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Occurrence and in-stream attenuation of wastewater-derived pharmaceuticals in Iberian rivers

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ABSTRACT

A multitude of pharmaceuticals enter surface waters via discharges of waste water treatment plants (WWTPs), and many rise environmental and health concerns. Chemical fate models predict their concentrations using estimates of mass loading, dilution and in-stream attenuation. However, current comprehension of the attenuation rates remains a limiting factor for predictive models. We assessed in-stream attenuation of 75 pharmaceuticals in 4 river segments, aiming to characterize in-stream attenuation variability among different pharmaceutical compounds, as well as among river segments differing in environmental conditions. Our study revealed that in-stream attenuation was highly variable among pharmaceuticals and river segments, and that none of the considered pharmaceutical physicochemical and molecular properties proved to be relevant in determining the mean attenuation rates. Instead, the octanol-water partition coefficient ($K_{ow}$) influenced the variability of rates among river segments, likely due to its effect on sorption to sediments and suspended particles, and therefore influencing the balance between the different attenuation mechanisms (biotransformation, photolysis, sorption, and volatilization). The magnitude of the measured attenuation rates urges scientists to consider them as important as dilution when aiming to predict concentrations in freshwater ecosystems.

Keywords: pharmaceuticals, water quality, water purification, natural contaminants attenuation, ecosystem services, and stream ecology.
GRAPHICAL ABSTRACT

[Image: Diagram showing the flow of various pharmaceuticals in a stream with statistical data in the background.]
1. INTRODUCTION

A multitude of organic compounds used in households, such as pharmaceuticals, enter surface waters mainly via point-source discharges of wastewater treatment plants (WWTPs) (Gros et al. 2007; Pal et al. 2010). Although most of these pharmaceuticals are present at low concentrations, many of them raise environmental and health concerns, and have become a key environmental problem (Schwarzenbach et al. 2006).

Several studies have predicted the expected concentrations of pharmaceuticals in surface waters using estimates of mass loading, dilution and in-stream attenuation, here understood as the reduction of the concentration of pharmaceuticals along the river segment by processes different than dilution (Huset et al. 2008; Alder et al. 2010). Reliability of these models is however constrained by the high variability in chemical emissions from WWTPs and attenuation in surface waters (Pistocchi et al. 2010). On the one hand, chemical emissions from WWTP effluents vary widely because of differences in regional usage of the compounds and efficiency of wastewater treatment (Pal et al. 2010). On the other hand, the processes that drive in-stream attenuation (i.e. biotransformation, photolysis, sorption, volatilization) depend in turn on the different pharmaceuticals characteristics as well as on a series of physicochemical and biological parameters of the river such as river flow rate, temperature, the vertical hydrological exchange between surface and subsurface compartments, turbidity, dissolved oxygen concentration, biofilm biomass, and pH (Gurr and Reinhard 2006; Kunkel and Radke 2008). Because these parameters vary at different spatial and temporal scales, in-stream attenuation rates might also show high variability and complicate the prediction of attenuation rates from one river to the next (Gurr and Reinhard 2006; Fenner et al. 2013). Most of the available information on in-stream attenuation of pharmaceuticals comes from a few field studies based on single stream segments and accounting for a limited number of compounds (Yamamoto et al. 2009; Kunkel and Radke 2011; Writer et al. 2012). This approach, though practical, limits our comprehension of the effect of variability in local environmental parameters on in-stream attenuation rates.
Laboratory controlled conditions allow the quantification of attenuation rates for a large number of pharmaceuticals in WWTP (Joss et al. 2006), and are used to rank them according to their biological degradation during wastewater treatment. Overall, our current comprehension of the in-stream attenuation rates of pharmaceuticals remains a limiting factor for the development and calibration of predictive models of the chemical fate of pharmaceuticals in rivers, thus hindering the development and implementation of more effective regulatory strategies.

Within this framework, we assessed the in-stream attenuation of 75 pharmaceutical compounds in 4 river segments downstream of 4 WWTPs, aiming to characterize in-stream attenuation variability among different pharmaceutical compounds, as well as for each pharmaceutical compound but among rivers differing in environmental conditions. In order to better understand the mechanisms driving the in-stream attenuation of the pharmaceuticals (i.e. biotransformation, photolysis, sorption, and volatilization), we examined the relationship between the attenuation rates of pharmaceuticals and those of dissolved nitrogen, phosphorus and carbon for which the attenuation mechanisms are well known.
2. MATERIAL AND METHODS

2.1 River segment selection and description. Field surveys were performed in 4 rivers segments within the Ebro basin (N Iberian Peninsula) affected by WWTP effluents on March 12-14, 2012. The studied river segments were selected to reflect different types of impact by WWTP on river ecosystems based on the magnitude and type of WWTP effluent, as well as on the dimensions of the receiving water body (Table 1). The study sites encompassed considerable ranges in terms of elevation, drainage area and slope (Table 1). Mean width of the studied river segments ranged from 5 to almost 15 m, whereas river flow rate ranged from 0.05 to 2.7 m$^3$ s$^{-1}$, and mean water velocity from 0.09 to 0.33 m s$^{-1}$ (Table 1). Additional criteria for the selection of study sites were homogeneity within each one of the river segments upstream and downstream from the WWTP in terms of geomorphology (e.g., no major changes in river width and depth), hydrology (e.g., no diversions or tributaries), and chemistry (e.g., no other effluents within the segment). Consequently, changes in the structure and function of the receiving freshwater ecosystems could be mostly attributed to the impact by the WWTP effluents.

2.2 Environmental factors. River flow rate was measured 100 m upstream and 100 m downstream from the discharge of the WWTP effluent(Gore and Hamilton 1996) using an acoustic Doppler velocity meter (ADV; Flowtracker, SonTek, San Diego, CA, U.S.A.). This required taking depth readings every 20 cm across the width of the channel, as well as velocity readings at 0.6 of the depth. Furthermore, river width was measured at all the sampling locations along each river segment. The potential dilution along the river segment by vertical (i.e. groundwater) and lateral (i.e. tributaries) inputs was determined by means of an end member mixing analysis (EMMA) using chloride and sulfate anions as conservative tracers (Christophersen et al. 1990). Specifically, EMMA was used to determine mean and standard deviation dilution coefficients along the studied river segments. Both mean and standard deviations in the dilution coefficients were based on the calculation of multiple dilution coefficients, by using random values within the ranges defined by the observed
chloride and sulfate concentrations and the predicted concentrations according to EMMA. These dilution coefficients were in turn used to remove the effect of dilution on the decline in the concentration of pharmaceuticals and nutrients along the river segments. Electrical conductivity, pH, and dissolved oxygen were measured in situ at the same locations using handheld probes (WTW, Weilheim, Germany).

2.3 Sample collection. Six locations were sampled within a 30 min time span in each river segment: the WWTP effluent itself (e), 100 m upstream from the discharge point of the WWTP effluent (u), and 4 locations at increasing distances from the discharge point of the WWTP effluent (d1, d2, d3, d4). These distances varied among river segments according to their hydrology, and were decided according to the exponential relationship between the river segment length and river flow rate, and therefore assuring complete mixing at the uppermost sampling location (Kilpatrick and Cobb 1985) (Table 1). Accordingly, sampling locations spanned 4500 m at the river segment A, 2000 m at B and C, and 3000 m at D. Differences between locations u and d1 were used to characterize the WWTP effluent impact on the river flow rate and chemical composition, whereas the variation of chemical concentrations along the river axis (locations d1 to d4) were used to calculate the in-stream attenuation rates of each nutrient and pharmaceutical compound (see section 2.5). Water samples for both pharmaceuticals and nutrients were collected in triplicate from the river Thalweg at approximately 10 cm below the water surface. Water samples for nutrients were filtered in situ through combusted and pre-weighted glass fiber filters (Whatman, U. K.), placed in previously rinsed polyethylene bottles and stored at -20 °C until analysis. Water samples for pharmaceuticals were collected in previously rinsed with ultrapure water amber glass bottles, and stored at -20 °C until analysis.

2.4 Analytical methods and quality control. Total suspended solids (TSS) were determined by drying (60 °C, 72 h) and weighting the used glass fiber filters; whereas ash-free dry mass of the suspended solids (AFDM) was determined by ashing (500 °C, 5 h), and re-weighting the same filters. The concentration of total dissolved phosphorus (TDP) was
measured as the concentration of phosphate ($\text{PO}_4^{3-}$) after acid digestion in a Selecta Presoclave-II 30L autoclave (JP Selecta S.A., Barcelona, Spain). The concentrations of soluble reactive phosphorus (SRP), ammonium ($\text{NH}_4^+$) and nitrate + nitrite ($\text{NO}_3^- + \text{NO}_2^-$) were determined colorimetrically using an Alliance-AMS Smartchem 140 (AMS France, Frepillon, France). The concentrations of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured on a Shimadzu TOC-V CSH coupled to a TNM-1 module (Shimadzu Corporation, Kyoto, Japan). The concentrations of chloride ($\text{Cl}^-$) and sulfate ($\text{SO}_4^{2-}$) were determined on a Dionex ICS-5000 ion chromatograph (Dionex Corporation, Sunnyvale, USA).

The analysis of pharmaceuticals was carried out using a method based on an off line solid phase extraction (SPE) followed by ultra high performance liquid chromatography - tandem mass spectrometry (UHPLC-ESI-MS/MS) (Gros et al. 2012). Details on sample pretreatment, SPE, chromatographic separation and MS/MS detection, as well as the validation parameters are given in Appendix A. Quantification was performed based on the internal standard approach by adding the corresponding deuterated compounds to all the samples and aqueous standards for the calibration curve at a concentration of 10 ng L$^{-1}$ before analysis. For quality assurance, three replicates per sample were analyzed to determine the analytical variability, and both MeOH and chromatographic blanks were used. Despite limits of detection (LODs) and limits of quantification (LOQs) are detailed elsewhere (Gros et al. 2012), new values have been estimated as method LODs and LOQs for the water samples analyzed in this study, as the minimum detectable amount of analyte with signal-to-noise ratios of 3 and 10, respectively. Likewise, the accuracy of the method was also evaluated for this study and given as the recovery values (R %) obtained after spiking the different surface waters ($N = 3$) with a standard mixture at 50 ng L$^{-1}$. Water samples from locations upstream the WWTP (u) were used for this purpose and previously analyzed in order to check for background concentration. Recovery values were higher than 50% for the majority of the target analytes. However, some compounds such as atenolol, cimetidine or atorvastatin
showed much lower recoveries, which could be attributed to the fact that conditions chosen
are not the most appropriate for those specific compounds. This is one of the drawbacks of
multi-residue methodologies, where a compromise has to be reached for analytes of very
different nature and sometimes not the best analytical conditions for all target analytes can
be achieved. Nevertheless, despite the low recoveries, the sensitivity achieved for these
compounds was still very good to carry out the analysis (see Table A.1 for LODs). Regarding
sensitivity, LODs were determined for each compound and sample, and given as average
values, which ranged from 0.001 to 2.07 ng L\(^{-1}\) for surface water, and from 0.002 to 4.30 ng
L\(^{-1}\) for WWTP effluent samples (Table A.1).

2.5 Calculations of in-stream attenuation rates. To estimate the in-stream attenuation
rates of pharmaceuticals and nutrients in several systems, we followed the intrinsic tracers
approach (Writer et al. 2011). We assessed in-stream attenuation by comparing the
decrease in pharmaceutical and nutrient concentrations relative to the dilution coefficients
determined from conservative elements by the EMMA. Key assumptions when using this
method include continuous and steady state loading from the WWTP and the absence of
factors that may substantially alter downstream hydrology, including groundwater influence,
water diversions, or precipitation events. The effect of these factors was minimized by
selecting homogeneous river segments (see section 2.1). Furthermore, the potential variation
of the load from the WWTP was assessed as the coefficient of variation of the discharge of
the WWTP effluent during the period integrated by the sampling in each river, that is, the
segment travel time (Table 1). The first-order decay constant of the chemical solutes along
the river segments downstream the WWTP \((k)\ (h^{-1})\) was calculated as:

\[
C_\tau = C_0 e^{-k\tau}
\]

(1)

where \(C_\tau\) and \(C_0\) are EMMA dilution-corrected concentrations of chemical solutes (ng L\(^{-1}\)),
and \(\tau\) the travel time along the river segment originating from the WWTP effluent (h), with
origin \(\tau = 0\) at the location of emission. Thus, EMMA dilution-corrected \(C_\tau\) values at
successive river locations (water samples \( d1 \) to \( d4 \)) and fitted to a straight line on a natural logarithmic scale provide a slope \( k \). Linear regression was used to determine the \( k \) values, but only those showing coefficients of determination \((r^2)\) higher than 0.75 were accepted. The obtained \( k \) values were then used to estimate the half-life times of each pharmaceutical and nutrient \((h)\). Furthermore, the mass transfer coefficient \((v_f)\) (O’Conner 1988) was estimated as:

\[
v_f = \frac{Q}{w v} k
\]  

(2)

where \( Q \) is mean river flow rate \((m^3 \text{ s}^{-1})\), \( w \) is mean width \((m)\), and \( v \) is mean velocity \((m \text{ s}^{-1})\). The \( v_f \) is a scale-free parameter that standardizes for river flow rate, velocity and river width, and thus allows comparing attenuation rates among rivers of different size (Stream Solute Workshop 1990). It can be either positive when there is net uptake or negative when there is net release, and can be viewed as a measure of attenuation efficiency (Davis and Minshall 1999).

2.6 Statistical analyses. Two statistical approaches were followed to assess the effects of physicochemical features and environmental variables on the estimated \( v_f \) values. On the one hand, we analyzed the relationship between the mean and the coefficient of variation (CV) of the estimated in-stream attenuation rates with the physicochemical features of the pharmaceutical compounds \((N = 34\) pharmaceuticals with estimated \( v_f \) values in at least 3 river segments). The considered physico-chemical features were the first and the second acid dissociation constants \((pK_{a1} \text{ and } pK_{a2})\), the molecular weight, and the octanol-water partition coefficient \((K_{ow})\) (Table A.2). On the other hand, we analyzed the relationship between the estimated in-stream attenuation rates from each river segment with the environmental factors \((N = 4\) river segments). Pearson-moment correlation analysis was used to identify the direction and strength of the relationships between variables, including the analyses between conservative solutes performed to identify mixing, dilution or concentration processes as described above for the EMMA. Normality of all variables was
checked with the Kolmogrov-Smirnov test, and variables were log-transformed when necessary. All analyses were considered significant at $P < 0.05$, and were performed with SPSS (version 17.0, SPSS Inc., Chicago, U.S.A.).
3. RESULTS

3.1 Environmental conditions. As expressed by the ratio Q_{e:w} (flow of the WWTP effluent versus river flow rate upstream the WWTP effluent), the WWTP effluents significantly increased river flow rate at sites C and D but not much at sites A and B (Table 1). In the case of the NH_{4}^{+} loads (ratio load NH_{4}^{+}_{e:w}), the contribution of the WWTP was the highest at site D and the lowest at site B. The WWTP effluent discharge coefficient of variation encompassed during the measurements ranged from 20 to 23 % (Table 1), with river segment A showing the minimum and B the maximum variations.

The river flow rate changed along the river segments, increasing due to vertical or lateral inputs between 11.0 ± 1.8% (C) and 57.0 ± 0.1 % (D) (Table 1). The travel times slightly differed among river segments, encompassing between 227 and 476 min (Table 1). All river segments showed similar dissolved oxygen concentrations and pH values, but sites C and D had much higher values of electrical conductivity and SO_{4}^{2-} concentration than sites A and B. This was also the case for Cl^{-} concentration, but only for site C (Table 1).

3.2 Nutrients and pharmaceuticals in WWTP effluents and receiving river waters. The concentration of NH_{4}^{+} upstream from the WWTP (locations u) was similar among sites, but not at downstream river segments (locations d1 to d4), where the concentration ranged from 0.21 to 3.4 mg N L^{-1}, with highest values at site A and lowest at sites B and D. SRP ranged at locations u from 0.004 to 0.04 mg P L^{-1}, NO_{3}^{-} from 0.63 to 2.5 mg N L^{-1}, and DOC from 2.2 to 6.7 mg L^{-1}.

Overall, of the 75 pharmaceuticals analyzed, a total of 25 were never detected above their LOQ (Table A.3). The numbers of quantified compounds was lowest at locations upstream from the WWTP (u), and highest right downstream the discharge of WWTP effluents (d1). Concentrations at sites u were below LOD for more than 50 % of the analyzed compounds, but there were variations among sites, as the percentage of non-detected compounds ranged from 45 % at site A to 75 % at site B. Also, a decrease in the number of compounds
detected along all studied impacted river segments was observed. In fact, the number of detected compounds decreased from sites $d1$ to $d4$ by 3 % at river segment A, 12 % at B, 45 % at C, and 35 % at D. Obviously, the same compounds found right downstream the discharge of WWTP effluents ($d1$) were also present in WWTP effluents ($e$) but at higher concentrations (Table A.3).

The highest concentrations in WWTP effluents were found for the antihypertensive drugs valsartan and ibersartan (> 4 μg L$^{-1}$), lipid regulator gemfibrozil and analgesic naproxen (>3 μg L$^{-1}$) and β-blockers atenolol and nadalol (> 2 μg L$^{-1}$). All of them were detected in river water downstream from the discharge of the WWTP effluent at levels frequently higher than 0.3 μg L$^{-1}$. Other compounds frequently detected in WWTP effluents and river water were the: analgesics codeine (up to 2 μg L$^{-1}$ in effluent and 0.12 μg L$^{-1}$ in river water), diclofenac (1.2 μg L$^{-1}$ in effluent and 0.2 μg L$^{-1}$ in river water), acetaminophen (0.53 μg L$^{-1}$ in effluent and 0.5 μg L$^{-1}$ in river water), and the diuretics hydrochlorothiazide (1.4 μg L$^{-1}$ in effluent and 0.4 μg L$^{-1}$ in river water) and furosemide (up to 1.4 μg L$^{-1}$ in effluent and 0.42 μg L$^{-1}$ in river water).

### 3.3 In-stream attenuation rates - Nutrients.

Mass transfer coefficients were the highest for NH$_4^+$ (1.16 ± 0.21 mm min$^{-1}$) and the lowest for NO$_3^-$ (-0.10 ± 0.26 mm min$^{-1}$). Furthermore, $v_f$ values were higher for the P compounds than for the N compounds, whereas DOC showed intermediate values between P and N (Figure 1). There were also differences between compounds in terms of variability among sites, as the coefficient of variation of $v_f$ - NH$_4^+$ was only 18 %, whereas it was 255 % for NO$_3^-$. Overall, $v_f$ - NH$_4^+$ showed high and similar values among sites, whereas $v_f$ - NO$_3^-$ showed negative and highly variable values among sites. Among study sites, $v_f$ values were higher at sites C and A. The values of $v_f$ were directly proportional to their respective background river concentrations for the cases of SRP and TDP ($r^2 > 0.95$, $P < 0.05$, $N = 4$), but not for DOC, TDN, NO$_3^-$ and NH$_4^+$. 
### 3.4 In-stream attenuation rates - Pharmaceuticals

Attenuation rates could be quantified in 134 cases of the possible 300 (resulting from 4 river segments x 75 analyzed pharmaceuticals) (Figure 2). Mean half-life times for pharmaceuticals ranged from 1.6 to 34.2 h (Table 2). Mean \( v_f \) were similar for pharmaceuticals than for nutrients, ranging between 0.37 and 2.06 mm min\(^{-1}\), and without major differences among therapeutic groups (Figure 1).

Differences among river segments for each pharmaceutical were not even, as reflected in the standard deviations (Table 2; Figure 1). Thus, some pharmaceuticals such as bezafibrate, ibersartan or propyphenazone showed similar \( v_f \) values among river segments (i.e. low CV), whereas others such as salicylic acid, metronidazole, hydrochlorothiazide or ketoprofen showed large differences (i.e. high CV). The mean \( v_f \) was not significantly correlated with physicochemical characteristics of the analyzed compounds (molecular weight, \( pK_{a1} \), \( pK_{a2} \), \( K_{ow} \)) \((P > 0.05, N = 34)\). Instead, the coefficient of variation of \( v_f \) was significantly and negatively correlated with \( K_{ow} \) \((r^2 = 0.40, P < 0.05, N = 32)\) (Figure 3), indicating that higher \( K_{ow} \) was related to lower variation among sites in terms of \( v_f \) for each compound.

When analyzing the relationship between the estimated \( v_f \) values for each compound and site with the local environmental characteristics at each site, only a few showed significant correlations. Thus, \( v_f \) of ibersartan and valsartan were significantly correlated with the \( v_f \) of SRP and TDP as well as with the background river concentration of SRP \((r^2 > 0.95, P < 0.05, N = 4)\), but not with the background river concentration of DOC, TDN, \( \text{NO}_3^- \) and \( \text{NH}_4^+ \).

Similarly, \( v_f \) of carbamazepine, citalopram, and venlafaxine were significantly correlated with the \( v_f \) of SRP, as well as with the background river concentrations of both SRP and TSS \((r^2 > 0.95, P < 0.05, N = 4)\). In contrast, none of the \( v_f \) values were related with environmental variables such as water temperature, dissolved oxygen concentration, pH, or conductivity \((P > 0.05, N = 4)\).
4. DISCUSSION

4.1 Considerations on the followed approach. We believe that our approach in estimating the in-stream attenuation rates following the intrinsic tracers approach is a realistic one. However, we are aware of that the followed intrinsic tracers approach is a serious study limitation. In fact, it is true that the assumption of steady state loading from the WWTPs may be inaccurate given the temporal variability in effluent pharmaceutical loads (Majewsky et al. 2011; Nelson et al. 2011) and the time encompassed by the measurements (i.e. reach travel times). In fact, variations in pollutant loads in the influent of the WWTPs are dampened in the effluent, since daily influent loads are distributed over more than one day in the effluent because of the particular WWTP hydrodynamics (Majewsky et al. 2011). Still, the reported variability of some compounds in the WWTP effluents (Nelson et al. 2011) was most likely reflected in the deviations from the ideal first-order decay of pharmaceuticals along the river segments (Figure 2). To characterize the possible effects of the non-steady loading from WWTPs, we estimated the residence time of water within the WWTP (hydraulic residence time), and the WWTP effluent discharge variation encompassed during the measurements (river segments travel time). In fact, the hydraulic residence time of the WWTPs ranged from 15.8 to 28.8 h (Table 1). In regards to the variation encompassed during measurements, it averaged 21 % (as coefficient of variation, Table 1), therefore indicating that the WWTP effluent discharges changed remarkably during the time it took for the water to travel from $d1$ to $d4$. These changes in the WWTP effluent discharge encompassed during the measurements pose a threat to the steady-state assumption, and the potential errors generated by the WWTP effluent discharge variation were accordingly assessed. Specifically, the effluent’s discharge temporal variation was propagated on the calculation of the $v_f$ values by applying Monte Carlo simulations ($N = 100$). For this uncertainty analysis, we only assessed changes in the WWTP effluent discharge, but not on the concentrations, so that changes in the pharmaceutical loads at the effluent were exclusively caused by discharge. Results of this uncertainty analysis are typified in Figure 4, which shows the
uncertainty in the \( v_f \) values of 6 pharmaceutical compounds in river segments A and B. Overall, discharge temporal variation differently affected the river segments because of the differences in the dilution capacity of the river (\( Q_{e:w} \)). As the variation in WWTP effluent discharge were smothered in river segments with lower \( Q_{e:w} \). Nevertheless, the overall patterns described in figures 1 and 3 were not masked by the uncertainty related with the WWTP effluent discharge variation.

**4.2 Pharmaceuticals in WWTP effluents and receiving river waters.** WWTPs were a significant source of pharmaceuticals in all river waters examined, as all pharmaceuticals found in river waters were also present in WWTP effluents, but at much higher concentrations. The pharmaceutical concentrations in the analyzed WWTP effluents fell within the range of concentrations reported in a recent review (Pal et al. 2010). In contrast, almost 40% of the analyzed pharmaceuticals (e.g., naproxen and diclofenac) in river waters were found at concentrations higher than those reported for other European rivers (Pal et al. 2010), but similar to those previously reported in the Ebro river (Gros et al. 2007). In fact, the levels of some pharmaceuticals close to the discharge of WWTP effluents (sites \( d1 \) and \( d2 \)) were almost an order of magnitude higher than those reported at study sites located downstream WWTP effluents in Swiss rivers (Ort et al. 2009). This result underlines the small effect of dilution on the concentration of wastewater derived pollutants in rivers with relatively low flow rate such as those found in our study catchment, and especially in arid or semi-arid regions. The antihypertensives valsartan and ibersartan, or the diuretic drug hydrochlorothiazide were detected in our study at concentrations higher than 400 ng L\(^{-1}\) right downstream the discharge of WWTP effluents in all studied river segments, and their mean concentrations along the river segments were always higher than 100 ng L\(^{-1}\) (Table A.3). Despite these elevated values, the reported concentrations were 100 to 1000-fold lower than those reported to cause toxicity in acute ecotoxicological tests (Fent et al. 2006). However, the margin of safety for some of the most abundant compounds producing chronic effects is narrower, as previous studies have reported that some pharmaceuticals such as the anti-
inflammatory diclofenac show chronic lowest-observed-effects concentrations for aquatic biota (fish, invertebrates) toxicity, well in the range of those observed at the river segments right downstream the WWTP effluents (Fent et al. 2006). In addition, potentially additive or synergetic effects of these compounds, when present in mixtures with other contaminants or chemical elements in water, can contribute to their environmental hazard (Tang et al. 2013).

4.3 In-stream attenuation rates. Our study revealed that dilution-corrected in-stream attenuation rates were highly variable among pharmaceuticals and river segments. This variability might be attributed to both the physicochemical properties of the pharmaceutical compounds and the local environmental conditions, similarly to what summarized for pesticides by Fenner et al. (2013). The first was reflected in differences in the mean values among pharmaceuticals as well as in the differences among river segments, whereas the second was reflected in the variability of $\nu_i$ among river segments for each pharmaceutical (Figure 1). Thus, differences in the mean values of $\nu_i$ among pharmaceuticals highlight the relevance of their physicochemical properties on their in-stream attenuation rates; however, none of the considered physicochemical properties showed significant relationships with the mean values of $\nu_i$. A previous study reported different attenuation rates among 10 pharmaceuticals in a river stretch downstream of a WWTP, and attributed differences to reactivity and physicochemical properties, but without pointing out which properties might be the most relevant (Kunkel and Radke 2012). In contrast, a modeling study exploring the relationship between attenuation rates and physicochemical properties such as the molecular weight and $K_{ow}$ of 225 anthropogenic organic chemicals, reported that attenuation was higher with low to medium volatility (-4 < log $K_{ow}$ < -2) and low hydrophobicity (0 < $K_{ow}$ < 4.5) (Gioia and Dachs 2012).

Besides physicochemical properties, molecular properties (functional groups and their positioning) are likely to determine rates of biotransformation and photolysis in case of compounds with chromophores. For example, a study on the removal of trace organics by membrane bioreactor treatment showed high removal efficiencies for compounds bearing
electron donating functional groups such as hydroxyl and primary amine groups (Tadkaew et al. 2011). Similarly, another study described the preferred biotransformation pathway of amide-containing compounds as a function of structural and electronic descriptors (Hebling et al. 2010). However, in our case, the categorization of studied compounds in terms of ring structure (heterocyclic/non-heterocyclic, mono or polynuclear) and functional groups (electron withdrawing/donating moieties) did not result in any meaningful correlation. Overall, none of the considered physicochemical and molecular properties were significantly correlated with the mean values of $v_f$, but with the variability of $v_f$ among river segments for each pharmaceutical. In fact, the $v_f$ values of some pharmaceuticals were similar among study sites, whereas others exhibited higher variability among sites. Those differences were apparently driven to some extent by physicochemical properties such as $K_{ow}$ (Figure 3). A plausible explanation for such a behavior is that in the case of hydrophobic compounds (with higher $K_{ow}$) sorption to suspended particles and sediments is a dominant process leading to in-stream attenuation by reducing the concentration in the aqueous phase along the river segment. In this way, those compounds become less exposed to other biotic (biotransformation) and abiotic (photolysis, volatilization) transformation processes and therefore become the least affected by the variation of environmental conditions between river segments. In this regard, a study using phthalate esters and their metabolites as test compounds showed that hydrophobic substances with high capacity to sorb to particulate organic matter were the least exposed to biodegradation, and were expected to have a low rate of biodegradation in natural sediments despite their inherent biodegradability (Kickham et al. 2012). Similarly, another study on in-stream attenuation of pharmaceuticals identified sorption as the predominant attenuation mechanism for 3 compounds with relatively high $K_{ow}$ (bezafibrate, metropolol and naproxen) (Riml et al. 2013), which were among the identified compounds with the lowest variability among river segments in our study. In contrast, hydrophilic (with low $K_{ow}$) compounds predominantly remain in the aqueous phase, and their observed in-stream attenuation is a result of transformation processes occurring in the
aqueous phase or at the water-air interface, where these compounds are likely affected by local environmental conditions (e.g. temperature, dissolved oxygen). Accordingly, higher differences in terms of the dominant attenuation processes are expected for hydrophilic compounds given the variability between river segments in terms of environmental conditions. The direct measurement of in-stream attenuation processes occurring in these compartments (i.e. biotransformation, photolysis, and volatilization) might help to understand the mechanisms behind the observed attenuations. In this sense, the measurement of other compound physicochemical parameters might have been relevant as well. Overall, and given the data set we have and the observed attenuations, we believe that the $K_{ow}$ of a given compound influences its in-stream attenuation by influencing the balance between the different attenuation mechanisms (biotransformation, photolysis, sorption, and volatilization).

Because of the differences in the sensitivity to the environment between compounds, one would expect compounds with low $K_{ow}$ to show not only more differences in attenuation rates between sites, but also more temporal differences (i.e. seasonal and day-night) within each site.

Despite the low number of river segments included in this study ($N = 4$), the environmental differences among sites introduced large variability in the $v_i$ of the pharmaceuticals. The differences between sites in the mass transfer coefficients of pharmaceuticals with low to mid $K_{ow}$ were associated with differences in P concentration and attenuation (i.e., SRP, TDP, $v_i$ - TDP) as well as with the concentration of TSS. In fact, some pharmaceuticals such as carbamazepine, citalopram, ibersartan, valsartan, and venlafaxine showed $v_i$ values closely related to those of SRP, indicating that attenuation of those pharmaceuticals were tightly associated to ecosystem processes responsible for P dynamics. The tight association between the attenuation rates of these pharmaceuticals and those of P and the concentration of TSS suggest that sorption, rather than biotransformation, photodegradation or other alternative processes, was the main mechanism driving the in-stream attenuation of these pharmaceuticals. Sorption of P typically occurs when dissolved P concentration in the river
water is not in equilibrium with dissolved P concentration in the river sediments or suspended solids (Reddy et al. 1999), which was probably the case in the studied river segments. The importance of sorption for P dynamics in wastewater-affected rivers has been previously demonstrated (Haggard et al. 2005). Unfortunately, we did not estimate the concentration of pharmaceuticals in sediments or suspended solids and thus we do not have direct evidence on the importance of sorption mechanisms. The tight association between attenuation rates of pharmaceuticals and attenuation rates of P has been also reported for WWTPs (Santos et al. 2009). The statistical analysis of 63 analyzed WWTPs indicated that removal efficiencies of pharmaceutical compounds such as diclofenac and ketoprofen were highly correlated to the removal rates of wastewater characterization parameters such as TSS, chemical oxygen demand, biological oxygen demand, pH, oil and grease content, TN and TP content within the WWTP.

The in-stream attenuation rates for pharmaceuticals reported in our study were similar to those reported in the literature (Fono et al. 2006; Joss et al. 2006; Lin et al. 2006; Writer et al. 2013). Our half-life times were comparable to those reported in a Californian stream (Lin et al. 2006), in a 300 km stretch of a river in Texas (Fono et al. 2006), or in a 5.4 km reach of Boulder creek in Colorado (Writer et al. 2013). In those studies, half-life times of ibuprofen were 2.7 and 4.6 h respectively, whereas in our study it averaged 2.01 ± 1.17 h. In the case of the Californian stream, the half-life times of ibuprofen, naproxen and gemfibrozil (2.4 - 4.6 h) were similar to those observed in our study segments (Table 2). In the case of Boulder creek, half-life time of carbamazepine was 21 ± 4.5h, whereas in our study river segments it averaged 4.1 ± 2.4 (Table 2). In fact, carbamazepine was the most persistent compound among the 14 neuro-active pharmaceuticals assessed in Boulder creek, but it was not among the most persistent compounds in our case. It is also possible to compare the pharmaceuticals load reduction, as this parameter is often reported in studies of pharmaceuticals attenuation in rivers and WWTP. For example, a study of the degradation of pharmaceuticals in WWTP reported that only 4 out of 35 pharmaceuticals were degraded by
more than 90 %, while 17 pharmaceuticals were removed by less than 50 % (Joss et al. 2006). In our studied river segments, when considering a river stretch of 1 km, 18 out of 42 pharmaceutical mean loads were reduced by more than 90 %, 10 were reduced between 50 and 90%, and 14 by less than 50 %. This comparison indicates that the dilution-corrected in-stream attenuation rates reported for river stretches of 1 km are similar in terms of percentage load removal to those reported for WWTP, and stresses that in-stream attenuation in river segments downstream of discharge of WWTP effluents is crucial to attenuate pharmaceuticals concentrations. However, observed in-stream attenuation rates must be considered as removal from the water column rather than true removal, as pharmaceuticals were not quantified in sediments, and therefore we cannot differentiate between temporal sorption in sediments or degradation within sediments. In fact, the sediments could be a source of contaminants in downstream river segments if resuspension of fine-grained bedded sediments occur, for instance during seasonal increases in flow rate or during flood events.

Some previous studies have shown that dilution is more relevant than in-stream attenuation for reducing pharmaceuticals concentrations in river water (Ort et al. 2009; da Silva et al. 2011). In contrast, our results point in the same direction of other studies (e.g., Kunkel and Radke, 2012) that provide strong evidence that in-stream attenuation is a significant process influencing the concentration of pharmaceuticals in river waters, and that it can be equally relevant to dilution in reducing river concentrations. In our study, dilution by vertical and horizontal inputs accounted for concentration reductions between 3.5 and 20 % in 1 km, whereas in-stream attenuation showed much higher values (Table 2). We suggest that reduction of pharmaceutical concentrations in rivers is a result of both dilution and in-stream attenuation, and that the latter process gains relative importance in regions subject to water scarcity where river flow rates are relatively low.
5. CONCLUSIONS

In-stream attenuation was highly variable among pharmaceuticals and river segments, and none of the considered physicochemical properties proved to be relevant in determining the mean attenuation rates. Instead, the octanol-water partition coefficient ($K_{ow}$) influenced the variability of rates among river segments, likely due to its effect on sorption to sediments and suspended particles.

Differences among sites in the in-stream attenuation rates of some pharmaceuticals were associated to the concentration of TSS, as well as to the concentration and attenuation of P, indicating a likely coupling to P attenuation and an important role of sorption.

The magnitude of the measured attenuation rates urges scientists to consider them as important as dilution when aiming to predict concentrations in freshwater ecosystems. Despite the importance of in-stream attenuation, the high variability observed among the in-stream attenuation rates of pharmaceuticals in our study and previous studies make it rather impracticable to use universal in-stream attenuation rates in spatially explicit models of pharmaceuticals to properly predict their dynamics.

The different processes responsible for in-stream attenuation (i.e. biotransformation, photolysis, sorption, and volatilization) should be better assessed in field conditions and explicitly considered in river water quality models when aiming to predict loads and concentrations of pharmaceuticals.
6. ACKNOWLEDGEMENTS

This research was supported by a Marie Curie European Reintegration Grant (PERG07-GA-2010-259219) and by a Marie Curie Career Integration Grant (PCIG9-GA-2011-293535) within the 7th European Community Framework Programme, as well as by the Spanish Ministry of Economy and Competitiveness through the projects SCARCE (Consolider-Ingenio 2010 CSD2009-00065) and ENDERUS (CTM-2009-13018), and the postdoctoral grants “Juan de la Cierva” (jci-2009-05604 and jci-2010-06397), and by the European Union through the European Regional Development Fund (FEDER). This work was partly supported by the Generalitat de Catalunya (Consolidated Research Group: Water and Soil Quality Unit 2009-SGR-965). Prof. Barceló acknowledges King Saud University for his visiting professorship.
7. APPENDICES

Appendix A. Details on the analytical method and quality control, and procedures to calculate dilution correction and attenuation.

Table A.1. Recovery rates (%), method limits of detection (LOD) and method limits of quantification (LOQ) for river and WWTP effluent water samples (ng L\(^{-1}\)).

Table A.2. Physico-chemical features of the analyzed pharmaceuticals.

Table A.3. Concentration of analyzed pharmaceuticals at the studied WWTP effluents and receiving river waters (ng L\(^{-1}\)) (concentrations corrected by the recoveries). Note that A-B-C-D refer to the studied river segments, \(u\) to upstream, \(e\) to effluent, and \(d\) to downstream sites.
8. REFERENCES


### 9. Tables

Table 1. Environmental characteristics of the river segments (labeled as A, B, C, and D), and impact of the WWTP on them, as ratios of river flow rate \( Q \) and load of \( \text{NH}_4^+ \) between the WWTP effluent \( (e) \) and the upstream river station \( (u) \). Note that means and standard deviations are based on 4 locations \((d1-d4)\), and that only one integrated sample was used for TSS.

<table>
<thead>
<tr>
<th></th>
<th>A-Puigcerdà</th>
<th>B-Gasteiz</th>
<th>C-Citruénigo</th>
<th>D-Alcanyís</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riv. slope (m ( \text{m}^{-1} ))</td>
<td>0.0078</td>
<td>0.0006</td>
<td>0.0042</td>
<td>0.0022</td>
</tr>
<tr>
<td>Riv. width (m)</td>
<td>13 ± 3.6</td>
<td>14 ± 3.9</td>
<td>5.1 ± 1.5</td>
<td>10 ± 4.5</td>
</tr>
<tr>
<td>Riv. Depth (m)</td>
<td>0.22 ± 0.06</td>
<td>2.19 ± 0.61</td>
<td>0.09 ± 0.02</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td>Elevation (m a.s.l.)</td>
<td>1108</td>
<td>496</td>
<td>357</td>
<td>284</td>
</tr>
<tr>
<td>Drainage area (km(^2))</td>
<td>287</td>
<td>822</td>
<td>1183</td>
<td>3438</td>
</tr>
<tr>
<td>Segment length (m)</td>
<td>4500</td>
<td>2000</td>
<td>2000</td>
<td>3000</td>
</tr>
<tr>
<td>Segment travel time (min)</td>
<td>227</td>
<td>370</td>
<td>303</td>
<td>476</td>
</tr>
<tr>
<td>Segment dilution ((d1 \text{ to } d4)) (%)</td>
<td>13.3 ± 0.21</td>
<td>18.3 ± 0.10</td>
<td>11.0 ± 1.83</td>
<td>57.0 ± 0.15</td>
</tr>
<tr>
<td>Riv. flow rate (m(^3) s(^{-1}))</td>
<td>0.961</td>
<td>2.771</td>
<td>0.053</td>
<td>0.121</td>
</tr>
<tr>
<td>Mean velocity (m s(^{-1}))</td>
<td>0.33</td>
<td>0.09</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>WWTP effluent discharge CV (%)</td>
<td>20.2</td>
<td>23.1</td>
<td>22.1</td>
<td>22.4</td>
</tr>
<tr>
<td>Hydraulic residence time (hours)</td>
<td>20.5</td>
<td>15.8</td>
<td>22</td>
<td>28.8</td>
</tr>
<tr>
<td>Temperature (ºC)</td>
<td>7.0 ± 1.9</td>
<td>12.4 ± 0.7</td>
<td>12.4 ± 0.7</td>
<td>13.5 ± 1.3</td>
</tr>
<tr>
<td>pH</td>
<td>7.8 ± 0.4</td>
<td>7.9 ± 0.2</td>
<td>7.8 ± 0.1</td>
<td>8.1 ± 0.4</td>
</tr>
<tr>
<td>Total suspended solids (mg L(^{-1}))</td>
<td>5.18</td>
<td>2.11</td>
<td>13.80</td>
<td>1.66</td>
</tr>
<tr>
<td>Dissolved oxygen (mg L(^{-1}))</td>
<td>11.0 ± 2.0</td>
<td>10.9 ± 1.4</td>
<td>11.2 ± 2.1</td>
<td>11.7 ± 2.2</td>
</tr>
<tr>
<td>Electrical conductivity (µS cm(^{-1}))</td>
<td>172 ± 28</td>
<td>597 ± 52</td>
<td>2876 ± 118</td>
<td>1591 ± 29</td>
</tr>
<tr>
<td>Cl(^-) (mg L(^{-1}))</td>
<td>11.4 ± 1.7</td>
<td>44.6 ± 9.5</td>
<td>287 ± 66.3</td>
<td>33.3 ± 8.2</td>
</tr>
<tr>
<td>SO(_4^{2-}) (mg L(^{-1}))</td>
<td>4.9 ± 0.3</td>
<td>14.2 ± 1.9</td>
<td>189 ± 52</td>
<td>162 ± 41</td>
</tr>
<tr>
<td>TDN (mg N L(^{-1}))</td>
<td>2.86 ± 1.63</td>
<td>7.48 ± 0.29</td>
<td>4.64 ± 1.65</td>
<td>2.93 ± 0.19</td>
</tr>
<tr>
<td>NH(_4^+) (mg N L(^{-1}))</td>
<td>1.6 ± 1</td>
<td>0.17 ± 0.04</td>
<td>0.39 ± 0.48</td>
<td>0.15 ± 0.09</td>
</tr>
<tr>
<td>NO(_3^-) (mg N L(^{-1}))</td>
<td>0.70 ± 0.15</td>
<td>6.34 ± 0.64</td>
<td>2.66 ± 0.60</td>
<td>2.14 ± 0.53</td>
</tr>
<tr>
<td>TDP (mg P L(^{-1}))</td>
<td>0.26 ± 0.14</td>
<td>0.22 ± 0.01</td>
<td>0.65 ± 0.46</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>SRP (mg P L(^{-1}))</td>
<td>0.24 ± 0.13</td>
<td>0.20 ± 0.01</td>
<td>0.52 ± 0.46</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>DOC (mg L(^{-1}))</td>
<td>2.3 ± 0.6</td>
<td>3.8 ± 0.1</td>
<td>8.2 ± 12.7</td>
<td>6.1 ± 4.9</td>
</tr>
<tr>
<td>Ratio ( Q_{eu} )</td>
<td>0.15</td>
<td>0.35</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Ratio load ( \text{NH}<em>4^+</em>{eu} )</td>
<td>29</td>
<td>9</td>
<td>57</td>
<td>106</td>
</tr>
</tbody>
</table>
Table 2. Mean and standard deviation of the half-life times of 34 pharmaceuticals along the river segments (N = 4).

<table>
<thead>
<tr>
<th>Therapeutic group</th>
<th>Pharmaceutical</th>
<th>Half-life time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analgesics and anti-inflammatories</td>
<td>Acetaminophen</td>
<td>1.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Codeine</td>
<td>3.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Diclofenac</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Hydrocodone</td>
<td>3.5 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Ketoprofen</td>
<td>4.0 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>Naproxen</td>
<td>8.3 ± 12.7</td>
</tr>
<tr>
<td></td>
<td>Phenazone</td>
<td>3.0 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>Propyphenazone</td>
<td>20.5 ± 29.4</td>
</tr>
<tr>
<td></td>
<td>Salicylic Acid</td>
<td>5.5 ± 3.3</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Dimetridazole</td>
<td>34.2 ± 57.7</td>
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<tr>
<td></td>
<td>Erithromycin</td>
<td>4.2 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>Metronidazole-OH</td>
<td>3.5 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Ronidazole</td>
<td>13.3 ± 11.1</td>
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<tr>
<td></td>
<td>Sulfamethoxazole</td>
<td>5.8 ± 4.9</td>
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<td>Trimethoprim</td>
<td>9.5 ± 14.4</td>
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<td>Antihelmintics</td>
<td>Levamisol</td>
<td>4.1 ± 4.3</td>
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<td>Antihypertensives</td>
<td>Ibersartan</td>
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<td></td>
<td>Losartan</td>
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<td>Valsartan</td>
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<td>Ca channel blockers</td>
<td>Verapamil</td>
<td>9.0 ± 12.8</td>
</tr>
<tr>
<td>Antiplatelet agents</td>
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<td>Diuretics</td>
<td>Furosemide</td>
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<td></td>
<td>Hydrochlorothiazide</td>
<td>2.1 ± 1.2</td>
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<td>Histamine receptor antagonists</td>
<td>Ranitidine</td>
<td>6.4 ± 4.9</td>
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<tr>
<td>Lipid regulators</td>
<td>Bezafibrate</td>
<td>2.9 ± 1.5</td>
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<td></td>
<td>Gemfibrozil</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>Psychiatric drugs</td>
<td>Carbamazepine</td>
<td>4.1 ± 2.4</td>
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<td></td>
<td>Citalopram</td>
<td>2.8 ± 1.5</td>
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<tr>
<td></td>
<td>Diazepam</td>
<td>28.1 ± 45.8</td>
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<td></td>
<td>Lorazepam</td>
<td>6.6 ± 6.1</td>
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<tr>
<td></td>
<td>Norfluoxetine</td>
<td>2.1 ± 0.7</td>
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<tr>
<td></td>
<td>Venlafaxine</td>
<td>2.7 ± 1.7</td>
</tr>
<tr>
<td>β–blockers</td>
<td>Atenolol</td>
<td>2.1 ± 1.4</td>
</tr>
</tbody>
</table>
10. FIGURE LEGENDS

Figure 1. Mean and standard deviation of the mass transfer coefficients ($v_f$) of 34 pharmaceuticals plus TDN, TDP and DOC in the river segments (N = 4).

Figure 2. Linear regressions between the travel times and the EMMA dilution-corrected concentrations (as LN ($C_t/C_0$) for 6 selected pharmaceuticals along the river segments (N = 4; see site codes in Table 1). The slope of the regressions is the first-order decay constant of the pharmaceuticals along the river segments downstream the WWTP ($k$; see Eq. 1).

Figure 3. Pearson-moment correlation between $K_{ow}$ and the coefficient of variation of the mass transfer coefficients ($v_f$), indicating that higher $K_{ow}$ was related to lower variation among river segments in terms of $v_f$. Dotted lines indicate 95% confidence intervals.

Figure 4. Vertical box-plot of the mass transfer coefficients ($v_f$) of 6 selected pharmaceutical compounds in river segments A and B after propagating the temporal variation of the WWTP effluent discharge on the calculation of $v_f$. Note that error bars indicate 5 and 95th percentiles.
Figure 1.
Figure 2.
Figure 3.
Figure 4.