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Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers

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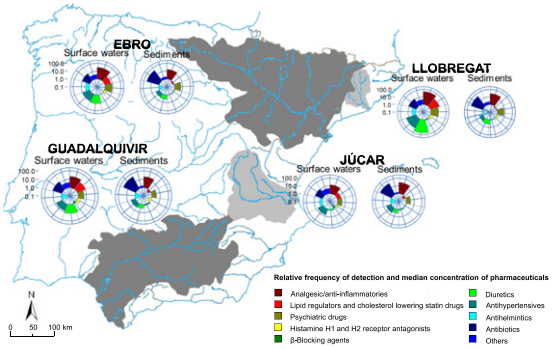
HIGHLIGHTS

- Spatial distribution of pharmaceuticals was assessed across 4 Iberian River basins.
- Ecotoxicological effects of pharmaceuticals to aquatic biota were estimated in SW.
- Hotspots of pharmaceuticals concentration and ecotoxicological risk were identified.
- Concentration and ecotoxicological risk was related to human/animal pressure.

GRAPHICAL ABSTRACT



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ABSTRACT

Considerable amounts of pharmaceuticals are used in human and veterinary medicine, which are not efficiently removed during wastewater and slurries treatment and subsequently entering continuously into freshwater systems. The intrinsic biological activity of these non-regulated pollutants turns their presence in the aquatic environment into an ecological matter of concern. We present the first quantitative study relating the presence of pharmaceuticals and their predicted ecotoxicological effects with human population and livestock units. Four representative Iberian River basins (Spain) were studied: Llobregat, Ebro, Júcar and Guadalquivir. The levels of pharmaceuticals were determined in surface water and sediment samples collected from 77 locations along their stream networks. Predicted total toxic units to algae, *Daphnia* and fish were estimated for pharmaceuticals detected in surface waters. The use of chemometrics enabled the study of pharmaceuticals for: their spatial distribution along the rivers in two consecutive years; their potential ecotoxicological risk to aquatic organisms; and the relationships among their occurrence and predicted ecotoxicity with human population and animal farming pressure. The Llobregat and the Ebro River basins were characterized as the most polluted and at highest ecotoxicological risk, followed by Júcar and Guadalquivir. No significant acute risks of pharmaceuticals to aquatic organisms were observed. However potential chronic ecotoxicological effects on algae could be expected at two hot spots of pharmaceuticals pollution identified in the Llobregat and Ebro basins. Analgesics/antiinflammatories, antibiotics and diuretics were the most relevant therapeutic groups across the four river basins. Among them, hydrochlorothiazide and gemfibrozil, as well as azithromycin and ibuprofen were widely spread and concentrated pharmaceuticals in surface waters and sediments, respectively. Regarding their predicted ecotoxicity, sertraline,

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gemfibrozil and loratidine were identified as the more concerning compounds. Significantly positive relationships were found among levels of pharmaceuticals and toxic units and population density and livestock units in both surface water and sediment matrices.

1. Introduction

Freshwaters receive considerable inputs of non-regulated pollutants like pharmaceutically active compounds (PhACs), which are consumed by human population and used in livestock farming (Kemper, 2008; Awad et al., 2014). Reliable information about PhACs consumption patterns in livestock farming and treatment of humans is scarce but a straightforward approach to indirectly assess them is their determination in PhAC-impacted surface waters. Up to now, the occurrence of more than 200 different PhACs has been reported in lakes, rivers and streams, for instance at concentrations of up to a maximum of 6.5 mg L^{-1} for the antibiotic ciprofloxacin (Petrie et al., 2015; Hughes et al., 2013). Of particular concern are antibiotics, which are used in great quantities in animal farming not only for therapeutic purposes (see Kools et al., 2008), but they are also administered to healthy livestock to promote growth (Van Boeckel et al., 2015). The second important source of PhACs in surface waters is expectedly the human population. The combined effects of improved health standards in developing countries with their rapidly growing populations and of aging populations in industrialized nations are anticipated to lead to an increase in the consumption of PhACs and ultimately their environmental burden. To date most publications on the environmental occurrence of PhACs study their presence in different matrices in conjunction with their spatial and temporal distribution. In many studies the sites with the highest levels of PhACs were located in the vicinity of big cities with high population densities (Fernández et al., 2010).

The intrinsic biological activity of PhACs turns their presence in the aquatic environment into an ecological matter of concern, since, despite intense research over the past 15 years, there are still substantial knowledge gaps in terms of chronic effects on non-target aquatic organisms and the effects on ecosystem functioning and biodiversity loss (Bartelt-Hunt et al., 2011; Hughes et al., 2013). Recently, several studies conducted at laboratory scale showed that some PhACs can act as endocrine disruptors suspected of causing intersex, while the widespread presence of antibiotics has been shown to lead to the selection of antibiotic resistant bacteria in the environment.

The application of chemometrics in environmental studies has facilitated the assessment of a huge volume of data and thus allowing statistically reliable conclusions (Mas et al., 2010). The more recent research on the environmental occurrence of PhACs has been carried out relying on chemometrics (Dai et al., 2015; Jia et al., 2011). The role of livestock and agricultural activities was proposed as a source of antibiotic contamination in the Huangpu River (Jiang et al., 2011). In other studies, however, the use of chemometrics allowed to statistically identify human discharge as the main source of antibiotic sulfonamides and other PhACs to Liaodong Bay and Beiyun River (China) (Jia et al., 2011; Dai et al., 2015). However to the best of our knowledge there are no quantitative studies in the literature relating their presence and their predicted ecotoxicity with human population and livestock. In this context, this study aimed (I) to determine the presence of the contaminants in four main river basins of the Iberian Peninsula, (II) to evaluate their spatial and temporal distribution between water and sediment compartments of the river along the four river basins, (III) to assess the ecotoxicological risk to aquatic organisms related to the PhAC presence in these freshwater systems and to correlate the predicted risk with sources of emission.

2. Materials and methods

2.1. River basins

Four representative Spanish River basins and 77 sampling sites located along their stream networks were studied: Llobregat (15 sites), Ebro (23), Júcar (15) and Guadalquivir (24) river basins (see Fig. S-1 in supporting material). These sampling sites were subjected to very different kind and degree of stresses, with some sites in clean headwater reaches and the others at various positions along the stream network. The Llobregat River (NE, Spain) is 156 km long and drains a 4957 km^2 catchment. This typically Mediterranean river is characterized by a highly variable hydrology, which is strongly influenced by seasonal rainfall. The Ebro River (NC-NE, Spain) is 910 km long and drains an area of 85,534 km^2. Due to its larger size, the river covers contrasting climates thus being characterized by a complex hydrological regime. The Júcar River (E, Spain) is 498 km long and drains a 21,632 km^2 catchment. Its hydrology is typically Mediterranean, with considerable hydrologic variability and rapid alternation of droughts and floods. The Guadalquivir River (S, Spain) is 657 km long and drains a 57,527 km^2 catchment. The entire basin is under a Mediterranean climate, receiving some influence from the Atlantic Ocean in the lowest part. Summer droughts are especially severe as a result of high temperature and lack of rain. These basins are characterized by a high population, agricultural and industrial pressure. As a consequence, water pollution is common all along these Iberian River basins. To test the relationship between the sources of PhACs, i.e. humans and livestock, and the occurrence of PhACs in the water and the sediments, we processed geographic data. Raster layers provided by the Food and Agriculture Organization of the United Nations (FAO, http://www.fao.org) were used to calculate the human population density and the livestock units (LSU) at each of the catchments. For the human population the 2015 estimate of global population map was used with a pixel size of 2.5 arc-minutes. The livestock densities were obtained for 2014 as separate layers for cattle, pigs, sheep, goats and chicken with a pixel size of 0.5 arc-minutes. The density values of those layers were multiplied by the coefficients specified in Eurostat (http://ec.europa.eu/eurostat) for each kind of animal: cattle = 1, pigs = 0.5, sheep and goat = 0.1 and chicken = 0.014. Those multiplied values were summed to obtain a new layer representing the livestock units (LSU), i.e. the cattle-equivalent density of domesticated animals at each pixel. This aggregation is based on the nutritional requirements of the animals, but we used it as an approach to represent the stockbreeding intensity as a source of PhACs in our sites. Both for the population density and for the LSU the average value of the pixels located in the upstream catchment for each of the sampling site was used as descriptor. The subcatchments in the four basins studied (Llobregat, Ebro, Júcar and Guadalquivir) spanned two orders of magnitude in terms of population density (from 1.8 to 208.7 human km^{-2}) and an order of magnitude for LSU (from 10.9 to 147.4 LSU km^{-2}) (Fig. 1). The population density was significantly higher in the catchments of Guadalquivir and Llobregat and lowest in Júcar, with Ebro showing values in between (ANOVA: $F_{3,73}$: 70.37, $p < 0.0001$) (Table S-1). On the other hand, the highest values for LSU were estimated for Llobregat, followed by Ebro and then by Guadalquivir and Júcar. Both variables were uncorrelated to each other (Pearson $r = 0.094$, $p = 0.42$).

2.2. Sampling campaign and sample analysis

Two extensive field campaigns were carried out in autumn 2010 (C1) and 2011 (C2) under different hydrological conditions. The

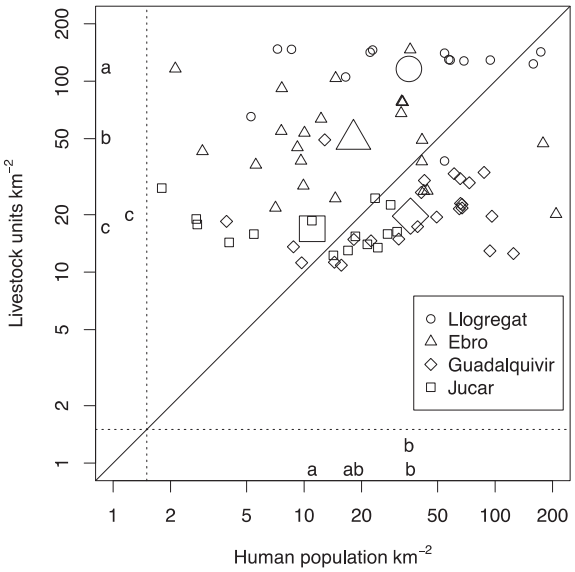

Fig. 1. Average livestock units (Y axis) versus average human population (X axis) calculated for every sub-catchment (km^2) that drained to each location studied: Llobregat (○); Ebro (△), Júcar (□) and Guadalquivir (◇). Geometric averages for population density and livestock unit using all the values at each of the four main basins are also shown. The diagonal line represents the 1:1 relation. Letters placed on the Y axis margin (a, b, c); and the X axis margin (a, b) represent the respective TukeyHSD posthoc differences for livestock units and human population density among the four river basins studied, respectively.

autumn of 2010 was characterized by intense precipitation, which resulted in the high flow of Iberian rivers, while the autumn 2011 was dry and the river flows were low. Grab surface waters (SW) and bed sediments were collected along the four river basins. Amber glass bottles pre-rinsed with ultrapure water were used for sample collection. Bottles were placed in a cooler (at 4 °C) and delivered to the laboratory within 2 days. Samples were immediately pre-treated and stored in a refrigerator (−20 °C) until analysis within one week. Due to logistic issues, SW samples from CAB2 (Júcar) and sediment samples from EBR5 and EBR8 (Ebro); JUC3 and CAB4 (Júcar) were not collected during the first sampling campaign. Over the second sampling campaign, only sediments from EBR8 were not collected. Procedures for analysis of water and sediments samples were previously described elsewhere (Jelic et al., 2009; Gros et al., 2012). Briefly, a) *SW samples* were filtered through 0.7-μm glass fiber filters followed by 0.45-μm nylon membrane filters (Whatman, U.K.). An aqueous solution of 5% Na_2EDTA was added to the SW samples to achieve a final concentration of 0.1% and surrogate standards were spiked at a final concentration of 50 ng L^{-1} in SW. Target compounds were extracted from SW samples by automatic Solid Phase Extraction (SPE) with a GX-271 ASPEC™ system (Gilson, Villiers le Bel, France) using Oasis HLB cartridges (200 mg, 6 mL). SPE cartridges were conditioned with 6 mL of methanol followed by 6 mL of HPLC grade water at a flow rate of 2 mL min^{-1}. 500 mL of SW were loaded onto the cartridge at a flow rate of 1 mL min^{-1}. After sample pre-concentration, cartridges were rinsed with 6 mL of HPLC grade water, at a flow rate of 2 mL min^{-1} and were dried with air for 5 min, to remove excess of water. Finally, analytes were eluted with 6 mL of pure methanol at a flow rate of 1 mL min^{-1}. The final volume of the extract was 1 mL methanol/water (10:90, v/v) and 10 μL of a 1 mg L^{-1} standard mixture of isotopically labeled standards. b) *Sediment samples*. 1 g of lyophilized sediment was spiked in the laboratory with perdeuterated PhACs as surrogate standards at 10 ng L^{-1} (see supporting material) and extracted by pressurized liquid extraction (PLE) using Dionex ASE 350 (Dionex; Sunnyvale, CA). Then, concentrated extracts were diluted in HPLC grade water to a methanol content of <5 vol.% and processed applying the same protocol used for SW samples. Afterwards, a selected list of 76 PhACs (Table S-2) was determined in SW and sediment extracts using a multi-residue analytical method based on ultrahigh performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS) (Gros et al., 2012) (see supporting material).

2.3. Chemicals and materials

The standards (see Table S-2 in supporting material) were purchased from Sigma-Aldrich (Steinheim, Germany); US Pharmacopeia (USP), European Pharmacopeia (EP), and Toronto Research Chemicals (TRC). Isotopically labeled compounds were used for internal standard calibration and as surrogate standards and were provided by Sigma-Aldrich (Steinheim, Germany), CDN isotopes (Quebec, Canada) and Toronto Research Chemicals (Ontario, Canada). All standards were of purity grade (>90%). Stock standard solutions were prepared on a weight basis in methanol, except ofloxacin and ciprofloxacin, which were dissolved in methanol adding 100 μL of NaOH 1 M, and cefalexin, which was solved in HPLC grade water. After preparation, standards were stored at −20 °C. Fresh stock solutions of antibiotics were prepared every three months while fluoroquinolone antibiotics were prepared monthly due to their limited stability. Stock solutions for the rest of substances were renewed every six months.

2.4. Calculation of toxic units

Toxic units (TU) were estimated on the basis of acute toxicity of PhACs to aquatic organisms. TU values were calculated as the ratio between concentrations and EC_{50} reported and estimated values, for three in vivo bioassays commonly used in environmental toxicology, namely, algae, *Daphnia* and fish (Table S-3). The ecotoxicity of PhACs to these aquatic organisms was assessed in SW of the entire four river basins, thus TU values estimated for each location studied. Acute toxicity values searched on the literature were only available for 55 compounds out of the 76 PhACs analyzed. Consequently, the study of the ecotoxicological effects to aquatic organisms was referred only to these 55 compounds (Table S-3). To assess the ecotoxicological risk of PhACs to aquatic organisms along the four river basins, we summed TU values of each compound at every site, on the basis of the concentration addition model for mixtures of substances (Ginebreda et al., 2014). Since the relative contribution of each PhAC to the ecotoxicity may vary according to its individual toxicity and concentration, to identify the PhACs that were contributing most to the total toxicity of the water at each site we divided the concentration: EC_{50} ratio for each compound by the total TU of the site and gave the result as percentage.

2.5. Statistical methods

ANOVA analyses followed by TukeyHSD pairwise comparisons were performed to compare human population density and LSU across the 4 basins. To avoid using multiple zeroes in the analyses derived from undetected PhACs we used the limits of detection (LOD) and limits of quantification (LOQ) of the analytical procedure (see Table S-4) in the datasets: Undetected compounds and compounds below LOQ were given the corresponding LOD/2 and LOQ/2 value in the datasets. As the distribution of the PhACs was extremely right-skewed and transformations were not able to approach it to normality we opted to use a non-metric Multidimensional Scaling (NMDS) to understand the distribution of the PhACs in the four basins with an ordination analysis. The NMDS was based on rank orders of Euclidean distances of log-transformed values of the PhAC concentrations. Permanova analyses (Euclidean pairwise distances and 10^6 permutations) with basins and sampling campaigns as fixed factors were performed to test for the overall differences of PhACs concentration in SW and sediment samples (Anderson, 2001). The PhACs were then compared among the four basins and the two sampling campaigns by means of ANOVA based on permutation (Anderson, 2001). As multiple univariate analyses were being

performed Bonferroni correction was applied to p-values to control familywise error rate (Dunn, 1961). We wanted to find the sites, basins and campaigns with outlying values above and below the average concentration across all the samples. We assumed that the distribution of the concentration of PhACs to be lognormal (Limpert et al., 2001) (i.e. approximate normal distribution after being log-transformed). Thus, outlying values were extracted from boxplots constructed with log-transformed concentration values. The concentrations above the value of adding 1.5 times the interquartile range to the 75 percentile (i.e. to the 3rd quartile) were considered "outlying high" concentrations. On the contrary, concentrations below 1.5 times the interquartile range below the 1st quartile were considered outlying low values. Thus, the outlying high and low values represent cases that showed outlying values from the distribution of the log-transformed concentrations for each particular PhAC. We counted the number of outlying concentrations observed per basin, site, campaign or PhAC to find the most problematic cases. To test whether the presence of outlying values was consistent across the four basins and the two sampling campaigns we performed a Fisher's exact test (Agresti, 1992). The relationship between mean concentration of PhACs, in the SW and the sediment, and the population density and the LSU were tested by means of linear mixed effect models (LME models) with sampling campaign as a random factor (Pinheiro and Bates, 2000). Mean concentration of PhACs used for human was tested against human population density, whereas mean concentration of PhACs used with livestock was tested against LSU (see Table S2 to see the use of the different PhACs). The relationship between the TU and, the population density and LSU were also tested by means of LME models. To test the effect of the variation of the discharge from the first to the second campaign we computed ratios using discharge and PhAC concentrations. If the discharge was the only relevant factor that varied between the sampling campaigns the discharge C2:C1 ratios and the PhAC concentration C2:C1 ratios would show the opposite trend. We tested this by a Permanova model with C2:C1 ratios of the PhAC concentrations as dependent variable and C2:C1 ratios for the discharge as independent. Both ratios were log-transformed for the analyses and Euclidean distance was used as dissimilarity index. All statistical analyses were performed in R using the package Vegan for NMDS analysis, LME4 for LME models (R Core Team, 2014).

3. Results

3.1. Occurrence of PhACs in water and sediments in the four river basins

The concentration of PhACs in SW varied from the low to high ng L^{-1} range (Table S-5). Llobregat and Ebro rivers were the most polluted in PhACs during C1, with corresponding total levels for the entire basin of 13,022 and 12,028 ng L^{-1}. These concentrations were substantially lower in Guadalquivir and Júcar: of 1702 and 759 ng L^{-1}, respectively. Diversely, Ebro presented the highest levels of drugs all along C2, followed by Llobregat, Guadalquivir and Júcar, with respective total concentrations of 7202, 4948, 4676, and 1638 ng L^{-1}. Among the three most concentrated therapeutics groups, per catchment and campaign, analgesics/antiinflamatories presented the highest average levels in all SW samples (see Table S-5 and also median concentrations averaged for both campaigns in Fig. 2). These levels were the highest in the Llobregat river, 193.88 and 109.21 ng L^{-1} for C1 and C2, respectively; followed by the Ebro, 147.52 and 90.04 ng L^{-1} for C1 and C2, respectively; the Guadalquivir, with respective 18.77 and 63.19 ng L^{-1} for C1 and C2; and the Júcar, with 9.36 and 27.99 ng L^{-1} in C1 and C2, respectively. Other concentrated therapeutic groups along the four river basins were: diuretics, in Llobregat (238.60 and 88.44 ng L^{-1} in C1 and C2, respectively) and Ebro (85.18 and 74.29 ng L^{-1} in C1 and C2, respectively) and Guadalquivir (29.67 ng L^{-1} in C2); antihypertensives in Ebro (94.73 and 43.65 ng L^{-1} in C1 and C2, respectively) and Llobregat (187.14 ng L^{-1} in C1) and Júcar (10.98 ng L^{-1} in C1); lipid regulators/cholesterol lowering drugs in Guadalquivir (11.91 and 41.62 ng L^{-1} in C1 and C2, respectively) and Llobregat (70.76 ng L^{-1} in C2); antibiotics in Júcar (11.28 and 24.99 ng L^{-1} in C1 and C2, respectively) and Guadalquivir (10.98 ng L^{-1} C1); and antihelmintics in Júcar (20.00 ng L^{-1} in C2). The individual compounds averaging highest levels per river basin (and campaign) were: iopromide in Llobregat (373.95 ng L^{-1} in C1), gemfibrozil in Llobregat (70.27 ng L^{-1} in C2) and Guadalquivir (11.46 and 40.98 ng L^{-1} in respective C1 and C2), hydrochlorothiazide in Ebro (72.22 and 61.33 in respective C1 and C2) and thiabendazole (6.31 ng L^{-1} in C1) and metronidazole in Júcar (22.40 ng L^{-1} in C2) (Table S-5). Other PhACs detected at high concentrations were: valsartan, furosemide, ibuprofen, ketoprofen, irbesartan, tetracycline, losartan, naproxen and indomethacine (Table S-5). Regarding their frequency of detection (average of both C1 and C2 is also shown in Fig. 2), about the 60% of the PhACs studied was present in at least half of the SW samples analyzed in both sampling campaigns. The 22% of these compounds were detected in all cases. PhACs were

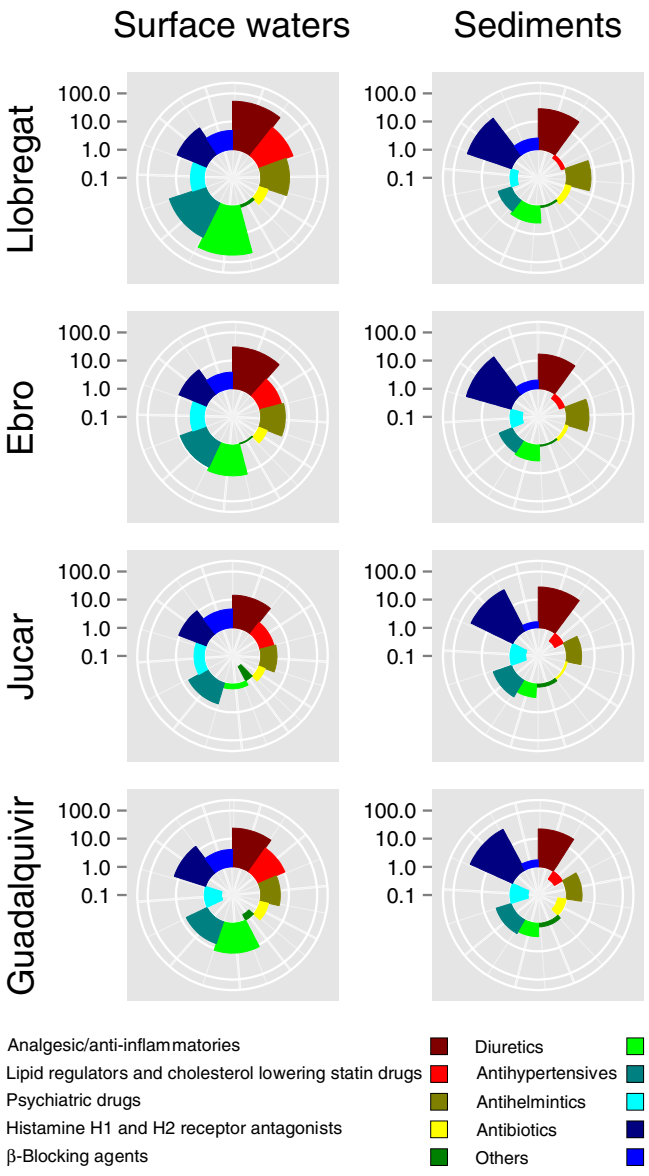

Fig. 2. Relative frequency of detection and median concentration of pharmaceuticals, classified by therapeutic families, in surface waters (left) and sediments (right) of the four river basins assessed: the Llobregat (on top), followed by the Ebro, the Júcar and the Guadalquivir (at the bottom). The circumference of each fan is scaled by the relative proportion of detections. Each point outward on the radial axis represents sequentially 0.1, 1, 10 and 100 ng L^{-1} of the median concentration of pharmaceuticals detected.

more frequently detected during C1 than over C2 in SW matrices. During C1 and C2 8 and 31 compounds respectively, were detected in less than 50% of SW samples. The major ubiquity of PhACs in SW was observed in the Llobregat river basin. Ebro, Júcar and Guadalquivir followed the frequency of detection rate for SW. Regarding individual compounds thiabendazole, hydrochlorothiazide and glibenclamide were present in all SW samples.

PhACs were found in sediments at the low ng g^{-1} level (Table S-6). Sediments from Guadalquivir and Ebro were the most concentrated, with respective total values for the entire basin of 1875 and 1596 ng g^{-1} in C1, and 1871 and 1601 ng g^{-1} in C2. Differently, levels determined in Llobregat and Júcar were lower and varied from respective values of 1323 and 1005 ng g^{-1} in C1 to 1051 and 1218 ng g^{-1} in C2. Among the three most concentrated therapeutics groups, per catchment and campaign, antibiotics and analgesics/antiinflamatories averaged the higher concentrations in all sediment samples (see Table S-6 and also median concentrations averaged for both campaigns in Fig. 2). These levels were the highest in Llobregat river, 22.97 and 15.08 ng g^{-1} for C1 and C2, respectively, followed by Ebro, 18.81 and 11.96 ng g^{-1} for C1 and C2, respectively, Júcar, with 21.74 and 33.31 ng g^{-1} in respective C1 and C2; and Guadalquivir, with respective 16.74 and 19.40 ng g^{-1} for C1 and C2. Other concentrated therapeutic groups were: psychiatric drugs in Llobregat (18.02 and 5.26 ng g^{-1} in respective C1 and C2) and Ebro (7.26 and 5.07 ng g^{-1} in respective C1 and C2) and Júcar (4.41 ng g^{-1} in C1), diuretics in Júcar (3.19 ng g^{-1} in C2) and Guadalquivir (3.32 ng g^{-1} in C2) and histamine receptor antagonists in Guadalquivir (6.89 ng g^{-1} in C1) (Table S-5, Fig. 2b). Concerning individual compounds, among the most concentrated (considering average highest levels) all along the catchments and over campaigns we found sertraline (12.08 ng g^{-1} in Llobregat C1), ketoprofen (7.13 ng g^{-1} in Llobregat C2), acridone (3.73 ng g^{-1} in Ebro C1), hydrochlorothiazide (3.01 ng g^{-1} in all cases), tetracycline (5.92 ng g^{-1} in all cases), codeine (11.58 ng g^{-1} in all cases), ibuprofen (12.56 ng g^{-1}) clarithromycin (12.72 ng g^{-1} in all cases) and azithromycin (23.92 ng g^{-1} in all cases). As for their frequency of detection (average of both C1 and C2 is also shown in Fig. 2), about the 60% of the PhACs studied were present in at least half of the sediment samples analyzed in both sampling campaigns. The 18% of these compounds were detected in all cases. PhACs were more frequently detected during C1 than over C2 in sediment matrices. 21 and 30 compounds were detected in less than 50% of sediment samples over C1 and C2, respectively. As it was observed in SW, the major ubiquity of PhACs in sediment matrices was observed in the Llobregat river basin. Júcar, Ebro and Guadalquivir river basins followed the frequency of detection rate. Regarding individual compounds, azithromycin and thiabendazole were the most ubiquitous compounds in sediments.

3.2. PhACs distribution differences over sampling campaigns and sites

The ordination of the sampled sites and the 76 PhACs by NMDS revealed a better fit for SW than for sediments (see lower stress value in Fig. 3). Llobregat and Ebro rivers showed more variability among sites than Júcar and Guadalquivir, in particular when considering SW samples (Fig. 3). The overlapping of C1 (continuous lines in Fig. 3) and C2 (broken lines) polygons for SW pointed out a large similarity between the two sampling campaigns, while temporal differences were clearly observed for sediment. Among the 20 compounds that showed the most differing patterns among sampling sites and campaigns we found atenolol, propyphenazone, phenazone or clarithromycin in SW (with high concentrations in the first campaign for Llobregat and Ebro, Fig. 4) and venlafaxine in sediment samples (with high concentrations in the first campaign for Llobregat, Fig. 5). These compounds differing the most among samples both for SW and sediments belonged to the therapeutic groups of analgesics/antiinflammatories, lipid regulators, psychiatric drugs, β-blocking agents, antihypertensives, x-ray contrast media, antihelmintics and antibiotics. Compounds classified as diuretics, prostatic hyperplasia and to treat asthma drugs were also among the ones showing the highest variations among samples in SW, while in sediments histamine receptor antagonists, synthetic glucocorticoid and calcium channel blocker were varying the most. According to the Permanova taking into account all the PhACs together (Table S-7), PhAC concentrations were significantly different among basins and campaigns, for both SW and sediment samples. Interestingly, a significant interaction between campaigns and basins was also found both for SW and sediment samples. Further univariate ANOVAs based on permutation for individual PhACs (Table S-8) revealed that on average 30% of the total variation in the concentration of PhACs in SW was explained by the factor Basin, 37% by the factor Campaign and a further 24% was explained the interaction between the two factors, with a 9% of the variation left unexplained, on average (Table S-8). For the concentration of PhACs in sediments those percentages were 22%, 50%, 21% and 7%, respectively. For both sample types the percentage of the variation that was able to explain each source of variation varied a lot, from a difference of 59% between the minimum and maximum variation explained for the Basin x Campaign interaction for SW to a difference of 100% for the factor Campaign for sediment samples (Table S-8). Levels of PhACs in SW where generally higher during C1 compared to C2, while sediments followed the opposite trend (Figs. 4 and 5). Nevertheless, the comparison of the C2:C1 ratios for

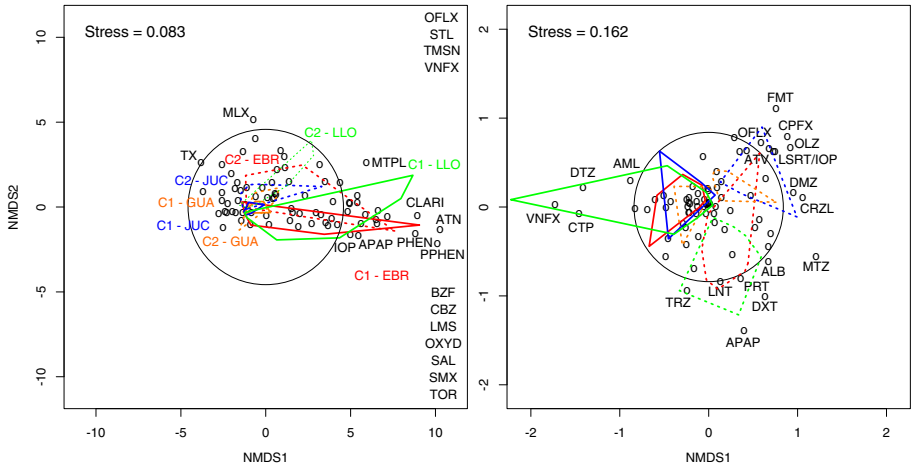

Fig. 3. NMDS ordination of the sites and the pharmaceuticals along the four river basins and the two sampling campaigns in surface waters (left) and sediments (right). The twenty pharmaceuticals that showed the most differing patterns across basins or sampling campaigns are outside the circles. Exact location of the pharmaceuticals in the ordination for the surface water samples is only given for some of them due to space constraints. Code for the short names of the pharmaceuticals can be seen in the supporting material (Table S-1).

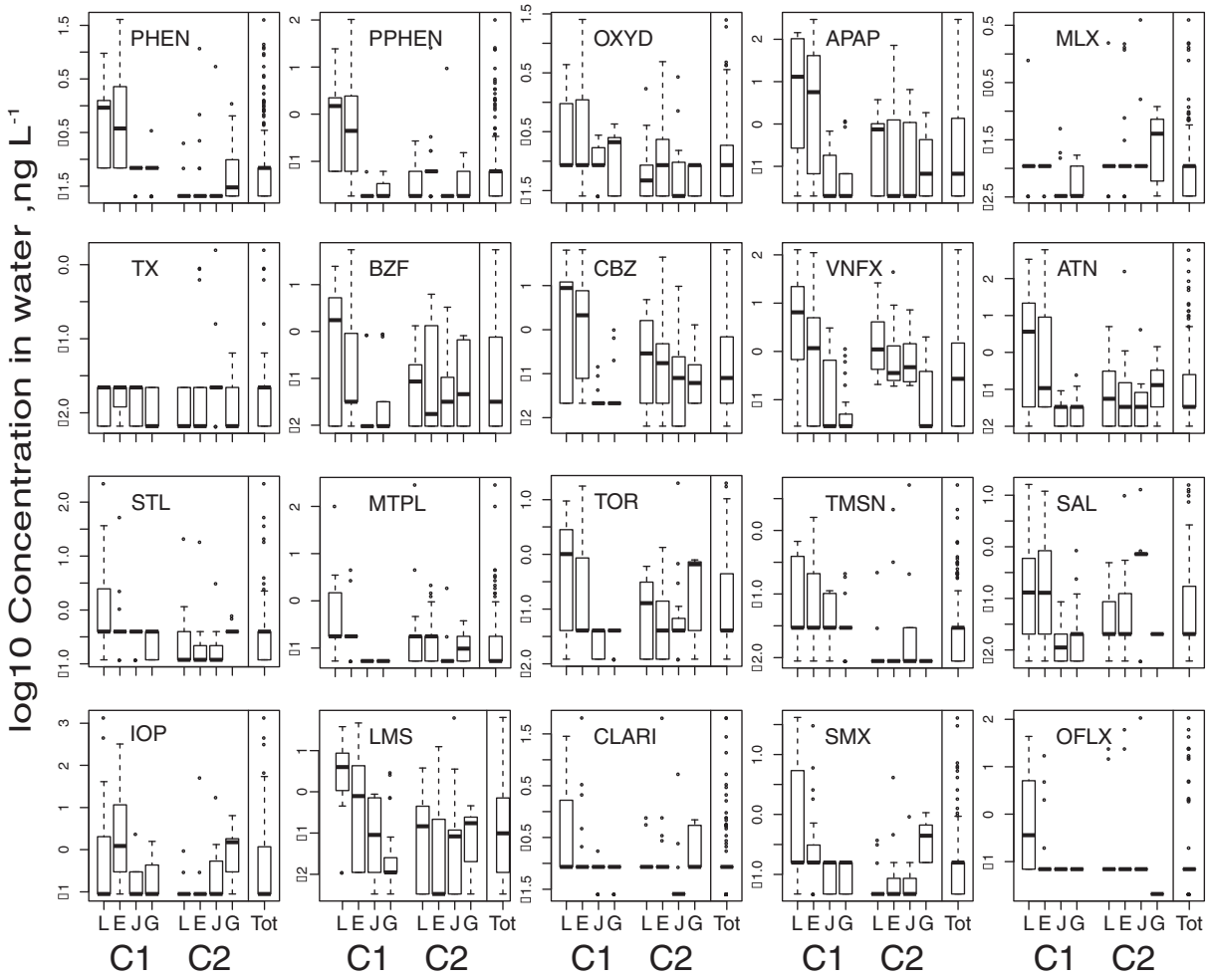

Fig. 4. Concentration boxplots of the twenty pharmaceuticals that showed the most differing pattern across basins or sampling campaigns in surface water samples. Boxplots for each basin at each campaign as well as a boxplot taking into account all the data are shown at each plot. Code for the short names of the pharmaceuticals can be seen in the supporting material (Table S-2).

PhACs and for the discharge with a Permanova did not reveal any significant pattern for SW and for sediment samples (Table S-9). The total levels of PhACs grouped by therapeutic class (Tables S-10 and S-11) for SW and sediments, respectively, show that the Llobregat and Cardener rivers followed a pronounced pollution gradient from headwaters to river mouth, mainly in C1, being LLO7 the most polluted site in both campaigns. By contrast, the most polluted site of the Anoia tributary was ANO2 in both C1 and C2. Within the Ebro river basin, the highest drug concentrations were observed at ARG, HUE and ZAD, whereas the least polluted site was GAL1 during both campaigns. In the Júcar catchment area, the most polluted site was JUC7 while the least polluted sites in both campaigns were CAB5, JUC5 and CAB4. Lastly, the Guadalquivir river basin showed maximum levels of PhACs in GUAA and the minimum levels were detected in GUA9 and GUA1 during both campaigns. Unlike the behavior observed in SW, PhACs did not show any pollution gradient nor any temporal pattern along the sediments of the Llobregat catchment in the different campaigns (see Table S-11).

3.3. Investigation on outlying cases of contamination and assessment of ecotoxicological risk

3.3.1. Outlying cases for each PhAC

Although some PhACs showed a distribution with a limited number of cases that were outside of the general pattern, most of the PhACs did not follow a log-normal distribution (Figs. 4 and 5). For SW only acetaminophen, bezafibrate, carbamazepine, venlafaxine and levamisole fitted to a log-normal distribution when taking into account all SW samples (Fig. 4). On the other hand, acetaminophen, carazolol, amlodipine, losartan, iopromide, albendazole, dexamethasone and metronidazole showed a fit to a log-normal distribution for sediment samples (Fig. 5). The PhACs showing "outlying high" values compared to the overall concentration across the sites and the campaigns were different for SW and sediment samples (Table S-12a). The PhACs showing the highest number of outlying high values in SW were phenazone and propyphenazone, while for sediments the compounds were trazodone and famotidine. On the other end, ibuprofen and pravastatin were among the compounds very frequently found at "outlying low" concentrations in SW, whereas for sediments these were nadolol and tetracycline (Table S-12b). Regarding the number of outlying high values, the Ebro and Júcar were identified as the basins showing the highest (Table S-13a) and the lowest ones (Table S-13b), respectively. On the other hand, Júcar and Ebro accounted for the highest number of outlying low values of PhACs in SW and sediment samples, respectively, whereas Llobregat and Júcar summed the lowest number in SW and sediments samples, respectively (Table S-13a). For SW samples C1 accounted for the highest number of cases with outlying high PhACs concentrations, while for sediments C2 showed the highest number (Table S-14b). On the contrary, C2 in SW and C1 in sediments summed the highest number of outlying low levels of PhACs (Table S-14a). Fisher's exact test revealed that the number of outlying high (Table S-15a) and low (Table S-15b) values of PhAC concentration in each basin varied significantly with the sampling campaign for both SW and sediments. Among the four catchments, the sampling

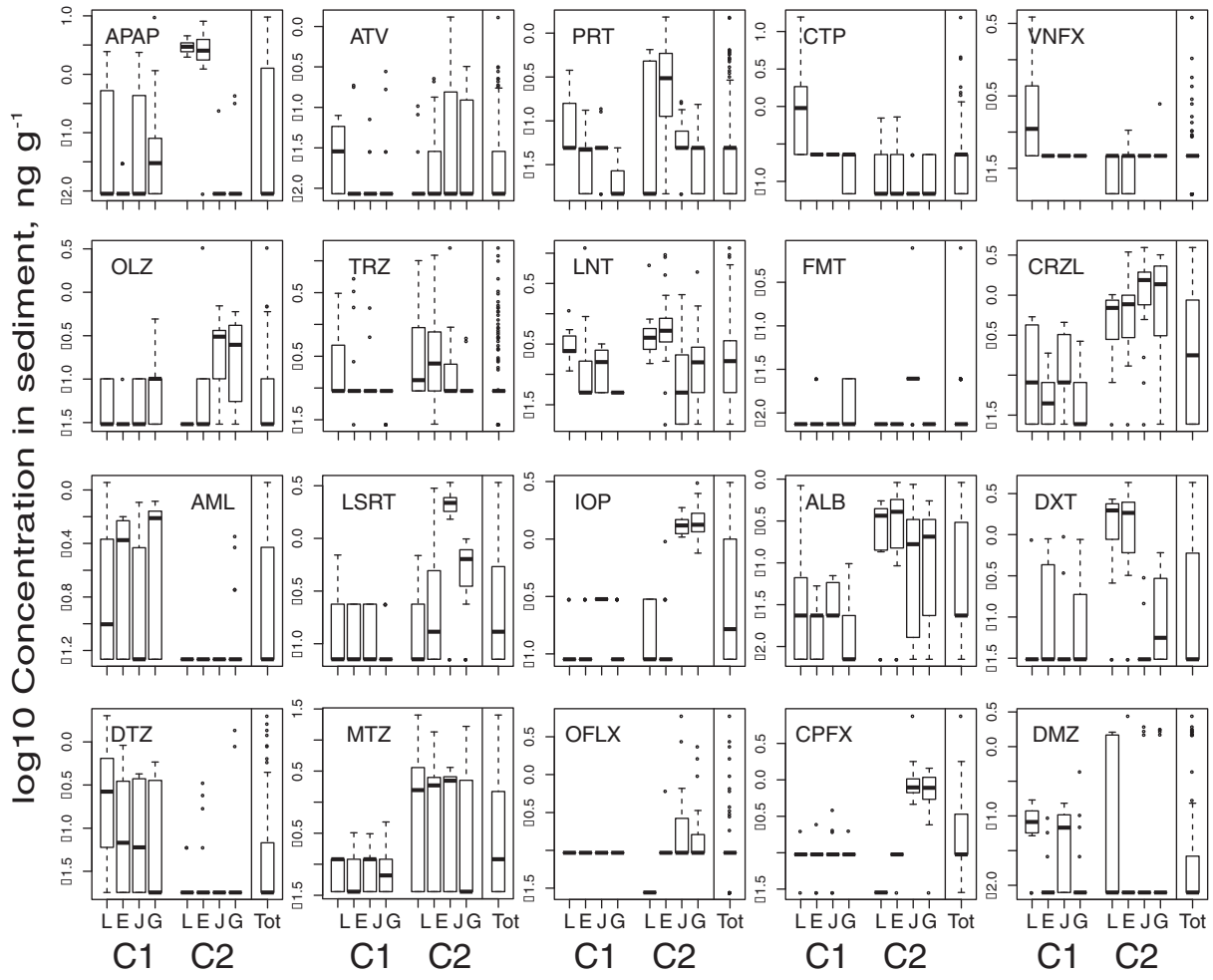

Fig. 5. Concentration boxplots of the twenty pharmaceuticals that showed the most differing pattern across basins or sampling campaigns in sediment samples. Boxplots for each basin at each campaign as well as a boxplot taking into account all the data are shown at each plot. Code for the short names of the pharmaceuticals can be seen in the supporting material (Table S-2).

sites having the highest number of PhACs detected at outlying high concentrations compared to the rest of the sites were ZAD, LLO7 and ANO2 in SW; while LLO7 and CAR4 were among the highest in sediments (Table S-16a). The SW and sediment samples from these sites were specially polluted by a high number of PhACs. Against expectations, all headwater reaches, such as in LLO1, EBR1, GUA1 and especially in JUC1, also showed outlying high levels of PhACs (Table S-16a). On the other end, for the locations close to the river mouth such as JUC8 (in SW samples) and EBR9 (in sediment samples), again against expectations, only a low number of PhACs showed outlying high concentrations. The locations with the most cases of PhACs at outlying low concentrations compared to the rest of the samples were completely different between SW and sediments (Table S-16b). The sites that displayed most outlying low levels of PhACs for SW were CAB5 and JUC5, whereas for sediments these were EBR1, LLO5 and RS. Unexpectedly again, some PhACs were found at outlying low concentrations at low reaches of the basins in SW, specially CAB5 (Table S-16b). Overall, fewer cases of outlying low concentrations of PhACs were detected in comparison to the cases of outlying high concentration of PhACs (Table S-16).

3.3.2. Ecotoxicological risk (toxic units): identification of sites, basins and campaigns associated with outlying high and low values and of compounds responsible for the ecotoxicological risk

TU values were highest for algae and lowest for fish, with *Daphnia* showing values in between (see Fig. 7 and Table S-17). TUs of PhACs to aquatic organisms estimated all over the four river basins spanned from 2.18E − 0.5 to 5.39E − 0.3 for algae, from 5.97E − 06 to 1.52E − 03 for *Daphnia* and from 2.91E − 06 to 8.39E − 04 for fish (Fig. 7 and Table S-17). More in detail, the locations where PhACs showed the minimum estimated ecotoxicological effects to aquatic organisms, per campaign were: CIN1 (3.37E − 05) in C1 and JUC5 (2.18 − 05) in C2 for algae, CIN1 (8.05E − 06 in C1) and CAB5 (5.97E − 06 in C2) for *Daphnia*, and GAL1 (5.17E − 03) in C1 and JUC5 (2.91E − 0.6) in C2 for fish. As for the maximum ecotoxicity observed, LLO7 was the location showing highest TU values in C1 for an all aquatic species (5.39E − 03 for algae, 1.52E − 03 for *Daphnia* and 8.39E − 04 for fish). In C2, the highest risk was shared between ZAD (3.45E − 03 for algae) and LLO7 (5.61E − 04 for *Daphnia* and 4.81E − 04 for fish). Among these locations, the presence of PhACs in LLO7 in C1 posed the highest ecotoxicological risk to all aquatic species (5.39E − 03 for algae, 1.52E − 03 for *Daphnia* and 8.39E − 04 for fish). A similar trend was observed in ZAD in C1, with TU values close to those estimated in LLO7 (4.67E − 03 for algae, 5.61E − 04 for *Daphnia* and 6.19E − 04 for fish). Generally, ecotoxicological effects estimated for PhACs were more important in C1 compared to C2 (TU values averaged 3.94E − 04, 7.84E − 05 and 7.94E − 05 in C1 and 1.98E − 0.4, 7.10E − 05 for corresponding algae, *Daphnia* and fish). As for the river basins, estimated average ecotoxicological risk to aquatic organisms was most relevant in Llobregat (2.50E − 04), closely followed by Ebro (2.28E − 04) and then Guadalquivir (6.35E − 05) and Júcar (3.97E − 05). TU calculated for algae showed the highest number of outlying high values with a total of 13 cases in SW samples (Table S-18). TU based on fish did not show outlying high values (Table S-18), as all the values estimated fitted within the whiskers of the boxplots created with log-

transformed TU values (not shown). Llobregat and Ebro showed the highest number of outlying high TU values, 6 taking into account the three kinds of TUs (Table S-19). The number of outlying high values was higher in C1 (8 cases) than in C2 (5 cases) (Table 20). LLO7 and ZAD were the sites with the highest number of outlying high TU values (4 cases) (Table S-21). No outlying low values were observed for TU. The distribution of outlying high values of TU across basins was not related to the sampling campaign (Fisher's exact test: $p = 0.53$ for SW and $p = 0.15$ for sediments) (Table S-22). The compounds that contributed at least 5% to the total predicted toxicity in the samples were sertraline, erythromycin, losartan and dimetridazole with values of 22, 20, 11 and 6%, respectively, when considering TU based on algae for SW (Table 23). For TU based on *Daphnia* there were again four PhACs reaching the 5%-threshold, namely, sertraline (29%), gemfibrozil (12%), loratidine (10%) and fluoxetine (5%). For TU based on fish gemfibrozil was found to be the PhAC that most contributed to the predicted toxicity of SW samples, 43% on average. Sertraline (11%), loratidine (10) and azithromycin (6) also showed predicted toxicities over 5% of the total TU of the sample (Table 23).

3.4. Relationship of PhACs pollution with population density and livestock units

Significant positive correlations were observed between mean PhACs concentrations in SW and both population density and LSU (mean concentrations of the PhACs that are used in each case, see subscripts in Table S-2), while for sediment a similar significant correlation was only observed for LSU (Fig. 6, Table S-24). For an increase in population density from 10 to 100 the mean PhAC concentration in SW was 4.2 times higher (Fig. 6). For the same increase in LSU the mean PhAC concentrations in SW and sediments were only 3.3 and 1.4 times higher, respectively. Moreover, the relationship between the TUs based on algae, *Daphnia* and fish and the population density and the LSU were all significant (Fig. 7, Table S-25). However, the relationships between TUs (for the three species) and population density were more pronounced than those observed with LSU: for an increase in population density from 10 to 100 the TU were 3.2, 3.3 and 5.4 times higher for algae, *Daphnia* and fish, whereas the same increment of LSU was associated with 2.0, 2.0 and 2.3 times higher predicted toxicity using the same indices (Fig. 7).

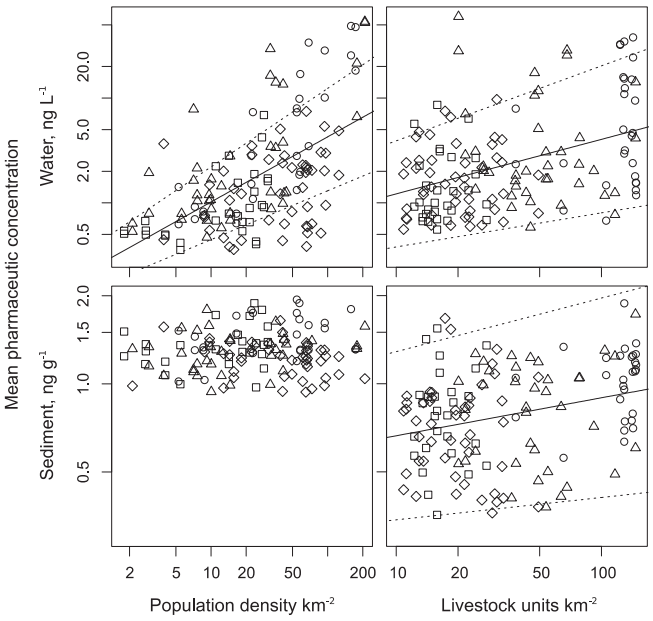

Fig. 6. Relationships of mean concentration of pharmaceuticals in surface waters (top) and sediments (bottom) with population density (left) and livestock units (right). Significant fixed effects of the fitted linear mixed effect models are displayed with continuous lines and 95% confidence intervals with dashed lines. Different symbols are used for the different catchments: Llobregat (○); Ebro (△), Júcar (□) and Guadalquivir (◇).

4. Discussion

4.1. Distribution and outlying cases of PhACs

PhAC concentrations varied four orders of magnitude in SW whereas they were much more constant in sediment. Similar results were previously observed in the Ebro river basin, in US streams, in Valencian wetlands (Spain) and the Túria river basin (Spain) (da Silva et al., 2011; Schultz et al., 2010; Vazquez-Roig et al., 2011, 2012; Carmona et al., 2014). We did not sample the supposedly most pristine points within the selected four basins, but with the extensive sampling in medium and low reaches of these streams we could have a useful measure of the maximum concentrations and estimated toxicity that we could detect in rivers of the Iberian Peninsula. The Llobregat and Ebro catchments displayed the highest ubiquity and concentrations of PhACs in SW, while sediments from Guadalquivir and Ebro were the most polluted ones. The presence of a wide diversity of PhACs had been previously confirmed in the water columns of the Llobregat and Ebro basins (Gros et al., 2007; da Silva et al., 2011; Osorio et al., 2012a, b). However, only three locations had been surveyed for PhACs levels in SW of the Guadalquivir River (Robles-Molina et al., 2014) and, to our knowledge, our study presents the first monitoring of PhACs in the Júcar river basin. The distribution of PhACs varied substantially across the Llobregat and Ebro river basins, in which the highest number of cases of outlying high concentrations and TU values of PhACs in SW were detected. In agreement with previous findings (da Silva et al., 2011; Osorio et al., 2012a,b; Carmona et al., 2014; Vazquez-Roig et al., 2011, 2012) and also following the global trend (Hughes et al., 2013) analgesics/anti-inflammatories, antibiotics, and diuretics were the most concentrated and frequently detected therapeutic groups in both SW and sediment samples. Therefore, our present database, together with previous research, reveals that analgesics/anti-inflammatories, antibiotics and diuretics are widespread and pseudo-persistent therapeutic groups in Spanish freshwater systems. The relevancy of other PhAC families varied across river basins and matrices, which could be due to regionally specific consumption patterns (Ortiz et al., 2013). Hydrochlorothiazide and gemfibrozil as well as azithromycin and ibuprofen were widely spread and concentrated PhACs in SW and sediments, respectively. Similar trends observed for hydrochlorothiazide, gemfibrozil and ibuprofen in published data (da Silva et al., 2011; Vazquez-Roig et al., 2011; Carmona et al., 2014) leads to consider these compounds as pseudo-persistent emerging pollutants in the national aquatic environment. The widely varying physicochemical properties of PhACs play an important role on their partitioning between sediments and the water column (Chen and Zhou, 2014). In agreement with a previous study (Osorio et al., 2012a) SW from the Llobregat catchment followed a PhAC pollution gradient from the headwaters to the river mouth, a pattern that was mimicked by the Cardener sub-catchment. Nevertheless, the remaining river basins did not follow any clear trend, with spots in which high concentration of PhAC spread across the four catchments. The highest levels of PhACs were detected in both SW and sediments of the sites ANO2, LLO7, ZAD and MAG. The locations LLO4 and LLO7 are well-known highly polluted sites of the Llobregat catchment (see MT and SJD corresponding to LLO4 and LLO7 in Osorio et al., 2012ab). Similarly, ZAD and ARG were previously identified as hotspots of PhACs (see T3 and T11 corresponding to ZAD and ARG in da Silva et al., 2011). Other troublesome locations identified were ARG, HUE, JUC7, and GUAA for SW; and LLO4, JUC1, GUA4 and BOR for sediments. On the other hand, the lower levels of PhACs in both SW and sediments were detected LLO1, LLO2, EBR1, GAL1, RS, CAB5, JUC5 and GUA1. On this basis, we would propose the water management

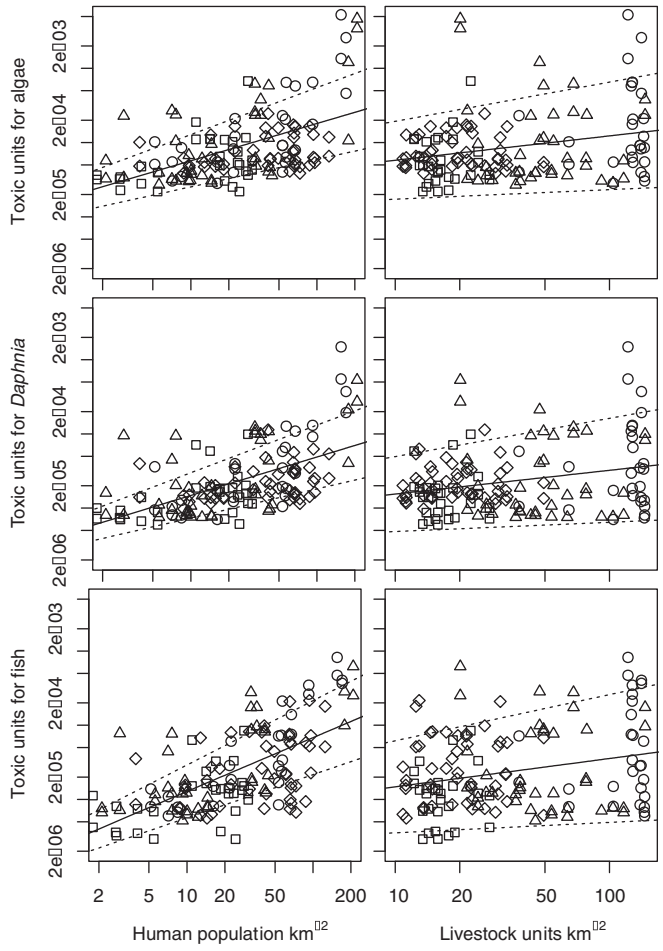

Fig. 7. Relationships of pharmaceutical toxic units for algae (top), *Daphnia* (middle) and fish (bottom) in surface waters with population density (left) and livestock units (right). Significant fixed effects of the fitted linear mixed effect models are displayed with continuous lines and 95% confidence intervals with dashed lines. Different symbols are used for the different catchments: Llobregat (○); Ebro (△), Júcar (□) and Guadalquivir (◇).

authorities to include the monitoring of PhACs in these locations as an indicator of the water quality status.

4.2. Effect of the changes of the discharge

There are studies that explain the seasonality of the concentration of PhACs by means of the more or less intensive use of those PhACs by the population (Moreno-González et al., 2015). Both sediment and water samples are subject to seasonal variations of the concentration of pollutants (Fairbairn et al., 2015). Influents of WWTP have also shown clear hourly and seasonal cycles that were also related to the consumption of those products by human (Coutu et al., 2013). In the work by Coutu et al. (2013) the relationship between PhAC concentration and discharge of the effluent was positive, i.e. the highest loads of PhAC were recorded in the first hours of morning or in winter. Nevertheless, the flow of the WWTP influents depends on the consumption of that water by human, whereas in lotic systems the relationship between PhAC concentration and the natural water discharge should be negative, as the latter dilutes the inputs from WWTP (see Hua et al., 2006; Kumar et al., 2011). Although some of the sites that showed outlying high values (ANO2, LLO7, ZAD, MAG2 for both SW and sediments) repeated from the first (high flows) to the second campaign (low flows), the overall relationship between PhACs and discharge C2:C1 ratios was non-significant. It seems that the hypothesized modulation of PhACs concentration under changing hydrological conditions observed in previous studies (Osorio et al., 2014) is not supported by our data, although we realize that with just two samplings we lack statistical power to be totally confident about any conclusion. The additional factors affecting the variation of PhAC concentration such as natural attenuation processes (mainly photodegradation and biodegradation) (Kümmerer, 2010), route that the PhACs use to reach the river (point emissions from WWTPs for human drugs; diffuse sources for veterinary drugs) and anthropogenic causes (human and animal drug consumption patterns, water use and a changing wastewater treatment efficiency) could counteract their natural dilution by the discharge in SW (Kümmerer, 2010; Vystavna et al., 2012). Besides, sediments can act as a reservoir of PhACs from where these substances can be re-dissolved into the aqueous phase under turbulent flow conditions in the river, thus modifying the concentration of PhACs in both phases of the water column (Nentwig et al., 2004). Moreover, the intrinsic physicochemical properties of PhACs, such as speciation or solubility, combined with the physicochemistry of the freshwater system, such as pH or total suspended solids, can affect the distribution of these substances along the water column, thus contributing to the variability of PhAC levels in SW and sediments (Carmona et al., 2014; Veach and Bernot, 2011).

4.3. Risk-based prioritization of locations and PhACs

Similar to what was observed for PhACs concentrations, the potential risk of PhACs to aquatic organisms increased downstream the Llobregat River, but no clear trend of increasing concern was observed for the remaining basins. The highest total predicted TUs of PhACs in SW were estimated at the sites LLO7, ZAD, MAG2, GUA6, GUA4, MAG1 and JUC7. None of the total TUs calculated at every site for algae, *Daphnia* and fish, exceeded the unit value, thus, according to standard thresholds (Malaj et al., 2014), no acute risk associated with PhACs was observed. However, though only for LLO7 and ZAD, the corresponding total TU values for algae were estimated above ~1E − 03 in both sampling campaigns, evidencing the potential long-term ecotoxicological effects on these primary producers (Malaj et al., 2014). On the other hand, CAB5, JUC5, LLO2, ESE, GUA1 and CIN1 were among the less worrisome locations. Similar findings were reported for the particular study cases of the Llobregat and Ebro river basins (Ginebreda et al., 2014; Damásio et al., 2011; Gros et al., 2010). Ginebreda et al. (2014) observed an increase of total TU estimated for algae and *Daphnia* downstream the Llobregat River as well (see the locations LL2 and LL7 in the referenced work, corresponding to LLO4 and LLO7 in the present study). Damásio et al. (2011) also observed the same trend for *Daphnia* (among other two invertebrate species) (see the locations L2 and L3 of the study cited, corresponding to LLO4 and LLO7 in the present one). Besides, the location LLO7 was also estimated at high risk of chronic ecotoxicological effects in both studies. Diversely to the current study, both works aforementioned assessed the apportionment of other pollutants such as pesticides (Ginebreda et al., 2014) and also metals and alkylphenols (Damásio et al., 2011) to total ecotoxicity. Indeed, Damásio et al. (2011) reported a marginal contribution of PhACs (4%) to the total predicted hazard to invertebrate species; while metals and pesticides accounted for 39% and 54%, respectively. These findings evidence the need to expand the ecotoxicological risk assessment to all kinds of pollutants that might be present in a complex environmental mixture, as it has been recently attempted by Kuzmanović et al. (2015). However, the set of biomarkers applied by Damásio et al. (2011) were not developed to evaluate the effects of PhACs, which indicates that further research on should rely on specific biochemical responses to these substances. The vast number of chemical products that society is using nowadays makes it difficult to find a way to decontaminate every one of them. Alternatively, a clear prioritization using the potential risk of the different chemicals should highlight the critical products among the rest. To create the prioritization the toxicity of the compound, its concentration in nature and the facility to transform into innocuous compounds needs to be taken into account. Our study

cannot address the last issue, but we present extensive data on the concentration of PhACs and estimate their contribution to the total toxicity in the field. In this sense, we have observed very low concentration of erythromycin that following literature seems to be a very toxic compound (VSDB), and on the other hand, we also have seen very high concentrations of atenolol and ketoprofen but their toxicity is very low (ECOTOX; Sanderson et al., 2003). Computing the relative contribution of the different substances to the total toxicity in the locations sampled we have been able to enumerate the critical PhACs in the waters of the catchments of the Iberian Peninsula None of the compounds found to contribute at least 5% to the total predicted toxicity were in agreement with those reported by Damásio et al. (2011). However, as well as the present work, Gros et al. (2010) reported fluoxetine as one of the major contributors to the ecotoxicological risk to *Daphnia* species. Importantly, although our ecotoxicological risk assessment was only focused on PhACs, the compounds identified as principal contributors to total predicted toxicity were classified by Kuzmanović et al. (2015) among the more relevant pollutants of the same River Basins (i.e. sertraline, erythromycin and losartan to algae; sertraline to *Daphnia*; and gemfibrozil for fish). All in all, we propose in essence that sertraline, gemfibrozil and loratidine are the PhACs into which a bigger effort should be concentrated if contamination of freshwater systems by PhACs needs to be controlled.

4.4. Effects of population density and livestock units

Our study shows a significant positive effect of the potential sources of PhACs, i.e. human population and livestock, on the concentration of PhAC in SW and sediments and the TU in SW. These relationships were stronger for SW and especially with the variation with population density. Given the very different use of the PhACs in terms of dosage, target population or seasonality (Ortiz et al., 2013; Veach and Bernot, 2011) it is remarkable to observe these significant relationships between the spatial information of the sources of PhACs and their average concentration and estimated toxicity on rivers. To our knowledge, this relationship has never been empirically proven beforehand, although other studies that find a relationship between the density of the population or the presence of activities involving livestock and the concentration of PhACs in river waters are quite common (Bartelt-Hunt et al., 2011; Murata et al., 2011; Osorio et al., 2012a; Fairbairn et al., 2015). Nevertheless, interestingly, in no case the differences of the population density or the LSU in the catchments are followed by a proportional increase of the concentration of PhAC in SW or in sediments. The highest increment of average PhAC concentration was observed for SW in relation to population density (X4.2 in PhAC concentration for a tenfold increase in population density). Although a higher density of population and LSU is linked to a higher use of PhACs (e.g. Kools et al., 2008) the water consumption also increases (Mekonnen and Hoekstra, 2012; Panagopoulos et al., 2012), diluting in part the PhAC spilled into nature. On the other hand, the activity of microorganisms in the water/sediment interface or the streambed sediments have been seen to be very relevant in the biodegradation of pharmaceuticals (e.g. Radke and Maier, 2014), what might partially explain the lower increment of the concentration of PhACs (about 40% of increase in concentration of PhACs for a tenfold increase in LSU) in sediment samples.

Pollutants can have contrasting effects on the biotic components of ecosystems and on the processes, and thus services, that the biota can drive (Flores et al., 2014). Given the individual toxicity of the PhAC and their concentrations in the field the TUs in our work were highest for algae and lowest for fish, with *Daphnia* showing values in between. This result suggests that toxicity from PhACs would harm the assemblage of primary producers more than other biota. Nevertheless, TUs for *Daphnia* and fish showed a stronger response, i.e. a steeper slope, to the increase of the population density and the LSU, suggesting that whereas the affections on ecosystem processes in which algae are important, as primary production, metabolism and autodepuration, would not change very much with population density of LSU, the assemblages representing the top of the food webs (invertebrates and fish) are going to become more impaired as pollutants are further concentrated in those ecosystems. Among the relevant functions, secondary production will be reduced as invertebrates and vertebrates are affected (Carlisle and Clements, 2003). The important but indirect role of invertebrates and fish in the regulation of other important processes as autodepuration (controlled by herbivory of primary consumers, Libourissen et al., 2005) or organic matter recycling (through the consumption of it or of its consumers, Woodward et al., 2008) makes the understanding of the effects of PhACs on different kind of organisms a critical step to predict alterations on ecosystem processes.

5. Conclusions

With this work we have demonstrated the ubiquity of PhACs in SW and sediments of Iberian rivers, although some sites have shown outlying concentrations of some PhACs and the total concentration of PhACs, which focuses the attention on specific sites and PhACs. Both average concentration of the PhACs and their estimated total toxicity, have shown to be positively related to the population density and the livestock units in the upstream sub-basin, thus responding to the anthropic pressures in the catchments. Although the contribution of the different PhACs to the estimated total toxicity of the SW is site dependent, five compounds (erythromycin, gemfibrozil, loratidine, losartan and sertraline) are responsible for more than 50% of the TU for algae, *Daphnia* or fish, and should therefore be specially addressed when dealing with SW pollution with emergent contaminants. Our study highlights that SW can receive relevant amounts of PhACs that might interfere with the natural organization of the biota and affect ecosystem processes and, thus, services.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.06.143.

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