This UV-filter was also identified at almost all SPs during the wet season, with a maximum concentration of 132 ng L^{-1}. Similar EHMC concentrations were reported in other studies from Brazil (n.d. to 150 ng L^{-1}), Spain (mean: 24.2 ng L^{-1}), Japan, China, USA, and Arctic (up to 150 ng L^{-1}) (Tsui et al., 2014; da Silva et al., 2015; Aparicio et al., 2017). The occurrence of EHMC in Hong Kong river water samples was reported at higher levels (4043 ng L^{-1}) (Tsui et al., 2014). Although UV-filters are widely used in personal care products to protect human skin from UV radiation, they are also applied in several materials, such as rubber, plastics, and paints, to prevent degradation (Tsui et al., 2014). The occurrence of EHMC in both seasons may be related to these applications. Downstream of SP6 in the Ave, a marked increase was observed for the concentration of many micropollutants (except for azithromycin, clarithromycin, and EHMC), which can be explained by the presence of two urban WWTPs, and other anthropogenic activities (e.g., agriculture and industry). Overall, the concentration of pollutants generally increased from the headwaters to the mouth of the Ave.

3.2.2. Antuã river

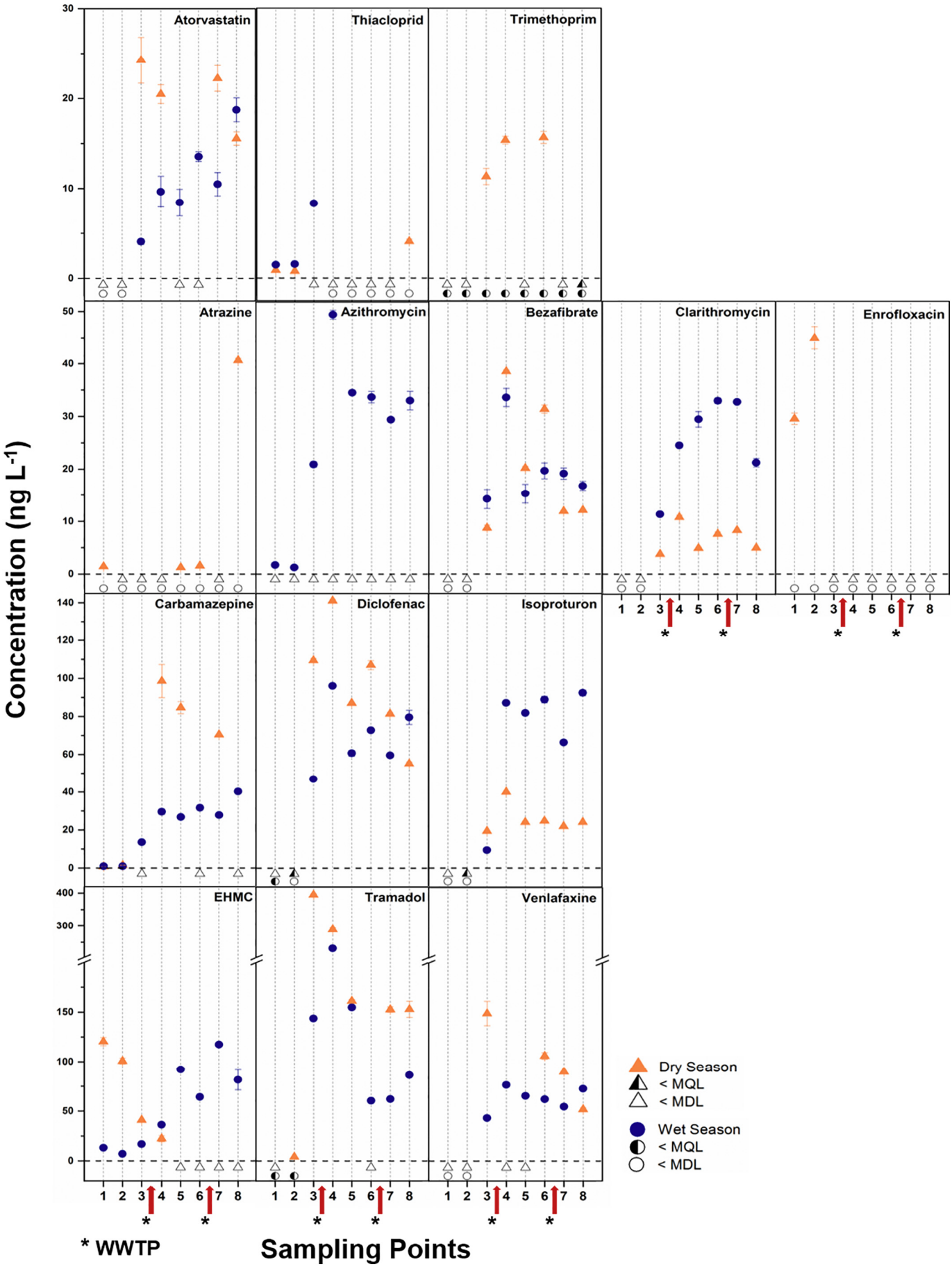
Twenty-two target organic micropollutants were found in the Antuã, including seventeen pharmaceuticals, three pesticides, a UV-filter, and an industrial compound (Figs. 3 and S2). Some of these analytes occur only in the dry season, namely the antibiotics enrofloxacin and trimethoprim, bezafibrate, metoprolol, and warfarin. The most frequently detected compounds (>50%) in Antuã river samples during the two campaigns were the pesticides isoproturon and thiacloprid and the pharmaceuticals atorvastatin, azithromycin, carbamazepine, clarithromycin, clindamycin, diclofenac, diphenhydramine, fluoxetine, tramadol, and venlafaxine. The highest concentration in this river was found for enrofloxacin in the dry season (343 ng L^{-1}), which may be due to the livestock production in the surrounding areas. In the wet season, venlafaxine registered the highest concentration at SP5 (199 ng L^{-1}). A significant increase in the concentrations of many contaminants was recorded downstream of Salgueiro WWTP (SP4). Several factors contribute to the variation of micropollutant concentrations along this river and between the different rivers investigated here. For instance, enrofloxacin and PFOS were determined in Antuã river and not detected in Ave river. Overall, flow rate, environmental factors (e.g., temperature, sunlight, nutrients), and fate/distribution mechanisms, such as adsorption to sediments or particulate matter, biodegradation, photodegradation, other abiotic processes, and uptake by biota, affect the concentrations of these compounds along the rivers of interest (Paíga et al., 2016).

3.2.3. Cértima river

In Cértima river (Figs. 4 and S3), twenty compounds were determined at concentrations up to 755 ng L^{-1} during the wet and dry season monitoring campaigns. The high concentrations observed in this river can be related to its low flow rate (ca. 0.13 m^3 s^{-1}) during the dry season. In fact, some target compounds, namely the pharmaceuticals diphenhydramine, propranolol, metoprolol, trimethoprim, enrofloxacin, ketoprofen, and venlafaxine and the pesticide isoproturon, were quantified only in the samples collected during the dry season. The most frequently detected (>50%) micropollutants in the Cértima varied by season: in the dry season, diphenhydramine, isoproturon, ketoprofen, the antibiotic enrofloxacin, and the neonicotinoid thiacloprid; in the wet season, azithromycin, bezafibrate, fluoxetine, and the pesticide simazine; and across both seasons, clindamycin, carbamazepine, diclofenac, EHMC, tramadol, and clarithromycin. The highest concentrations in this river were recorded for the neonicotinoid thiacloprid in the wet season (755 ng L^{-1}), a finding which may be due to agricultural leaching caused by precipitation events, and for the anti-inflammatory ketoprofen in the dry season (702 ng L^{-1}). With the exception of thiacloprid and PFOS, the concentrations of organic pollutants increased at SP2. The higher concentrations of most micropollutants in this area can be explained by the presence of Mealhada WWTP.

3.2.4. Leça river

The levels of organic micropollutants determined in the eight SPs of Leça river are reported in Figs. 5 and S4. Twenty-one compounds were observed, including the antibiotics clindamycin, enrofloxacin, and trimethoprim, metoprolol, and the pesticides atrazine and simazine


Atorvastatin
Thiacloprid
Trimethoprim
Atrazine
Azithromycin
Bezafibrate
Clarithromycin
Enrofloxacin
Carbamazepine
Diclofenac
Isoproturon
EHMC
Tramadol
Venlafaxine
Concentration (ng L-1)
Dry Season
< MQL
< MDL
Wet Season
< MQL
< MDL
* WWTP
Sampling Points

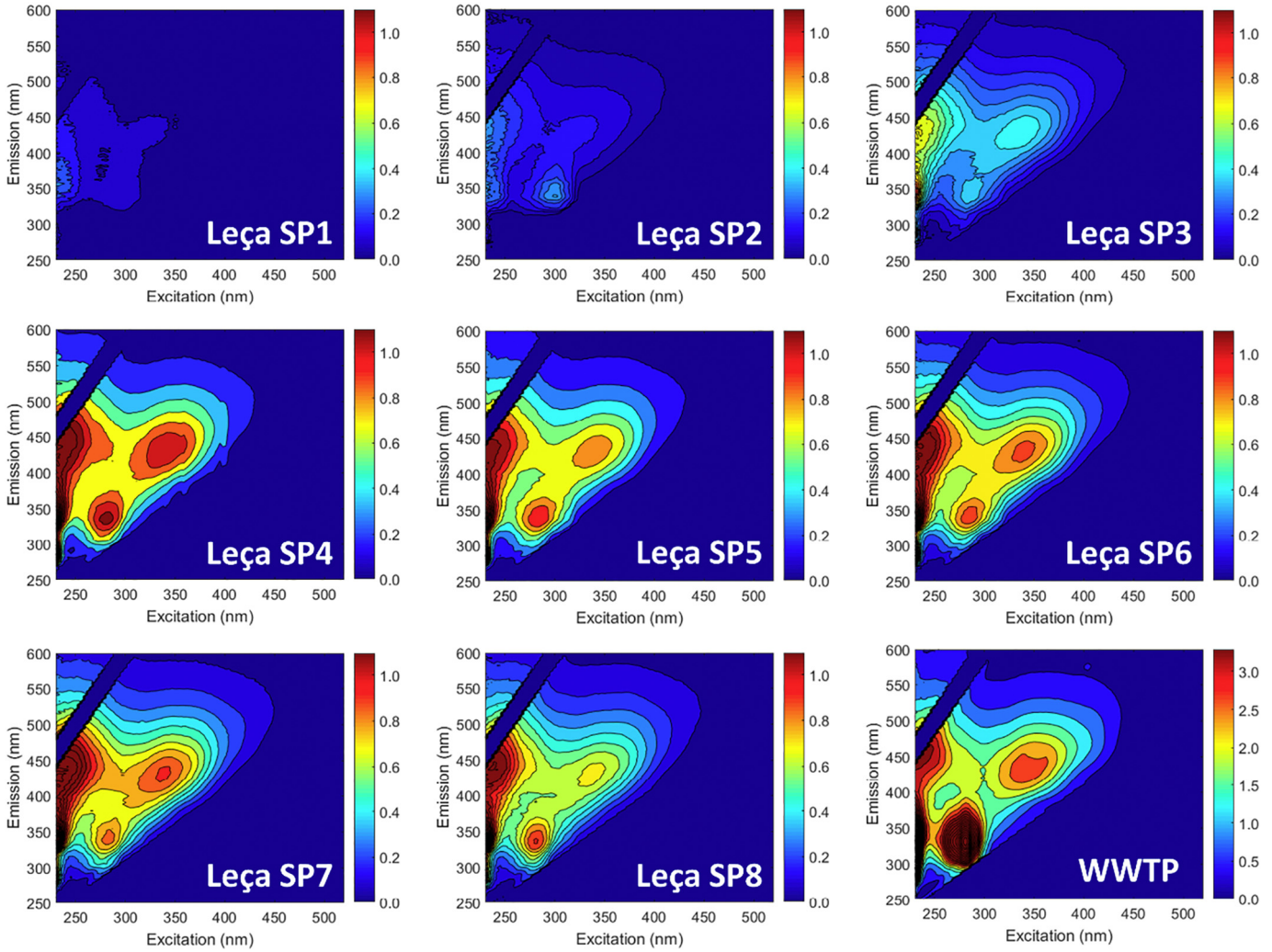


Fig. 6. Fluorescence EEMs for the eight Leça river samples and one WWTP sample.

quantified only during the dry season, and azithromycin found only in the wet season. Twelve analytes, namely atorvastatin, diphenhydramine, fluoxetine, propranolol, bezafibrate, clarithromycin, carbamazepine, diclofenac, isoproturon, EHMC, tramadol, and venlafaxine, were detected in >50% of the river samples across both seasons. During the dry and wet seasons, tramadol was found at the highest concentration, with a maximum of 396 ng L^{-1} and 233 ng L^{-1}, respectively. This analgesic was frequently detected in all four rivers up to hundreds of ng L^{-1}, as also recently reported by Burns et al. (2018) for two rivers in York (UK). EHMC, isoproturon, carbamazepine, diclofenac, and venlafaxine were also found at high concentrations during the two sampling campaigns and, together with tramadol, these micropollutants exhibited the highest detection frequencies and concentrations.

Parada and Ponte de Moreira WWTPs (upstream of SP4 and downstream of SP6, respectively) and the associated industrialized areas (SP4 and SP7) seemed to directly influence the levels of organic micropollutants in Leça river, increasing the concentration of most compounds. These data were well correlated with the fluorescence EEMs of the surface water and WWTP effluent samples (Fig. 6). The WWTP effluent sample exhibited a strong fluorescence response in all regions, and the fluorescence signature was similar to previous reports for wastewater (Baker, 2001; Sgroi et al., 2017). The fluorescence signatures of SP1 and SP2 were minimal. SP3 showed minor fluorescence in regions III (fulvic acid-like) and V (humic acid-like). The fluorescence response increased in all regions at SP4, downstream of the first WWTP. The SP5–8 samples showed similar fluorescence signatures in all regions, although the signal was slightly lower than at SP4. However, slight increases in the fluorescence response were observed at SP6, which is influenced by a WWTP, and SP7, which is surrounded by a highly industrialized area.

To better highlight the changes in fluorescence with sample location, the regional volumes of the environmental samples were normalized by the corresponding regional volumes from the WWTP sample. As indicated in Fig. 7, the relative presence of aromatic protein-, fulvic acid-, soluble microbial product-, and humic acid-like fluorescence increased between SP3 and SP4. Downstream of SP4, the fluorescence response associated with regions I and II decreased, presumably due to biotic/abiotic degradation mechanisms. The fluorescence in regions III, IV, and V remained fairly consistent from SP5 to SP8.

The similarity of CEC concentrations and regional EEM volumes along the Leça river is shown in Fig. 7. Azithromycin and carbamazepine concentrations were significantly, positively correlated with the EEM volumes for regions I–V; furthermore, significant correlations were observed for EHMC concentrations with fluorescence signatures from regions III, IV, and V (Table S7 in the SI). Significant correlations were also observed for all three CECs with the total fluorescence response,

Fig. 5. Spatial distribution and concentrations of micropollutants in Leça river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in Leça river, please see Fig. S4).

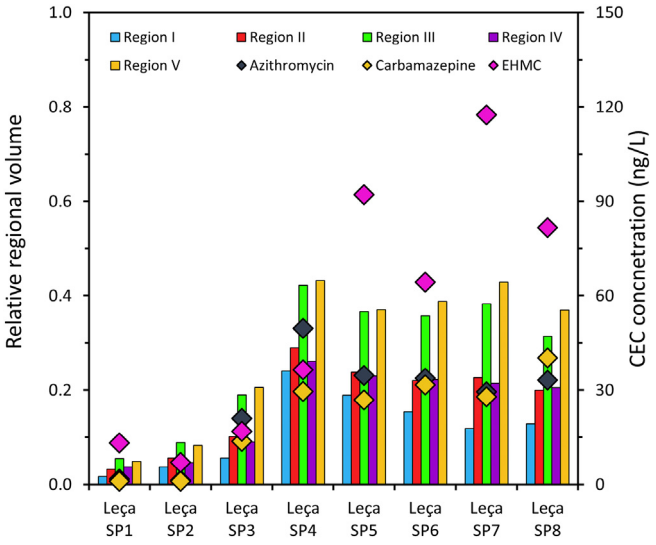


Fig. 7. The relative regional volume of environmental samples (Leça SP1–8) as a function of the WWTP sample for region I (tyrosine-like fluorescence), region II (tryptophan-like fluorescence), region III (fulvic acid-like fluorescence), region IV (soluble microbial product-like fluorescence), and region V (humic acid-like fluorescence). The average azithromycin, carbamazepine, and EHMC concentrations are plotted to highlight the correlation in EEM results and CECs concentrations.

which was calculated as the sum of EEM volumes from regions I–V. Based on these results, the fluorescence intensity in regions III, IV, and V may serve as useful screening tool to inform the presence of PSs/CECs in the Leça river. As DOM signatures and sources vary in other river systems, the EEM-based screening tool needs to be evaluated and refined for other watersheds.

While previous reports Yang et al. (2013) have found correlations between the total and summed (e.g., region I, II, and IV) EEM volumes with particular CECs, the more specific correlations with particular regions identified in this study may better reflect CECs fate and transport along spatiotemporal gradients. In particular, strong correlations were observed for azithromycin, carbamazepine, and EHMC concentrations with the region V fluorescence response, suggesting that these CECs exhibit similar fate and transport behavior as humic acid-like substances. Yan et al. (2017) highlighted the use of parallel factor (PARAFAC) analysis to not only deconvolute fluorescence EEMs into a finite number of components, but also predict CEC degradation. This approach may provide further insights into the fate and transport of PSs/CECs in Portuguese rivers.

3.3. Occurrence of target micropollutants in surface waters

The comparison of many target micropollutants found in this study with results from other reports on seasonal surface water monitoring (Table S6) is complex since the production and usage of industrial products, the application of pesticides in agricultural activities, and the consumption of pharmaceutical compounds is different between locations. However, the macrolide antibiotic azithromycin, the anti-inflammatory chemicals diclofenac and ketoprofen, and the antidepressants fluoxetine and venlafaxine have been reported in surface water at levels similar to those found in the present work. For instance, azithromycin, fluoxetine, and venlafaxine were found in Lis river (Portugal) up to 30 ng L^{-1}, 20 ng L^{-1}, and 159 ng L^{-1}, respectively (Paíga et al., 2016). Diclofenac was quantified in surface water collected from China at a maximum of 170 ng L^{-1} (Dai et al., 2015). Ketoprofen was detected in Spain up to 225 ng L^{-1} (Moreno-González et al., 2014). These findings indicate that a similar consumption pattern of these pharmaceutical compounds may occur in different regions of the world.

The pesticides atrazine and simazine were quantified in other studies at maximum levels well above those determined here. For example, in three different monitoring studies performed in Spain (Herrero-Hernández et al., 2017), Brazil (Machado et al., 2017), and Thailand (Sangchan et al., 2014), atrazine was found at maximum concentrations of 333, 320, and 800 ng L^{-1}, respectively. These concentrations are more than one order of magnitude higher than those determined in the present study (e.g., 41 ng L^{-1}). Simazine was found in Australia at concentrations between 50 and 670 ng L^{-1} (Allinson et al., 2014) and in Spain up to 207 ng L^{-1} (Herrero-Hernández et al., 2017). The intense agricultural activity in those regions may explain the presence of triazine herbicides in surface water even after these chemicals were phased out since they can be illegally acquired and/or released from existing sediments/soils. In contrast, the other target pesticide, isoproturon, was determined at higher concentrations in the present study compared to values found in the literature (Palma et al., 2014; Papadakis et al., 2015).

The UV-filter EHMC was determined in this work at a maximum value of 562 ng L^{-1} and a similar level was reported in Brazil (669 ng L^{-1}) (da Silva et al., 2015). This compound was also detected in surface water samples in Hong Kong at concentrations one order of magnitude higher, i.e., 4043 ng L^{-1} (Tsui et al., 2014), than those determined in the present study. Importantly, this compound has also been shown to accumulate in aquatic and marine organisms (He et al., 2017), raising concerns about the high aqueous-phase concentrations detected here.

The concentrations of bezafibrate, carbamazepine, clarithromycin, and thiacloprid described in the literature are slightly higher than those reported in the target Portuguese rivers. The beta-blocker metoprolol was found at a maximum concentration of 25 ng L^{-1} in Ave river, while it was determined up to 448 ng L^{-1} in Beiyun river of Beijing, China (Dai et al., 2015; Ma et al., 2017). The maximum concentration of the antibiotic trimethoprim in the current study was 64 ng L^{-1} in Antuã river in the dry season, which was comparable to the maximum concentration (36 ng L^{-1}) found in Llobregat river, Spain (Osorio et al., 2012) and lower than the maximum concentration (180 ng L^{-1}) reported in a monitoring study performed in the Los Angeles and San Gabriel rivers (Sengupta et al., 2014).

4. Conclusions

In this first simultaneous survey of specific PSs and CECs defined by EU documents in four stressed rivers (i.e., Ave, Leça, Antuã, and Cértima) in Portugal, 26 out of 39 target micropollutants were found at least in one of the selected rivers. Of the detected compounds, thirteen were consistently determined in all four rivers: azithromycin; carbamazepine; clarithromycin; clindamycin; diclofenac; diphenhyhydramine; EHMC; fluoxetine; isoproturon; metoprolol; thiacloprid; tramadol; and, venlafaxine. The highest concentrations were verified for ketoprofen in Ave river, tramadol in Leça river, enrofloxacin in Antuã river, and thiacloprid in Cértima river. These data highlight the different land-use patterns and contaminant sources found in the targeted rivers, with the occurrence and concentration distributions along particular rivers depending on location and seasonal variations. The increase in fluorescence response profiles for specific locations of the Leça river matched the distribution of micropollutants along this river. Although some of these compounds are already prioritized or defined in the Watch List, larger monitoring programs are needed for further prioritization and risk assessment of such contaminants. Given the significant correlations found for EEM regional volumes with CEC concentrations in the Leça river, preliminary EEM analysis may help to inform the design of future monitoring studies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.06.355.

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