

Suspended solids moderate the degradation and sorption of waste water-derived pharmaceuticals in estuarine waters

Aminot Yann ¹, Fuster Laura ¹, Pardon Patrick ¹, Le Menach Karyn ¹, Budzinski H el ene ^{1,*}

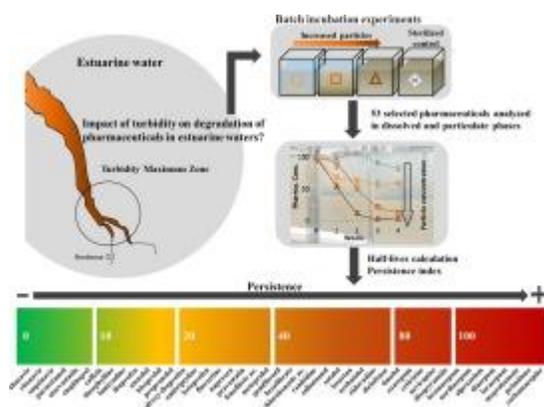
¹ Univ Bordeaux, EPOC, UMR 5805, LPTC, 351 Cours Lib erat, F-33400 Talence, France.

* Corresponding author : H el ene Budzinski, email address : h.budzinski@epoc.u-bordeaux1.fr

Abstract :

This study focuses on the fate of pharmaceuticals discharged into an estuarine environment, particularly into the Turbidity Maximum Zone (TMZ). Batch experiments were set up to investigate the factors regulating the degradation of 53 selected pharmaceuticals. Treated effluents from Bordeaux city (France) were mixed with water from the estuarine Garonne River during 4 weeks under 6 characterized conditions in order to assess the influence of suspended particulates, sterilization, untreated wastewater input and dilution on the degradation kinetics. Of the 53 pharmaceuticals monitored, 43 were quantified at the initial time. Only 7 exhibited a persistent behavior (e.g. carbamazepine, meprobamate) while biotic degradation was shown to be the main attenuation process for 38 molecules (e.g. abacavir, ibuprofen highly degradable). Degradation was significantly enhanced by increasing concentrations of suspended solids. A persistence index based on the half-lives of the compounds has been calculated for each of the 43 pharmaceuticals to provide a practical estimate of their relative stability. The stability of pharmaceuticals in estuarine environments is likely to be highly variable and attenuated primarily by changes in suspended solid concentration.

Graphical abstract



Highlights

► Wastewater derived pharmaceuticals were incubated in estuarine waters. ► Dissolved and particulate concentrations were monitored over 4 weeks. ► Only 7/43 pharmaceuticals were persistent. ► Degradation rates were enhanced by increasing particle concentrations. ► Limited degradation in sterilized conditions

Keywords : Pharmaceuticals, Degradation, Persistence, Wastewater, Estuarine waters, Adsorption

36 1 Introduction

37 Since pharmaceuticals were identified as contaminants of emerging concern (Daughton and Ternes,
38 1999), their occurrence in urban and natural aquatic systems has been increasingly studied. Multi-
39 residue screenings have confirmed their presence in wastewater (López-Serna et al., 2010; Rosal et al.,
40 2010), surface water (Baker and Kasprzyk-Hordern, 2013; Silva et al., 2011), seawater (Benotti and
41 Brownawell, 2007; Vidal-Dorsch et al., 2012) and groundwater (Hass et al., 2012; Vulliet and Cren-
42 Olivé, 2011).

43 After discharge into a water body, concentrations of pharmaceuticals in the dissolved phase are governed
44 by physical processes such as dilution, diffusion and transport as well as by chemical (abiotic) or
45 biochemical (biotic) processes. While the physical processes are likely to be similar between all
46 contaminants, physico-chemical and biochemical processes will differ according to molecular structures
47 (Fatta-Kassinos et al., 2011). In environmental waters, physico-chemical processes relate mainly to
48 photodegradation and sorption. Photodegradation is well documented, with many studies for each
49 carbamazepine, diclofenac, sulfamethoxazole and propranolol (Challis et al., 2014; Trawiński and
50 Skibiński 2017). Concerning sorption to suspended solids (SS) and sediments, pharmaceuticals have
51 received less attention owing to their perceived hydrophilic nature. However, historical records of
52 pharmaceutical contamination have been recently detected in an urban impacted estuary (Lara-Martín
53 et al., 2015) and some authors have reported significant partitioning to sediment of compounds such as
54 psychotropics and β -blockers (Aminot et al., 2015; Baker and Kasprzyk-Hordern, 2011; Burke et al.,
55 2013).

56 To date, most of the studies on pharmaceutical biodegradation focus on their fate through wastewater
57 treatment and during biological secondary treatment (Lahti and Oikari, 2011; Pomiès et al., 2013).
58 However, despite their continuous input to surface waters through treated urban effluents and/or
59 combined sewers overflows (Verlicchi et. 2012), little is known of the parameters governing the fate of
60 pharmaceuticals after discharge. Biodegradation can be investigated through in-stream studies
61 (Aymerich et al., 2016; Kunkel and Radke, 2011; Writer et al., 2013) and laboratory experiments

62 (Baena-nogueras et al. 2017 ; Benotti and Brownawell, 2009; Bradley et al., 2007; Grenni et al., 2013;
63 Yamamoto et al., 2009). Even if laboratory experiments do not strictly represent natural aquatic systems
64 (Kunkel and Radke, 2011) they can provide important information concerning the factors governing in-
65 stream attenuation. Previous studies (Bradley et al., 2007; Radke and Maier, 2014) have evaluated the
66 ability of river sediments to biodegrade pharmaceuticals. Other incubation experiments (Benotti and
67 Brownawell, 2009) have revealed important differences in the biodegradation rates of studied
68 compounds e.g. a paracetamol half-life of less than 1 day compared to a half-life of carbamazepine
69 which is greater than 100 d. The authors also observed that in coastal waters kinetics of degradation
70 were faster under eutrophic conditions.

71 In this context, and as numerous cities like Bordeaux in France, are located along estuaries subject to
72 tidal cycles, there is a real need to investigate the fate of pharmaceuticals in such environments (Zhao
73 et al., 2015). Previous research evidenced a removal of some compounds within the Garonne estuary,
74 with an increase of the attenuation rates in low flow summer periods (Aminot et al., 2016). Water
75 dynamics in tidal estuaries are complex and a zone of high turbidity, known as the Turbidity Maximum
76 Zone (TMZ), is generally observed at the freshwater/seawater interface. In this area, the number of
77 freely suspended bacteria and their growth rate are small compared to those living on the particles
78 (Plummer et al., 1987, Servais and Garnier, 2006), so the particles of the TMZ are expected to play a
79 key role on the biochemical processes governing the water quality, in particular the organic contaminant
80 concentration (Abril et al., 1999; Lanoux et al., 2013).

81 Up to now, the transport and reactivity of emerging contaminants in estuarine environments are poorly
82 understood, yet it closely relates to their effects in such coastal ecosystems. In particular, it remains
83 unclear if the estuarine TMZ acts as a passive vector of contaminants from land to sea or as an active
84 incubator, and, if so, whether sorption or biodegradation is the dominant transformation process. This
85 study, therefore, aims to fill in an important gap in our knowledge by identifying in which way selected
86 pharmaceuticals and estuarine particles characteristic of the TMZ interact. Laboratory batch
87 experiments simulating mixing conditions of the discharge of wastewater into a turbid estuary were

88 performed to assess the influence of suspended solid concentration, type of effluent and dilution on a
89 selection of 53 pharmaceuticals present in waste water from the city of Bordeaux.

90 2 Experimental methods

91 2.1 Estuarine river water and waste water characteristics

92 River water (approx. 100 L) was collected in 20 L HDPE (High Density PolyEthylene) flasks from the
93 estuarine Garonne River adjacent to the city of Bègles (coordinates 44°47'58.31"N; 0°31'37.99"W). This
94 hydrosystem is a macrotidal estuary characterized by a tidal cycle dependent TMZ (Lanoux et al., 2013).
95 Water was sampled at mid-ebb to ensure the highest SS concentration. Three 20 L flasks were subject
96 to magnetic stirring to prevent particle settlement whilst two others were left unagitated for three days
97 at room temperature in the dark. This treatment provided samples from the same water body under three
98 different suspended solid conditions: unagitated flask supernatants, stirred waters and unagitated flask
99 concentrates from the settled particles at respectively low (0.1 g.L^{-1}), intermediate (1 g.L^{-1}) and high
100 (10 g.L^{-1}) SS concentration. Water salinity was representative of TMZ particularity (0.5 ‰) (Lanoux et
101 al. 2013).

102 A few hours before the start of the experiment, large volume wastewater grab samples (approx. 80 L
103 effluent and 20 L influent) were collected in 20 L HDPE flasks from one of the two major waste water
104 treatment plants (WWTP) of the Bordeaux urban area in October 2012 (*Clos de Hilde* WWTP). This
105 WWTP served 264 600 inhabitants (estimate of *Lyonnaise des Eaux*, manager). The WWTP is equipped
106 with biofilters as a secondary treatment.

107 2.2 Chemicals and selection of 53 pharmaceuticals

108 Fifty-three commonly used pharmaceuticals were chosen using multistep selection based upon sales
109 statistics, occurrence and fate in aquatic environment. Selected pharmaceuticals belong to various
110 therapeutic classes and physicochemical properties and were quantified in the studied wastewater
111 effluent in preliminary studies. Details on pharmaceuticals and chemicals used are given elsewhere and

112 in Table I (Aminot et al., 2015). Mercury (II) chloride (99 %) was purchased from Sigma-Aldrich (Saint
113 Quentin Fallavier, France).

114 **2.3 Incubation experiment set-up**

115 Incubation experiments were adapted from previous works on the characterization of organic matter
116 degradation in TMZ (Lanoux, PhD, 2013).

117 Cubic 30 L glass aquariums were filled with river water and wastewater under the 6 following conditions
118 (Figure 1): *low SS (LSS)* 12.5 L effluent, 12.5 L river water supernatant; *intermediate SS (MSS)* 12.5 L
119 effluent, 12.5 L stirred river water; *high SS (HSS)* 12.5 L effluent, 12.5 L river water concentrate;
120 *untreated wastewater (Unt)* 12.5 L influent, 12.5 L stirred river water; *sterilized condition (HgCl₂)*
121 12.5 L effluent, 12.5 L stirred river water, mercury (II) chloride at 100 mg.L⁻¹; *higher dilution (10xD)*
122 2.5 L effluent, 22.5 L stirred river water.

123 Continuous mixing was performed by homemade glass rotors mounted on overhead stirrers while air
124 was bubbled in through immersed frits at an approximate 1 L.min⁻¹ rate. The 6 experimental devices
125 remained in an air-conditioned room (room temperature varied between 18 and 22.5 °C) in the dark.

126 The ambient pharmaceutical concentrations in wastewater effluent samples mixed with estuarine water
127 were sufficient that additional spiking was not required (no introduction of carrying solvent). The
128 dilution rates were chosen as a compromise of environmental relevant levels and to ensure the detection
129 of the analytes on their whole degradation kinetics. Tenfold wastewater dilution (10xD) is comparable
130 to an effluent discharge into a small river. To compensate for this higher dilution, 7 selected compounds
131 (abacavir, carbamazepine, fenofibric acid, ibuprofen, naproxen, paracetamol, sotalol) were spiked into
132 this aquarium to achieve a target concentration of 500 ng.L⁻¹ (Figure 1).

133 Poisoning with mercury (II) chloride has already been used efficiently for soil sterilization prior to PAH
134 analysis (Wang et al., 2011), pharmaceuticals analysis (Yu et al. 2006) and nutrient analysis (Fitzhugh
135 et al., 2003; Wolf et al., 1989) as well as for nutrient analyse of marine waters (Kattner, 1999). Regarding
136 waste waters, it was observed that complete inhibition of microbiological growth was achieved when

137 preserved with 40 mg.L⁻¹ of mercuric chloride, provided that total organic carbon (TOC) was below 20
138 mg.L⁻¹ (Krawczyk, 1975). With average levels of TOC in the effluent of 21.5 mg.L⁻¹ (Lanoux, 2013)
139 and of 5.7 mg.L⁻¹ (Abril et al., 2002) in the estuarine waters, the chosen HgCl₂ level of 100 mg.L⁻¹ is
140 adequate.

141 **2.4 Sampling and analysis**

142 Sampling was performed 10 min after water mixing (T0) and after 7, 14, 21 and 28 days in parallel with
143 conductivity, pH, dissolved O₂ (percentage) and water temperature measurements (note that the
144 sterilized condition was not monitored to prevent probe damage and cross-contamination). Water
145 samples were filtered through glass microfiber filters, GF/F (0.7 µm) (Whatman, supplied by Fisher
146 Bioblock Scientific, Illkirch, France), 4 filters were kept for particle analysis and samples were stored
147 at -20 °C.

148 Water samples were extracted in triplicate by Solid Phase Extraction (SPE) and filters of suspended
149 solids by focused microwave assisted extraction (MAE). Analysis was performed by LC-MS/MS.
150 Protocol details and performance can be found in a previous work (Aminot et al., 2015). Briefly, accurate
151 quantification was ensured by the use of 32 labeled internal standards (given in Table I), spiked in the
152 samples prior to extraction. One processed spiked sample and one procedural blank sample were
153 included in each batch of 12 samples (18 control points for waters and 6 for particles). The LC-MS/MS
154 injections were conducted in one batch, with instrumental calibrants injected every 20 injections and
155 instrumental blanks in between triplicates. Procedural and instrumental blanks revealed no
156 contamination during sample preparation and analysis. By using numerous internal standards
157 compensating for potential preparation losses and matrix effect, the procedural recoveries were in an
158 acceptable range of 80–120 % for 47 (SPE) and 45 (MAE) of the studied compounds (the compounds
159 with lower recoveries were 4-chlorobenzoic acid, ranitidine, losartan, salbutamol, terbutaline for SPE
160 and MAE, plus indinavir for SPE, and lamivudine, caffeine and disopyramide for MAE). Limits of
161 detection did not exceed 1 ng.L⁻¹ for 40 compounds (6 ng.L⁻¹ for the 13 remaining).

162 **2.5 Physico-chemical parameters**

163 The evolution of conductivity, salinity, pH and dissolved oxygen during the 4-week incubation is
164 presented in Figure S1. Initial conductivity was around $1200 \mu\text{S}\cdot\text{cm}^{-1}$ in conditions *LSS*, *MSS*, *HSS* and
165 *Unt* (50:50 dilution rate) and reached $1300 \mu\text{S}\cdot\text{cm}^{-1}$ in condition *10xD* due to the higher brackish water
166 content. In the 5 monitored conditions, conductivity showed a progressive 5 to 10 % increase every
167 week. This increase was attributed to a slight evaporation of the water in the air-conditioned laboratory.
168 This was also reflected with persistent contaminants like carbamazepine, as detailed further in 3.2. pH
169 values ranged between 7.8 and 8.8 with similar tendencies among the experimental conditions: an initial
170 2-week decrease followed by a 2-week increase, probably in association with the assumed evaporation.
171 Rapid ammonia oxidation can be accountable for the initial pH decrease. Except after water mixing
172 (T0), dissolved oxygen was close to 100 %, indicating that the air-bubbling was adequate to maintain
173 aerobic conditions. SS initial concentrations and relative changes during the experiment are available in
174 Table S1, S2 and Figure S2. Tested SS concentrations varied between conditions by a factor of 50 from
175 0.08 to $4 \text{ g}\cdot\text{L}^{-1}$ which are environmentally relevant levels in estuarine waters. After an initial decrease
176 related to the observable sedimentation, this parameter followed the global increase trend attributed to
177 evaporation.

178 **2.6 Data analysis**

179 **2.6.1 Normalization of pharmaceutical concentrations**

180 The slight evaporation over the 4 weeks of incubation caused a concentration increase. Considering
181 carbamazepine's high stability (Benotti and Brownawell, 2009; Chenxi et al., 2008; Kunkel and Radke,
182 2012) and its good analytical robustness (Aminot et al., 2015), other analytes were normalized to
183 carbamazepine concentration in each treatment and sampling time (with carbamazepine concentration
184 set constant at 100 %). The concentrations of carbamazepine with no adjustment are given in Figure S3.

185 **2.6.2 Half-lives and persistence indices**

186 Half-lives were extrapolated from the experimental data (Table 2) by linear regression (detailed in
187 supporting information "half-life calculation"). The application of a finer model would have required
188 additional sampling points in the vicinity of the lag phase and more complex mathematical tools (Chong,

189 2009), outside the scope of this study. Analytes showing a concentration higher than 80 % of the initial
190 concentration after 4 weeks were considered as stable. Concerning compounds undetected after 1 week,
191 calculation gives a 3.5 d half-life but the actual half-life can be somewhat shorter.

192 In order to give a practical relative comparison of the compound degradabilities (including abiotic), a
193 persistence index based on the compound half-lives was calculated. It consists of grading each
194 pharmaceutical in each treatment where it was quantified. Marks depend on half-life values: < 7 d = 0;
195 from 7 to 14 d = 20; from 14 to 21 d = 40; from 21 to 28 d = 60; > 28 d = 80; not calculable because of
196 stable concentrations = 100. The average mark gives the persistence index (Table 2).

197 3 Results and discussion

198 Concentrations are given as total, *i.e.* the sum of SS- and dissolved-phase concentrations (measured
199 separately). Of the 53 monitored analytes, 43 were quantified after initial water mixing (T0) in at least
200 one treatment and 26 in the 6 treatments (Table S3).

201 3.1 Behavior of the pharmaceuticals

202 3.1.1 Impact of sterilization

203 To evaluate if mercury (II) chloride poisoning affected the analytes, initial concentrations in the
204 sterilized condition were compared to the average concentrations in conditions *LSS*, *MSS* and *HSS* which
205 are similar in terms of effluent type and dilution. Agreement between these conditions, plotted in Figure
206 S4, indicates that out of the 40 molecules quantified above their limit of quantification (equal to 3.3
207 times the limit of detection) in conditions *LSS*, *MSS* and *HSS*, 26 were considered unaffected by HgCl₂,
208 while 8 were partially affected ($C_{\text{HgCl}_2} < 0.8 \cdot C_{\text{LSS, MSS, HSS}}$ for lamivudine, ritonavir, alprazolam, 4-
209 chlorobenzoic acid, primidone, theophylline, losartan, disopyramide) and 6 were highly affected
210 ($C_{\text{HgCl}_2} < 0.2 \cdot C_{\text{LSS, MSS, HSS}}$ for abacavir, bromazepam, atorvastatin, ranitidine, salbutamol). Appropriate
211 responses for the internal standards (abacavir d4, bromazepam d4, atorvastatin d5, primidone d5)
212 preclude any analytical artefacts. These losses were rapid for some compounds (e.g. abacavir) with the
213 analytes not being detected a few minutes after water mixing at T0. This sterilization method has

214 previously been applied without significantly altering the organic matter of soils (Fitzhugh et al., 2003;
215 Wolf et al., 1989). However, a 2-36 % loss of PAH has already been observed following mud
216 sterilization (Wang et al., 2011). $HgCl_2$ has also been shown to be capable of rapidly degrading the
217 booster biocide Irgarol 1051 at environmental pH by hydrolysis of the cyclopropylamine group (Liu et
218 al., 1999). Hydrolysis of abacavir, with a similar functional group, could account for its disappearance,
219 although further investigations are required to evaluate the mechanism.

220 Focusing only on the 26 unaffected analytes, the condition $HgCl_2$ can be considered as an abiotic batch
221 control experiment. Steady concentrations were observed for 13 pharmaceuticals (lamivudine,
222 ketoprofen, naproxen, ibuprofene, hydroxy-ibuprofene, gemfibrozil, bezafibrate, 4-chlorobenzoic acid,
223 fenofibric acid, pravastatin, metoprolol, sotalol, losartan) over the 21 days of incubation in this condition
224 only (all data supplied in the Supporting Information, Figure S5).

225 3.1.2 Degradation and the influence of suspended solids

226 Considering conditions *LSS*, *MSS*, *HSS* and the sterilized condition $HgCl_2$, 4 specific behaviors were
227 noticeable (Figure 2, all data are plotted in Figure S5). The meprobamate-type compounds (Figure 2.a)
228 exhibited constant concentrations (> 80 % T_0) in all conditions over the 4 weeks (bromazepam,
229 nordiazepam, alprazolam, lorazepam, meprobamate, primidone, and carbamazepine). The bezafibrate-
230 type compounds (Figure 2.b) showed constant concentrations in the sterilized condition but decreasing
231 concentrations under the biotic conditions with faster kinetics observed for higher SS concentrations
232 (ketoprofen, naproxen, diclofenac, ibuprofene, hydroxy-ibuprofene, gemfibrozil, bezafibrate, 4-
233 chlorobenzoic acid, fenofibric acid, pravastatin, metoprolol, sotalol, cetirizine, losartan, disopyramide).
234 The atenolol-type (Figure 2.c) concentration decrease was more rapid than for the bezafibrate-type and
235 included some degradation under sterilized conditions (lamivudine, zidovudine, atenolol, bisoprolol,
236 propranolol, caffeine, theophylline, abacavir, acebutolol, ranitidine). The ritonavir-type compounds
237 (Figure 2.d) exhibited rapid decreasing concentrations in all the conditions with similar kinetics between
238 sterilized and biotic conditions (ritonavir, oxazepam, amitriptyline, fluoxetine, clopidogrel). All the non-
239 persistent molecules exhibited an initial slow concentration decrease (lag period) followed by an
240 acceleration of the kinetics (Figure 2.b).

241 Suspended solids are known to play a crucial role in biogeochemical processes between water, sediments
242 and microorganisms (Turner and Millward, 2002). The degradation observed was mainly biotic
243 (bezafibrate- and atenolol-type) as the sterilized condition remained higher or even constant. Only for
244 the 5 molecules in the ritonavir-type category the similarity between sterilized and biotic conditions
245 implied abiotic processes as the major degradation pathway. An overall increase in the biodegradation
246 rate was measured for increasing concentrations of SS. Bacterial activity is largely dominated by
247 bacteria living on particles in estuarine waters: Plummer and co-workers (Plummer et al., 1987)
248 measured a contribution of freely suspended bacteria as little as 15 % of the whole bacteria enumeration
249 and activity in the Tamar TMZ (UK) while Servais and Garnier (2006) showed that the growth rates of
250 attached bacteria were, on average, three times higher than those of free-living ones. Consequently,
251 additional bacteria are brought with increasing SS concentrations and the biochemical processes are
252 promoted, in agreement with our findings. This is also in agreement with the increased microbial
253 respiration measured as the depletion of dissolved oxygen in the TMZ of the Gironde estuary, France
254 (Lanoux et al., 2013).

255 The observed kinetics is inconsistent with a first-order reaction, even though it was reported in previous
256 studies (Li et al., 2015). The initial lag phase has also been identified during degradation by activated
257 sludge of dissolved organic matter (Galvez et al., 1996), ibuprofen and ketoprofen (Almeida et al., 2013)
258 as well as for bisphenol A, estradiol and ethinylestradiol degradation in the marine environment (Ying
259 and Kookana, 2003). This evolution has been attributed to the acclimation and development of the
260 microbial populations in general (Almeida et al., 2013; Chong, 2009; Ying and Kookana, 2003) and
261 sigmoidal functions were previously proposed to model the kinetics. Biodegradation of amino acids in
262 estuarine waters, in the absence of wastewater, also showed a delayed degradation after the initial
263 compound spiking (Tappin et al., 2010). These studies and our observed kinetics suggest that a
264 development and/or acclimation of the microbial populations occurred after mixing estuarine water with
265 wastewaters. This supports the conclusions that the biodegradation was mainly the consequence of the
266 degrading microbes from the turbid river water, un-acclimated yet to the wastewater born

267 pharmaceuticals, and not the consequence of the wastewater effluent microorganisms, as the wastewater
268 dilution rate showed no influence on the kinetics.

269 **3.1.3 Influence of effluent treatment**

270 Comparing conditions *MSS* and *Unt*, respectively comprising a WWTP effluent and influent, affords
271 consideration of both the type of effluent and the SS concentration/nature (Figure 3). Analytes exhibited
272 slightly faster degradation under condition *Unt*, with half-lives a few days shorter (2.5 d and 10 d in the
273 case of naproxen and zidovudine, respectively, Figure 3.a and b). Only in the case of fenofibric acid
274 (Figure 3.c), a significantly slower degradation was observed with influent wastewater. Potentially high
275 concentrations of fenofibrate (the unmonitored parent compound of fenofibric acid in human
276 metabolism) in the influent could account for this result through degradation into fenofibric acid.
277 However, studies on such a transformation have not been reported in literature.

278 **3.1.4 Influence of dilution rate**

279 Conditions *MSS*, *HSS* - composed of 50 % vol. effluent - and *10xD* -composed of 10 % vol. effluent-
280 were compared to explore the impact of dilution on the degradation kinetics. SS concentrations in
281 condition *10xD* were included between conditions *MSS* and *HSS* (Table S1). For all the degradable
282 molecules in these 3 conditions, the kinetics was function of the SS concentration and no atypical
283 behavior emerged from condition *10xD*.

284 **3.2 Sorption of pharmaceutical to suspended solid**

285 The number of detected pharmaceuticals was dependent on the suspended solid concentration of the
286 treatment considered. In condition *MSS*, with intermediate SS concentrations, up to 25 molecules were
287 quantified on SS while 41 were found in dissolved phase. The evolution of the analyte concentrations
288 on SS and in the dissolved phase were similar for all detected compounds (Figure S6).

289 When comparing the experimental conditions *LSS*, *MSS* and *HSS* which were similar in terms of dilution
290 rate and effluent type, the highest pharmaceutical concentrations on particles were observed for the
291 lowest SS concentrations. It was found that the partition coefficient K_d decreased with SS concentration

292 with a difference up to 2 log between the lowest and highest SS conditions (Figure 4). This observation
293 was not due to a change in organic content of SS as log K_{oc} exhibited a similar trend. Average partition
294 coefficients measured in the intermediate condition MSS are available in the supporting information for
295 every pharmaceutical detected in both the dissolved and particulate phases at least twice in the 4 weeks
296 (Table S4). Ritonavir, amitriptyline and propranolol have the highest affinity with SS, as previously
297 observed (Aminot et al., 2015).

298 The partitioning coefficients K_d and K_{oc} , ranging from 0.6 to 3.7 and 0.5 to 3.0 respectively, in the
299 intermediate SS concentration condition (MSS), were low to moderate (Table S4) and in agreement with
300 previously reported values (Al-Khazrajy and Boxall, 2016; Aminot et al., 2015). Poor correlation
301 ($R^2=0.07$) was obtained when attempting to correlate log K_d with log D at pH 8 (Figure S7). As an
302 example, beta-blockers, all containing one (propranolol, metoprolol, bisoprolol) to two (sotalol,
303 acebutolol) secondary amines moieties and positively charged at pH 8 showed an affinity to SS 1 to 2
304 orders of magnitude higher than diclofenac, fenofibric acid and bezafibrate, containing a carboxylic acid
305 function and negatively charged at pH 8, despite a similar log D at pH 8. It was previously showed that
306 compounds with basic characteristics, protonated under natural water pH, tend to show higher affinity
307 to the negatively charged SS (Schaffer et al., 2012; Silva et al., 2011). Variabilities in the sorption of
308 pharmaceuticals and other organic contaminants between different substrates are also attributed to
309 factors like their organic carbon content and quality, mud/clay content or inorganic cation content
310 (Aminot et al., 2015; Belles et al., 2016; Schaffer et al., 2012; Silva et al., 2011). Interestingly, the
311 partitioning coefficients were found to be dependent on the SS concentration. Non-constant K_d indicate
312 a non-linear adsorption isotherm, which could be better described by more complex adsorption models,
313 outside the scope of this study. In our case, the type of particle is the same across experimental conditions
314 and only its concentration varied. Similar behaviour was observed for carbamazepine, propranolol and
315 diclofenac on SS in Kent River, UK (Zhou and Broodbank, 2014). The authors proposed a power law
316 to describe decreasing K_d for increasing SS and attributed this observation to a combination of multiple
317 factors including a higher sorbing power of fine and organic-rich SS at low SS concentrations, increasing

318 desorption at high SS concentrations due to more frequent interactions of SS, and potentially higher
319 colloids being produced at high SS concentrations competing with SS.

320 **3.3 Half-lives and persistence indices and pharmaceutical degradability**

321 Half-lives as a function of SS concentrations (Figure 5) followed a decreasing exponential form. It
322 indicates that a similar variation in SS concentrations will have a higher impact on the degradation
323 kinetics at low SS values compared to high SS values. Between conditions *10xD* and *HSS*, a 2-fold SS
324 concentration increase has little effect on half-lives. Kinetics were different in condition *Unt* (influent
325 wastewater) for losartan, gemfibrozil and bezafibrate, giving a point slightly aside of the exponential
326 trend.

327 In order to compare relative compounds degradabilities, a persistence index was calculated (Table 2).
328 Of the 43 molecules, 6 (paracetamol, abacavir, ritonavir, saquinavir, atorvastatine, clopidogrel) were
329 considered as very degradable with an average score of 0 whilst 8, all psycholeptics (bromazepam,
330 nordiazepam, alprazolam, diazepam, lorazepam, meprobamate, primidone, carbamazepine), were very
331 persistent (score 100). Oxazepam scored 80 but exhibited a very slight decrease with a half-life > 60 d.

332 Up to 14 analytes (/43 detected) were considered as stable in biotic conditions (bromazepam,
333 nordiazepam, alprazolam, lorazepam, meprobamate, primidone, carbamazepine, ranitidine, acebutolol,
334 diclofenac, timolol, cetirizine, nevirapine and disopyramide in the “LSS” condition). Relative
335 persistence is consistent with those reported in literature: e.g. naproxen < gemfibrozil (Grenni et al.,
336 2013); paracetamol << carbamazepine (Yamamoto et al., 2009); paracetamol < caffeine < ketoprofene
337 < salbutamol \approx ranitidine < carbamazepine (Benotti and Brownawell, 2009). The relative persistence
338 and half-lives values calculated in the highest SS condition (HSS) are in agreement with those calculated
339 at the water sediment interface in a previous study (Li et al., 2015). Psycholeptics compounds like
340 benzodiazepines showed minor to no degradation. Diazepam was found to be refractory in the absence
341 of sunlight in a previous incubation of estuarine waters (Tappin et al., 2014). Oxazepam persistence in
342 estuarine conditions is consistent with its stability through wastewater treatment (González Alonso et
343 al., 2010; Yuan et al., 2013) and in fresh waters (Hass et al., 2012). Our findings emphasize the concerns

344 on this pharmaceutical, recently reported as bioaccumulative (Lagesson et al., 2016) and toxic (Brodin
345 et al., 2013).

346 **3.4 Implications on pharmaceutical degradability in estuaries**

347 Macrotidal estuaries are characterized by their TMZ in which river water and its organic contaminants
348 from upstream meet high SS concentrations in the freshwater/surface water Interface. In the estuarine
349 Garonne River, the SS concentration close to the discharge point of Bordeaux city effluents has seasonal
350 variations from less than 50 mg.L⁻¹ during high flows to over 10 g.L⁻¹ during low flow periods (Etcheber
351 et al., 2011). Additionally, intra-day variations are based on the tidal cycle with a maximum SS
352 concentration reached at mid-ebb where a tenfold increase can be observed within 3 h. Considering the
353 longitudinal transport of contaminants, when approaching the TMZ from upstream, contaminants are
354 exposed to increasing SS concentrations whilst after the TMZ and along the salinity gradient, the SS
355 concentration decreases. In agreement with the conclusions of our experiments, the rise in SS is expected
356 to enhance the degradation rate of pharmaceuticals inducing high spatial and temporal variations on the
357 compounds degradation rates. The seasonal removal of pharmaceuticals, previously demonstrated in the
358 Garonne estuary (Aminot et al., 2016), is likely not only to be due to increased water residence time but,
359 also a consequence of more turbid waters during the low flow summer period. A recent study also
360 observed that river waters could show higher attenuation efficiencies than WWTPs for a same residence
361 time, confirming that environmental degradation processes are significant and not only controlled by
362 residence time (Aymerich et al., 2016).

363 When taking into account the numerous water physicochemical parameters that may influence
364 degradation, the understanding of the processes governing in-stream attenuation becomes excessively
365 complex and *in-vitro* experimentation is necessary. Besides, additional work on the microbial fauna is
366 required to understand the degrading power of the different bacterial communities that may be associated
367 with freshwater, TMZ and marine waters. In addition to SS, it has been shown that pharmaceuticals tend
368 to degrade faster in more eutrophic waters, or waters more concentrated in biodegradable dissolved
369 organic carbon (Benotti and Brownawell, 2009; Lim et al., 2008). Our degradation experiments have

370 been conducted under aerobic conditions. Previous studies (Ying and Kookana, 2003) demonstrated that
371 the stability of organic micropollutants (steroids, alkylphenols, bisphenol A) in seawater was
372 significantly increased under anaerobic conditions. Similarly, enhancement in biodegradation rates was
373 observed after introducing oxygen to an anoxic water/sediment system (Radke and Maier, 2014). In the
374 estuarine Garonne River, dissolved oxygen can reach 30 % at 1 m under the surface in summertime
375 (Lanoux et al., 2013) while anoxic conditions have been observed in the fluid mud (SS concentrations
376 $> 140 \text{ g.L}^{-1}$) (Abril et al., 1999). Persistence of the contaminants is then expected to be enhanced under
377 such conditions.

378 4 Conclusions

379 The quantification of 43 of the 53 screened pharmaceuticals enabled the evaluation of their stability.
380 Persistent behaviour was observed for 7 molecules during the 4 weeks of experiment, as indicated by
381 the persistence index proposed (bromazepam, nordiazepam, alprazolam, diazepam, lorazepam,
382 meprobamate, primidone, carbamazepine). By quantifying the analytes in the dissolved and particulate
383 phases and comparing total concentrations to a sterilized condition, we provided evidence that biotic
384 degradation and not sorption to particles was the main attenuation process. This biodegradation was
385 enhanced by increasing concentrations of SS: half-lives were reduced by up to 6-fold by a 50-fold SS
386 increase. The influence of the type of effluent as well as its mixing proportion appeared to be minor.
387 When considering dissolved and particulate phases separately, it was found that the equilibrium between
388 these compartments was a function of the SS concentration, although most of the targeted analytes
389 exhibited low to moderate affinity towards particles, as per the low $\log K_d$ calculated.

390 In natural aquatic systems and in particular in estuaries where the penetration of light is limited by the
391 turbidity of the waters, biodegradation is expected to be a major removal process for pharmaceuticals.
392 However, the kinetics of this attenuation is water body-dependent and its moderation by different
393 bacterial communities or by variations in organic carbon particle compositions, in salinities, in oxygen
394 rates etc. can be significant and requires further investigations.

395 Acknowledgements

396 This work was supported by the Etiage program (Agence de l'Eau Adour-Garonne, the CUB
397 (Communauté Urbaine de Bordeaux) and Lyonnaise des Eaux), the Aquitaine Region and the European
398 Union (CPER A2E project). Europe is moving in Aquitaine with the European Regional Development
399 Fund. This study has been carried out in the framework of the Cluster of Excellence COTE. Professor
400 James W. Readman from Plymouth University is also acknowledged for his helpful suggestions that
401 improved the manuscript.

402

403 **Tables**

404 **Table I.** Selected physicochemical properties of the studied pharmaceuticals, CAS number, associated
 405 internal standard. The partitioning coefficient log Kow, log D pH 2 and log D pH 8 were calculated using
 406 Chemaxon log D predictor tool (<https://disco.chemaxon.com/apps/demos/logd>). *pKa values were
 407 summarized by Shalaeva et al. 2007; Takayanagi et al. 2015; Barbic et al. 2007; Escher et al. 2010;
 408 Verlicchi et al. 2012.

Analyte	Therapeutic classes	CAS n°	Molecular weight (g/mol)	log Kow	log D pH 2	log D pH 8	pKa*	Associated internal standard	Ionisation mode
abacavir	Antiretroviral	136470-78-5	286.33	0.39	-1.58	0.38	15.41/5.77	abacavir d4	ESI pos
indinavir	Antiretroviral	150378-17-9	613.79	2.81	-1.56	2.79	13.19/7.37	indinavir d6	ESI pos
lamivudine	Antiretroviral	134678-17-4	229.26	-1.1	-1.1	-1.1	14.29	lamivudine 15N2-13C	ESI pos
nefinavir	Antiretroviral	159989-64-7	567.78	4.72	1.46	4.52	9.32/8.18	nevirapine d3	ESI pos
nevirapine	Antiretroviral	129618-40-2	266.30	2.49	0.11	2.49	10.37/5.06	nevirapine d3	ESI pos
ritonavir	Antiretroviral	155213-67-5	720.94	5.22	4.49	5.22	13.68/2.84	nevirapine d3	ESI pos
saquinavir	Antiretroviral	127779-20-8	670.84	2.58	-0.36	2.56	5.11/8.31	nevirapine d3	ESI pos
zidovudine	Antiretroviral	30516-87-1	267.24	-0.3	-0.41	-0.42	9.96	zidovudine d3	ESI neg
bromazepam	Psycholeptic	1812-30-2	316.15	2.54	1.85	2.54	12.24/2.68	bromazepam d4	ESI pos
nordiazepam	Psycholeptic	1088-11-5	270.71	1.32	2.31	3.21	-	nordiazepam d5	ESI pos
alprazolam	Psycholeptic	28981-97-7	308.77	2.37	-0.79	3.02	18.3/5.08	diazepam d5	ESI pos
diazepam	Psycholeptic	439-14-5	284.74	3.08	2.11	3.08	2.92	diazepam d5	ESI pos
oxazepam	Psycholeptic	35295-88-6	286.71	2.92	2.92	2.92	10.61/-1.5	oxazepam d5	ESI pos
lorazepam	Psycholeptic	846-49-1	321.16	3.53	3.53	3.53	10.61/-2.2	diazepam d5	ESI pos
clonazepam	Psycholeptic	106955-87-7	315.71	3.15	2.92	3.15	11.89/1.86	diazepam d5	ESI pos
meprobamate	Psycholeptic	57-53-4	218.25	0.93	0.93	0.93	15.17	meprobamate d3	ESI pos
ketoprofen	Analgesic	172964-50-0	254.28	3.61	3.61	0.18	3.88/-7.5	ketoprofen d3	ESI neg
naproxen	Analgesic	23981-80-8	230.26	2.99	2.98	-0.36	4.19/-4.8	naproxen d3	ESI neg
diclofenac	Analgesic	15307-86-5	296.15	4.26	4.25	0.85	4/-2.1	diclofenac d4	ESI neg
ibuprofen	Analgesic	58560-75-1	206.28	3.84	3.84	0.85	4.85	ibuprofen d3	ESI neg
2-hydroxy-ibuprofen	Analgesic	51146-55-5	222.28	2.37	2.37	-0.77	-	OH ibuprofen d6	ESI neg
paracetamol	Analgesic	2248282	151.16	0.91	0.91	0.89	9.46/	paracetamol d4	ESI pos
gemfibrozil	Lipopenics	25812-30-0	250.33	4.39	4.39	1.14	4.42/-4.8	gemfibrozil d6	ESI neg
bezafibrate	Lipopenics	41859-67-0	361.82	3.99	3.98	0.55	3.83/-0.84	bezafibrate d6	ESI pos
4-chlorobenzoic acid	Lipopenics	74-11-3	156.57	2.23	2.23	-1.15	-	diclofenac d4	ESI neg
fenofibric acid	Lipopenics	42017-89-0	318.75	4.36	4.33	0.85	-4.9	fenofibric acid d6	ESI neg
clofibric acid	Lipopenics	882-09-7	214.65	2.9	2.88	-0.6	0	clofibric acid d4	ESI neg
pravastatin	Lipopenics	81093-37-0	424.53	1.65	1.64	-1.69	4.21/	pravastatin d3	ESI neg
atorvastatin	Lipopenics	134523-00-5	558.64	5.39	5.39	2.09	4.33/-2.7	atorvastatin d5	ESI neg
atenolol	β-blocker	60966-51-0	266.34	0.43	-2.82	-1.24	14.8/9.67	atenolol d7	ESI pos
bisoprolol	β-blocker	66722-44-9	325.443	2.2	-1.05	0.53	14.09/9.67	propranolol d7	ESI pos
metoprolol	β-blocker	37350-58-6	267.36	1.76	-1.48	0.09	14.09/9.67	propranolol d7	ESI pos
propranolol	β-blocker	13013-17-7	259.34	2.58	-0.66	0.92	14.09/9.67	propranolol d7	ESI pos
sotalol	β-blocker	27948-47-6	272.36	-0.4	-3.19	-1.56	10.07/9.43	sotalol d7	ESI pos
timolol	β-blocker	131628-37-0	316.42	1.34	-1.91	-0.42	14.08/9.76	propranolol d7	ESI pos
acebutolol	β-blocker	37517-30-9	336.43	1.53	-1.71	-0.03	13.91/9.57	propranolol d7	ESI pos
imipramine	Antidepressant	50-49-7	280.41	4.28	0.77	3.06	9.2	amitriptyline d6	ESI pos
doxepin	Antidepressant	1668-19-5	279.38	3.84	0.34	2.08	9.76	amitriptyline d6	ESI pos
amitriptyline	Antidepressant	50-48-6	277.40	4.81	1.31	3.05	9.76	amitriptyline d6	ESI pos
fluoxetine	Antidepressant	57226-07-0	309.33	4.17	0.93	2.38	9.8	fluoxetine d5	ESI pos
primidone	Anticonvulsant	125-33-7	218.25	1.12	1.12	1.12	11.5/	primidone d5	ESI pos
carbamazepine	Anticonvulsant	298-46-4	236.27	2.77	2.77	2.77	15.96	carbamazepine d10	ESI pos
cetirizine	Antihistaminic	83881-51-0	388.89	0.86	-0.24	0.4	3.6/7.79	cetirizine d8	ESI pos
ranitidine	Antihistaminic	66357-35-5	314.40	0.98	-3.6	0.78	8.08	diazepam d5	ESI pos
clenbuterol	β2 agonist	37148-27-9	277.19	2.33	-1	0.71	14.06/9.63	diazepam d5	ESI pos
caffeine	Stimulant	71701-02-5	194.19	-0.55	-0.55	-0.55	-	caffeine d9	ESI pos
theophylline	Bronchodilator	58-55-9	180.16	-0.77	-0.77	-1.11	7.82	caffeine d9	ESI pos
sildenafil	PDE-5-inhibitor	139755-83-2	474.58	1.35	-1.51	0.92	7.27/5.97	sildenafil d3	ESI pos
losartan	Antihypertensive	114798-26-4	422.91	5.08	2.95	2.81	7.4/4.12	diazepam d5	ESI pos
salbutamol	Bronchodilator	18559-94-9	239.31	0.34	-2.36	-0.77	10.12/9.4	diazepam d5	ESI pos
clopidogrel	Antiplatelet agent	113665-84-2	321.82	4.03	1.05	4.03	5.14	diazepam d5	ESI pos
terbutaline	Bronchodilator	46719-29-3	225.28	0.44	-1.89	-0.19	8.86/9.76	diazepam d5	ESI pos
disopyramide	Antiarrhythmics	3737-09-5	339.47	3.47	-0.73	1.08	16.19/10.42	diazepam d5	ESI pos

409

410

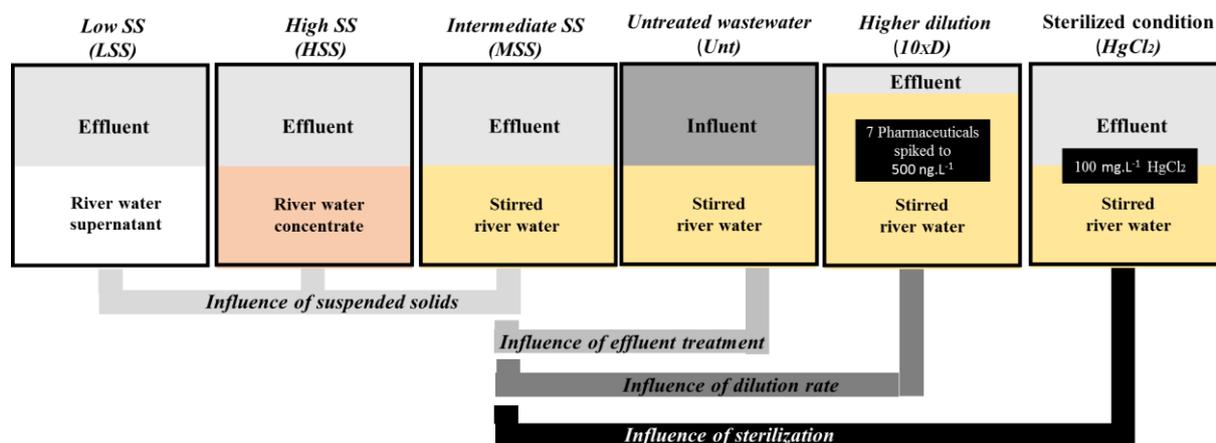
411

412
413**Table II. Calculated half-lives (conditions sorted by increasing SS) and persistence indices. Average values \pm uncertainties (n=3). Calculations are given in supplementary information. NC: not calculable as undetected.**

Analyte	Half-lives (d)						Persistence index
	<i>LSS</i>	<i>MSS</i>	<i>Unt</i>	<i>10xD</i>	<i>HSS</i>	<i>HgCl2</i>	
abacavir	3.6 \pm 0.2	3.7 \pm 0.5	3.6 \pm 0.2	3.5 \pm 0.4	3.5 \pm 0.5	NC	0
ritonavir	3.8 \pm 0.4	3.8 \pm 1	3.9 \pm 0.4	3.7 \pm 0.5	3.7 \pm 1.1	5.7 \pm 0.9	0
saquinavir	NC	NC	4.1 \pm 0.5	NC	3.5 \pm 0.2	5 \pm 0.8	0
paracetamol	NC	NC	3.5 \pm 0.2	3.5 \pm 0.6	NC	NC	0
atorvastatin	NC	3.5 \pm 2.5	3.6 \pm 3	NC	NC	NC	0
clopidogrel	4.5 \pm 1.2	5.5 \pm 1.3	4.1 \pm 0.4	4 \pm 1	4.9 \pm 1.2	4.5 \pm 1.2	0
caffeine	4.5 \pm 0.7	3.8 \pm 1.5	3.5 \pm 0.2	3.9 \pm 0.9	5 \pm 0.7	39 \pm 22	13
theophylline	5.6 \pm 1.1	5.2 \pm 3.8	3.6 \pm 0.1	4 \pm 0.6	3.6 \pm 2	31 \pm 24	13
lamivudine	3.5 \pm 0.1	4.8 \pm 0.5	5.2 \pm 0.7	3.5 \pm 0.4	3.5 \pm 0.5	stable	17
ibuprofen	6.5 \pm 1	4.3 \pm 0.4	3.5 \pm 0.3	3.6 \pm 0.2	3.5 \pm 0.5	stable	17
atenolol	5.6 \pm 1	7.2 \pm 1.3	5 \pm 0.7	3.8 \pm 0.8	3.7 \pm 0.6	41 \pm 37	17
bisoprolol	13 \pm 3	6.7 \pm 1.2	4.7 \pm 0.5	5.5 \pm 1.3	4.9 \pm 0.6	47 \pm 57	17
propranolol	6.9 \pm 1.2	7.6 \pm 1.6	5.2 \pm 0.5	6.2 \pm 1.3	4.9 \pm 0.6	56 \pm 139	17
hydroxy-ibuprofen	9.5 \pm 0.3	6.2 \pm 0.5	3.5 \pm 0.2	5.1 \pm 1.4	4.9 \pm 0.4	stable	20
amitriptyline	4 \pm 0.9	6.3 \pm 2.1	steady at 55%	NC	10.4 \pm 2.6	3.9 \pm 0.6	20
ketoprofen	8.1 \pm 0.4	6.1 \pm 0.9	9.4 \pm 1.4	6.8 \pm 2.6	6.1 \pm 0.5	stable	23
fluoxetine	3.6 \pm 0.4	6.3 \pm 1	8.1 \pm 4.8	NC	stable	5.7 \pm 1	24
naproxen	16 \pm 1	7.9 \pm 0.7	5.4 \pm 0.7	3.6 \pm 0.4	3.5 \pm 0.2	stable	27
pravastatin	19 \pm 4	7.7 \pm 1.4	3.5 \pm 0.3	3.5 \pm 0.1	3.6 \pm 0.3	stable	27
fenofibric ac.	10 \pm 0	8.7 \pm 0.4	14 \pm 1	5.5 \pm 0.8	5 \pm 0.6	stable	30
metoprolol	24 \pm 47	7.6 \pm 1.6	5.5 \pm 0.9	7.4 \pm 0.2	5.2 \pm 0.4	stable	33
gemfibrozil	19 \pm 3	13 \pm 2	18 \pm 3	11 \pm 4	10 \pm 1	stable	40
bezafibrate	22 \pm 3	14 \pm 2	8.7 \pm 1.3	11 \pm 2	9.2 \pm 0.8	stable	40
4-chlorobenzoic ac.	17 \pm 13	9.8 \pm 0.9	NC	NC	3.5 \pm 0.3	stable	40
ranitidine	stable	12 \pm 14	13 \pm 5	NC	8.4 \pm 4.9	NC	40
salbutamol	33 \pm 35	8.9 \pm 2.2	NC	NC	8.5 \pm 3.5	NC	40
sotalol	steady at 60%	14.2 \pm 3.1	12.9 \pm 9.6	10 \pm 2	4.9 \pm 0.6	stable	43
losartan	28 \pm 7	17 \pm 2	19 \pm 5	10 \pm 2	8.7 \pm 0.8	stable	47
acebutolol	stable	18 \pm 6	19 \pm 7	26 \pm 22	11 \pm 3	NC	52
zidovudine	49 \pm 223	24 \pm 10	14 \pm 5	NC	8.2 \pm 1.9	46 \pm 137	56
diclofenac	stable	23 \pm 2	14.6 \pm 4.5	11.2 \pm 2.2	8.9 \pm 0.3	stable	57
oxazepam	96 \pm 38	97 \pm 46	165 \pm 228	72 \pm 36	58 \pm 23	65 \pm 30	80
timolol	stable	30 \pm 22	43 \pm 45	NC	15 \pm 13	stable	80
cetirizine	stable	stable	stable	37.1 \pm 14.6	30 \pm 18	stable	93
nevirapine	stable	stable	stable	stable	30 \pm 22	stable	96
disopyramide	stable	stable	stable	stable	41 \pm 167	stable	97
bromazepam	stable	stable	stable	stable	stable	stable	100
nordiazepam	stable	stable	stable	stable	stable	stable	100
alprazolam	stable	stable	NC	NC	NC	NC	100
diazepam	NC	NC	stable	stable	NC	stable	100
lorazepam	stable	stable	stable	stable	stable	stable	100
meprobamate	stable	stable	stable	stable	stable	stable	100
primidone	stable	stable	stable	stable	stable	stable	100
carbamazepine	stable	stable	stable	stable	stable	stable	100
indinavir	NC	NC	NC	NC	NC	NC	NC
nelfinavir	NC	NC	NC	NC	NC	NC	NC
clonazepam	NC	NC	NC	NC	NC	NC	NC
clofibrac ac.	NC	NC	NC	NC	NC	NC	NC
imipramine	NC	NC	NC	NC	NC	NC	NC
doxepine	NC	NC	NC	NC	NC	NC	NC
clenbuterol	NC	NC	NC	NC	NC	NC	NC
sildenafil	NC	NC	NC	NC	NC	NC	NC
terbutaline	NC	NC	NC	NC	NC	NC	NC

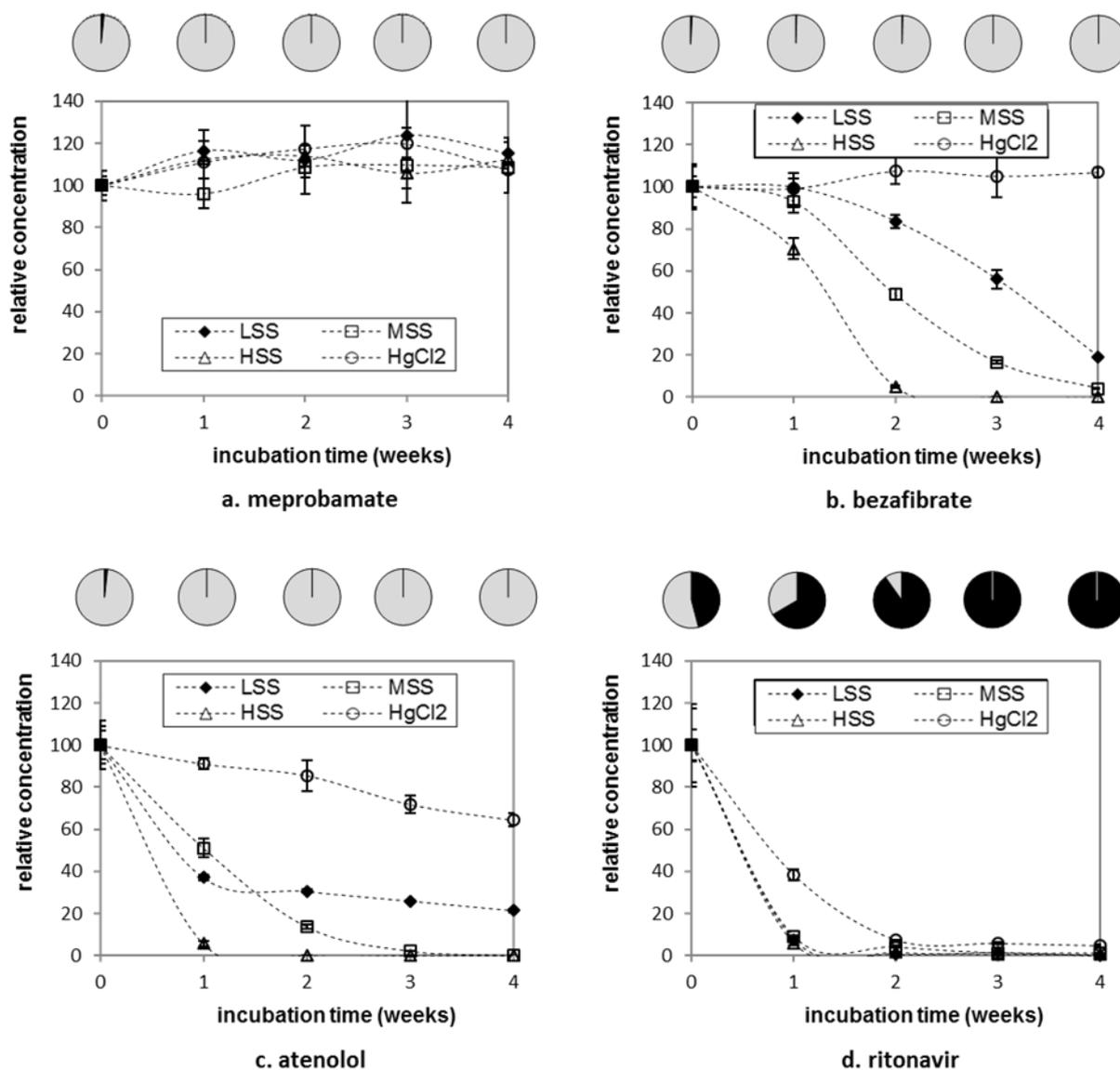
414

415 **Figures**



416

417 **Figure 1. Experimental setup.**



418

419 **Figure 2. Evolution of the relative concentrations for 4 molecules selected for the representativeness of the behaviours**

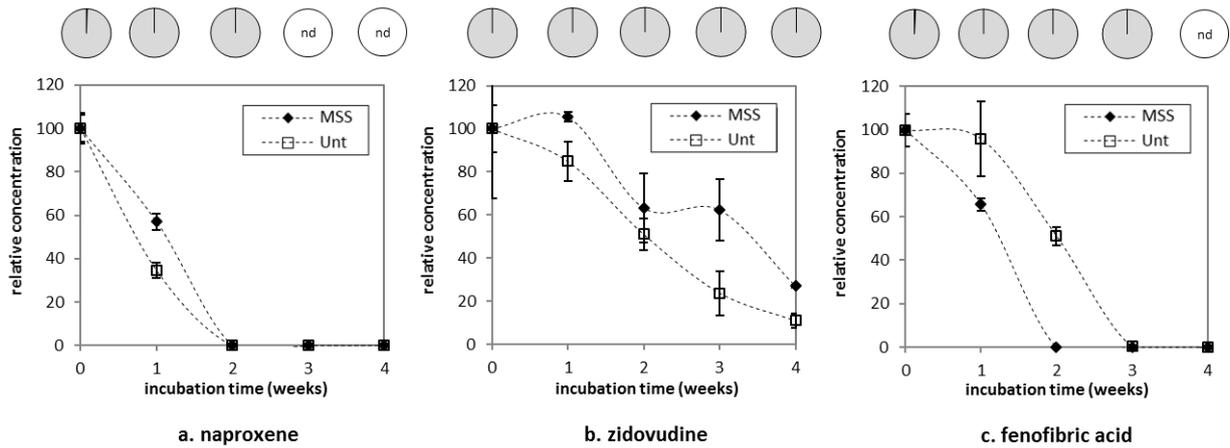
420 **observed. LSS: low SS, MSS: intermediate SS, HSS: high SS, Unt: untreated waste water influent, HgCl₂: abiotic**

421 **reference, 10xD: higher WW dilution rate. The pie charts indicate the mass balance between the dissolved (grey) and**

422 **particulate (black) phases in the condition MSS with intermediate particle concentration. Average values \pm standard**

423 **deviation (n=3).**

424



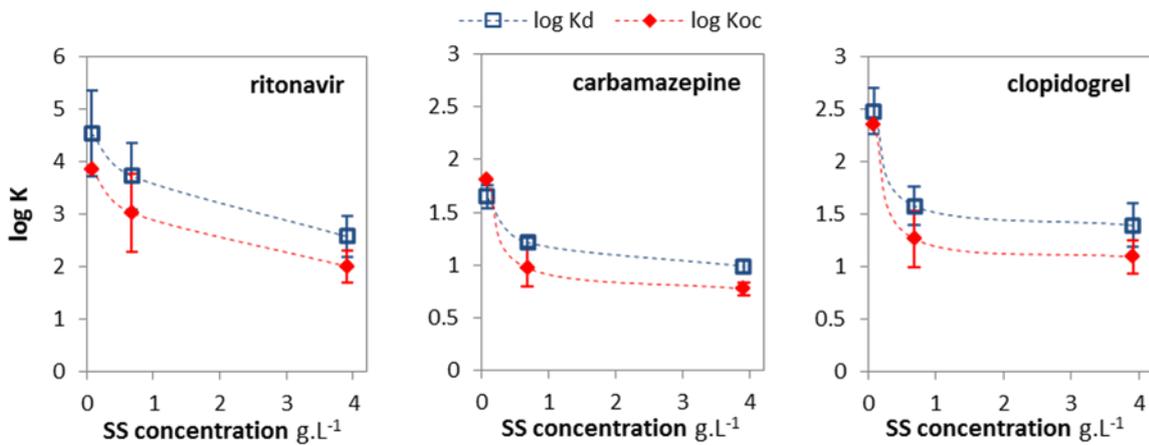
425

426 **Figure 3. Changes in the relative concentrations under conditions *MSS* (treated effluent) and *Unt* (untreated effluent)**

427 **during the degradation experiment for 3 selected-molecules. The mass balance between particulate (dark) and**

428 **dissolved (clear) phases is given in the pie charts for the condition *MSS* at each sampling time. Details of the**

429 **conditions are given in table 1. Average values \pm standard deviation ($n=3$).**



430

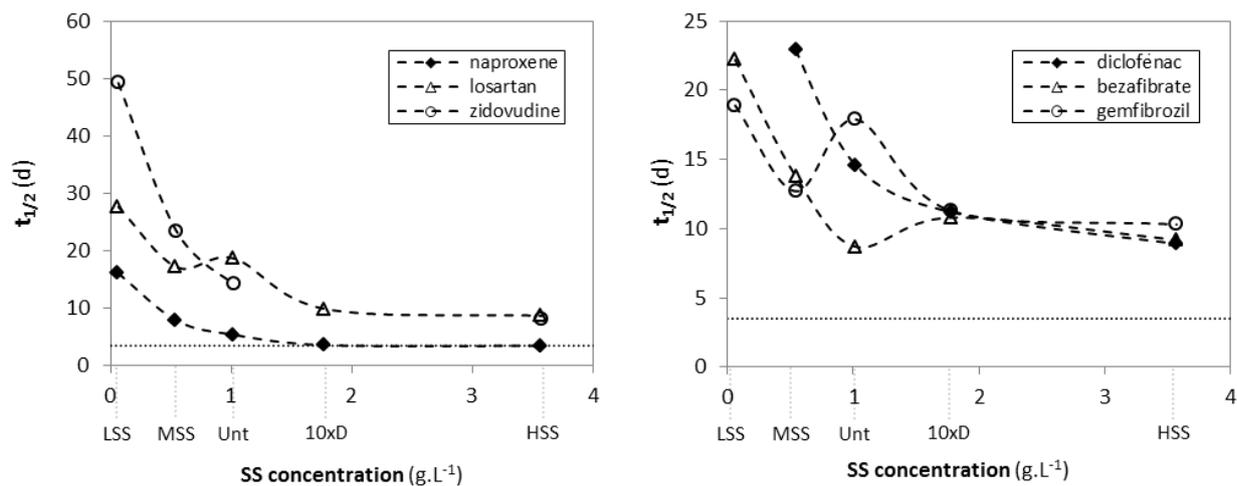
431 **Figure 4. Partition coefficient K_d and partition coefficient normalized by organic carbon content K_{oc} for 3 selected**

432 **analytes as a function of SS concentration in conditions *LSS*, *MSS* and *HSS*. Average values \pm standard deviation, $n=5$**

433 **(time points).**

434

435



436

437 **Figure 5. Relationship between half-lives and SS concentration for 6 selected analytes in the biotic conditions. Note**
 438 **that zidovudine was not quantified in condition 10xD and diclofenac was stable in condition LSS. The minimal**
 439 **calculable half-life (3.5 d) is represented by a dotted line.**

440

441 Bibliographic references

- 442 Abril, G., Etcheber, H., Hir, P.L., Bassoullet, P., Boutier, B., Frankignoulle, M., 1999. Oxic/anoxic
443 oscillations and organic carbon mineralization in an estuarine maximum turbidity zone (The
444 Gironde, France). *Limnol. Oceanogr.* 44, 1304–1315. doi:10.4319/lo.1999.44.5.1304
- 445 Abril, G., Nogueira, M., Etcheber, H., Cabecadas, G., Lemaire, E., Brogueira, M.J., 2002. Behaviour of
446 Organic Carbon in Nine Contrasting European Estuaries. *Estuar. Coast. Shelf Sci.* 54, 241–262.
447 doi:10.1006/ecss.2001.0844
- 448 Al-Khazrajy, O.S.A., Boxall, A.B.A., 2016. Impacts of compound properties and sediment
449 characteristics on the sorption behaviour of pharmaceuticals in aquatic systems. *J. Hazard.*
450 *Mater.* 317, 198–209. doi:10.1016/j.jhazmat.2016.05.065
- 451 Almeida, B., Oehmen, A., Marques, R., Brito, D., Carvalho, G., Barreto Crespo, M.T., 2013. Modelling
452 the biodegradation of non-steroidal anti-inflammatory drugs (NSAIDs) by activated sludge and
453 a pure culture. *Bioresour. Technol.* 133, 31–37. doi:10.1016/j.biortech.2013.01.035
- 454 Aminot, Y., Le Menach, K., Pardon, P., Etcheber, H., Budzinski, H., 2016. Inputs and seasonal removal
455 of pharmaceuticals in the estuarine Garonne River. *Mar. Chem.*, 13th International Estuarine
456 Biogeochemistry Symposium (IEBS) - Estuaries Under Anthropogenic Pressure 185, 3–11.
457 doi:10.1016/j.marchem.2016.05.010
- 458 Aminot, Y., Litrico, X., Chambolle, M., Arnaud, C., Pardon, P., Budzinski, H., 2015. Development and
459 application of a multi-residue method for the determination of 53 pharmaceuticals in water,
460 sediment, and suspended solids using liquid chromatography-tandem mass spectrometry. *Anal.*
461 *Bioanal. Chem.* 407, 8585–8604. doi:10.1007/s00216-015-9017-3
- 462 Aymerich, I., Acuña, V., Barceló, D., García, M.J., Petrovic, M., Poch, M., Rodriguez-Mozaz, S.,
463 Rodríguez-Roda, I., Sabater, S., von Schiller, D., Corominas, L., 2016. Attenuation of
464 pharmaceuticals and their transformation products in a wastewater treatment plant and its
465 receiving river ecosystem. *Water Res.* 100, 126–136. doi:10.1016/j.watres.2016.04.022
- 466 Baena-Nogueras, R.M., González-Lazo, E., Lara-Martín, P.A., 2017. Degradation kinetics of
467 pharmaceuticals and personal care products in surface waters : photolysis vs biodegradation.
468 *Science of the Total Environment* 591, 643–654. doi.org/10.1016/j.scitotenv.2017.03.015.
- 469 Baker, D.R., Kasprzyk-Hordern, B., 2013. Spatial and temporal occurrence of pharmaceuticals and illicit
470 drugs in the aqueous environment and during wastewater treatment: New developments. *Sci.*
471 *Total Environ.* 454–455, 442–456. doi:10.1016/j.scitotenv.2013.03.043
- 472 Baker, D.R., Kasprzyk-Hordern, B., 2011. Multi-residue determination of the sorption of illicit drugs
473 and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid
474 extraction, solid phase extraction and liquid chromatography coupled with tandem mass
475 spectrometry. *J. Chromatogr. A* 1218, 7901–7913. doi:10.1016/j.chroma.2011.08.092.
- 476 Barbic, S., Horvat, A.J.M., Mutavdzic, P., Kastelan-Macan, M., 2007. Determination of pKa values of
477 active pharmaceutical ingredients. *Trends in Analytical Chemistry* 26, 1043-106.
478 doi:10.1016/j.trac.2007.09.004
- 479 Belles, A., Alary, C., Mamindy-Pajany, Y., Abriak, N.-E., n.d. Relationship between the water-
480 exchangeable fraction of PAH and the organic matter composition of sediments. *Environ.*
481 *Pollut.* doi:10.1016/j.envpol.2016.05.077
- 482 Benotti, M.J., Brownawell, B.J., 2009. Microbial degradation of pharmaceuticals in estuarine and
483 coastal seawater. *Environ. Pollut.* 157, 994–1002. doi:10.1016/j.envpol.2008.10.009
- 484 Benotti, M.J., Brownawell, B.J., 2007. Distributions of Pharmaceuticals in an Urban Estuary during both
485 Dry- and Wet-Weather Conditions. *Environ. Sci. Technol.* 41, 5795–5802.
486 doi:10.1021/es0629965
- 487 Bradley, P.M., Barber, L.B., Kolpin, D.W., McMahon, P.B., Chapelle, F.H., 2007. Biotransformation
488 of caffeine, cotinine, and nicotine in stream sediments: Implications for use as wastewater
489 indicators. *Environ. Toxicol. Chem.* 26, 1116–1121. doi:10.1897/06-483R.1
- 490 Brodin, T., Fick, J., Jonsson, M., Klaminder, J., 2013. Dilute Concentrations of a Psychiatric Drug Alter
491 Behavior of Fish from Natural Populations. *Science* 339, 814–815.
492 doi:10.1126/science.1226850

- 493 Burke, V., Treumann, S., Duennbier, U., Greskowiak, J., Massmann, G., 2013. Sorption behavior of 20
494 wastewater originated micropollutants in groundwater - Column experiments with
495 pharmaceutical residues and industrial agents. *J. Contam. Hydrol.* 154, 29–41.
496 doi:10.1016/j.jconhyd.2013.08.001
- 497 Challis, J.K., Hanson, M.L., Friesen, K.J., Wong, C.S., 2014. A critical assessment of the
498 photodegradation of pharmaceuticals in aquatic environments: defining our current
499 understanding and identifying knowledge gaps. *Environ. Sci. Process. Impacts* 16, 672–696.
500 doi:10.1039/C3EM00615H
- 501 Chenxi, W., Spongberg, A.L., Witter, J.D., 2008. Determination of the persistence of pharmaceuticals
502 in biosolids using liquid-chromatography tandem mass spectrometry. *Chemosphere* 73, 511–
503 518. doi:10.1016/j.chemosphere.2008.06.026
- 504 Chong, N.-M., 2009. Modeling the acclimation of activated sludge to a xenobiotic. *Bioresour. Technol.*
505 100, 5750–5756. doi:10.1016/j.biortech.2009.06.071
- 506 Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment:
507 Agents of subtle change? *Environ. Health Perspect.* 107, 907–938.
- 508 Etcheber, H., Schmidt, S., Sottolichio, A., Maneux, E., Chabaux, G., Escalier, J.-M., Wennekes, H.,
509 Derriennic, H., Schmeltz, M., Quémener, L., Repecaud, M., Woerther, P., Castaing, P., 2011.
510 Monitoring water quality in estuarine environments: lessons from the MAGEST monitoring
511 program in the Gironde fluvial-estuarine system. *Hydrol Earth Syst Sci* 15, 831–840.
512 doi:10.5194/hess-15-831-2011.
- 513 Escher, B.I., Baumgartner, R., Koller, M., Trayer, K., Lienert, J., McArdell, C.S., 2010. Environmental
514 toxicology and risk assessment of pharmaceuticals from hospital wastewater. *Water Research*, 45,
515 75–92. doi.org/10.1016/j.watres.2010.08.019.
- 516 Fatta-Kassinos, D., Meric, S., Nikolaou, A., 2011. Pharmaceutical residues in environmental waters and
517 wastewater: current state of knowledge and future research. *Anal. Bioanal. Chem.* 399, 251–
518 275. doi:10.1007/s00216-010-4300-9
- 519 Fitzhugh, R.D., Lovett, G.M., Venterea, R.T., 2003. Biotic and abiotic immobilization of ammonium,
520 nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New
521 York, USA. *Glob. Change Biol.* 9, 1591–1601. doi:10.1046/j.1365-2486.2003.00694.x
- 522 Galvez, L., Hu, Y., Audic, J.M., Block, J.C., 1996. Cinétiques de biodégradation par boues activées de
523 la matière organique soluble d'un effluent synthétique. *Rev. Sci. Eau* 9, 207.
524 doi:10.7202/705249ar
- 525 González Alonso, S., Catalá, M., Maroto, R.R., Gil, J.L.R., de Miguel, Á.G., Valcárcel, Y., 2010.
526 Pollution by psychoactive pharmaceuticals in the Rivers of Madrid metropolitan area (Spain).
527 *Environ. Int.* 36, 195–201. doi:10.1016/j.envint.2009.11.004
- 528 Grenni, P., Patrolecco, L., Ademollo, N., Tolomei, A., Barra Caracciolo, A., 2013. Degradation of
529 Gemfibrozil and Naproxen in a river water ecosystem. *Microchem. J.*, XIV Hungarian - Italian
530 Symposium on Spectrochemistry: Analytical Techniques and Preservation of Natural
531 Resources, Sumeg (Hungary), October 5-7, 2011 107, 158–164.
532 doi:10.1016/j.microc.2012.06.008
- 533 Hass, U., Duennbier, U., Massmann, G., 2012. Occurrence and distribution of psychoactive compounds
534 and their metabolites in the urban water cycle of Berlin (Germany). *Water Res.* 46, 6013–6022.
535 doi:10.1016/j.watres.2012.08.025
- 536 Kattner, G., 1999. Storage of dissolved inorganic nutrients in seawater: poisoning with mercuric
537 chloride. *Mar. Chem.* 67, 61–66. doi:10.1016/S0304-4203(99)00049-3
- 538 Krawczyk, D.F., 1975. Preservation of wastewater effluent samples for forms of nitrogen and
539 phosphorus. *ASTM Spec. Tech. Publ.* 152–163.
- 540 Kunkel, U., Radke, M., 2012. Fate of pharmaceuticals in rivers: Deriving a benchmark dataset at
541 favorable attenuation conditions. *Water Res.* 46, 5551–5565. doi:10.1016/j.watres.2012.07.033
- 542 Kunkel, U., Radke, M., 2011. Reactive Tracer Test To Evaluate the Fate of Pharmaceuticals in Rivers.
543 *Environ. Sci. Technol.* 45, 6296–6302. doi:10.1021/es104320n
- 544 Lagesson, A., Fahlman, J., Brodin, T., Fick, J., Jonsson, M., Byström, P., Klaminder, J., 2016.
545 Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web -
546 Insights from a field experiment. *Sci. Total Environ.* 568, 208–215.
547 doi:10.1016/j.scitotenv.2016.05.206

- 548 Lahti, M., Oikari, A., 2011. Microbial Transformation of Pharmaceuticals Naproxen, Bisoprolol, and
549 Diclofenac in Aerobic and Anaerobic Environments. *Arch. Environ. Contam. Toxicol.* 61, 202–
550 210. doi:10.1007/s00244-010-9622-2
- 551 Lanoux, A., 2013. Caractérisation et rôle respectif des apports organiques amont et locaux sur
552 l'oxygénation des eaux de la Garonne estuarienne. Bordeaux 1.
- 553 Lanoux, A., Etcheber, H., Schmidt, S., Sottolichio, A., Chabaud, G., Richard, M., Abril, G., 2013.
554 Factors contributing to hypoxia in a highly turbid, macrotidal estuary (the Gironde, France).
555 *Environ. Sci. Process. Impacts* 15, 585–595. doi:10.1039/C2EM30874F
- 556 Lara-Martín, P.A., Renfro, A.A., Cochran, J.K., Brownawell, B.J., 2015. Geochronologies of
557 Pharmaceuticals in a Sewage-Impacted Estuarine Urban Setting (Jamaica Bay, New York).
558 *Environ. Sci. Technol.* 49, 5948–5955. doi:10.1021/es506009v
- 559 Li, Z., Sobek, A., Radke, M., 2015. Flume Experiments To Investigate the Environmental Fate of
560 Pharmaceuticals and Their Transformation Products in Streams. *Environ. Sci. Technol.* 49,
561 6009–6017. doi:10.1021/acs.est.5b00273
- 562 Lim, M.-H., Snyder, S.A., Sedlak, D.L., 2008. Use of biodegradable dissolved organic carbon (BDOC)
563 to assess the potential for transformation of wastewater-derived contaminants in surface waters.
564 *Water Res.* 42, 2943–2952. doi:10.1016/j.watres.2008.03.008
- 565 Liu, D., Pacepavicius, G.J., Maguire, R.J., Lau, Y.L., Okamura, H., Aoyama, I., 1999. Mercuric
566 chloride-catalyzed hydrolysis of the new antifouling compound irgarol 1051. *Water Res.* 33,
567 155–163. doi:10.1016/S0043-1354(98)00186-9
- 568 López-Serna, R., Pérez, S., Ginebreda, A., Petrović, M., Barceló, D., 2010. Fully automated
569 determination of 74 pharmaceuticals in environmental and waste waters by online solid phase
570 extraction–liquid chromatography–electrospray–tandem mass spectrometry. *Talanta* 83, 410–
571 424. doi:10.1016/j.talanta.2010.09.046
- 572 Plummer, D.H., Owens, N.J.P., Herbert, R.A., 1987. Bacteria-particle interactions in turbid estuarine
573 environments. *Cont. Shelf Res.* 7, 1429–1433. doi:10.1016/0278-4343(87)90050-1
- 574 Pomiès, M., Choubert, J.-M., Wisniewski, C., Coquery, M., 2013. Modelling of micropollutant removal
575 in biological wastewater treatments: A review. *Sci. Total Environ.* 443, 733–748.
576 doi:10.1016/j.scitotenv.2012.11.037
- 577 Radke, M., Maier, M.P., 2014. Lessons learned from water/sediment-testing of pharmaceuticals. *Water*
578 *Res.* 55, 63–73. doi:10.1016/j.watres.2014.02.012
- 579 Rosal, R., Rodríguez, A., Perdígón-Melón, J.A., Petre, A., García-Calvo, E., Gómez, M.J., Agüera, A.,
580 Fernández-Alba, A.R., 2010. Occurrence of emerging pollutants in urban wastewater and their
581 removal through biological treatment followed by ozonation. *Water Res.* 44, 578–588.
582 doi:10.1016/j.watres.2009.07.004
- 583 Schaffer, M., Börnick, H., Nödler, K., Licha, T., Worch, E., 2012. Role of cation exchange processes
584 on the sorption influenced transport of cationic β -blockers in aquifer sediments. *Water Res.* 46,
585 5472–5482. doi:10.1016/j.watres.2012.07.013
- 586 Servais, P., Garnier, J., 2006. Organic carbon and bacterial heterotrophic activity in the maximum
587 turbidity zone of the Seine estuary (France). *Aquat. Sci.* 68, 78–85. doi:10.1007/s00027-005-
588 0809-y
- 589 Shalaeva, M., Kenseth, J., Lombardo, F., Bastin, A., 2007. Measurement of Dissociation Constants (
590 pKa Values) of Organic Compounds by Multiplexed Capillary Electrophoresis Using Aqueous
591 and Cosolvent Buffers. Wiley Interscience, 11–14. doi: 10.1002/jps.21287.
- 592 Silva, B.F. da, Jelic, A., López-Serna, R., Mozeto, A.A., Petrovic, M., Barceló, D., 2011. Occurrence
593 and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro
594 river basin, Spain. *Chemosphere* 85, 1331–1339. doi:10.1016/j.chemosphere.2011.07.051.
- 595 Takayanagi, T.T., Amiya, M., Shimakami, N., Yabutani, T., 2015. Determination of Acid Dissociation
596 Constant of Pravastatin under degraded conditions by Capillary Zone Electrophoresis. , The
597 japan society for analytical chemistry, 31, 1193-1196.
- 598 Tappin, A.D., Loughnane, J.P., McCarthy, A.J., Fitzsimons, M.F., 2014. Bacterio-plankton
599 transformation of diazepam and 2-amino-5-chlorobenzophenone in river waters. *Environ. Sci.*
600 *Process. Impacts* 16, 2227–2236. doi:10.1039/c4em00306c
- 601 Tappin, A.D., Millward, G.E., Fitzsimons, M.F., 2010. Particle–water interactions of organic nitrogen
602 in turbid estuaries. *Mar. Chem.* 122, 28–38. doi:10.1016/j.marchem.2010.08.006

- 603 Trawinski, J., Skibinski, R., 2017. Studies on photodegradation process of psychotropic drugs : a review.
604 Environ. Sci. Poll. Res. 24, 1152–1199. doi:10.1007/s11356-016-7727-5.
- 605 Turner, A., Millward, G.E., 2002. Suspended Particles : Their Role in Estuarine Biogeochemical Cycles.
606 Estuarine, Coastal and Shelf Science, 55, 857–883. doi: 10.2006/ecss.2002.1033.
- 607 Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban
608 wastewater : Removal , mass load and environmental risk after a secondary treatment — A review.
609 Science of the Total Environment, 429, 123–155. doi.org/10.1016/j.scitotenv.2012.04.028.
- 610 Vidal-Dorsch, D.E., Bay, S.M., Maruya, K., Snyder, S.A., Trenholm, R.A., Vanderford, B.J., 2012.
611 Contaminants of emerging concern in municipal wastewater effluents and marine receiving
612 water. Environ. Toxicol. Chem. 31, 2674–2682. doi:10.1002/etc.2004
- 613 Vulliet, E., Cren-Olivé, C., 2011. Screening of pharmaceuticals and hormones at the regional scale, in
614 surface and groundwaters intended to human consumption. Environ. Pollut. 159, 2929–2934.
615 doi:10.1016/j.envpol.2011.04.033
- 616 Wang, C.-Y., Wang, F., Wang, T., Yang, X.-L., Bian, Y.-R., Kengara, F.O., Li, Z.-B., Jiang, X., 2011.
617 Effects of Autoclaving and Mercuric Chloride Sterilization on PAHs Dissipation in a Two-
618 Liquid-Phase Soil Slurry. Pedosphere 21, 56–64. doi:10.1016/S1002-0160(10)60079-3
- 619 Wolf, D.C., Dao, T.H., Scott, H.D., Lavy, T.L., 1989. Influence of sterilization methods on selected soil
620 microbiological, physical, and chemical properties. J. Environ. Qual. 18, 39–44.
- 621 Writer, J.H., Antweiler, R.C., Ferrer, I., Ryan, J.N., Thurman, E.M., 2013. In-Stream Attenuation of
622 Neuro-Active Pharmaceuticals and Their Metabolites. Environ. Sci. Technol. 47, 9781–9790.
623 doi:10.1021/es402158t
- 624 Yamamoto, H., Nakamura, Y., Moriguchi, S., Nakamura, Y., Honda, Y., Tamura, I., Hirata, Y., Hayashi,
625 A., Sekizawa, J., 2009. Persistence and partitioning of eight selected pharmaceuticals in the
626 aquatic environment: Laboratory photolysis, biodegradation, and sorption experiments. Water
627 Res. 43, 351–362. doi:10.1016/j.watres.2008.10.039
- 628 Ying, G.-G., Kookana, R.S., 2003. Degradation of Five Selected Endocrine-Disrupting Chemicals in
629 Seawater and Marine Sediment. Environ. Sci. Technol. 37, 1256–1260. doi:10.1021/es0262232
- 630 Yu, J.T., Bouwer, E.J., Coelhan, M., 2006. Occurrence and biodegradability studies of selected
631 pharmaceuticals and personal care products in sewage. Agricultural water management 86, 72–
632 80. doi.org/10.1016/j.agwat.2006.06.015
- 633 Yuan, S., Jiang, X., Xia, X., Zhang, H., Zheng, S., 2013. Detection, occurrence and fate of 22 psychiatric
634 pharmaceuticals in psychiatric hospital and municipal wastewater treatment plants in Beijing,
635 China. Chemosphere 90, 2520–2525. doi:10.1016/j.chemosphere.2012.10.089
- 636 Zhao, H., Zhou, J.L., Zhang, J., 2015. Tidal impact on the dynamic behavior of dissolved
637 pharmaceuticals in the Yangtze Estuary, China. Sci. Total Environ.
638 doi:10.1016/j.scitotenv.2015.06.055
- 639 Zhou, J., Broodbank, N., 2014. Sediment-water interactions of pharmaceutical residues in the river
640 environment. Water Res. 48, 61–70. doi:10.1016/j.watres.2013.09.026
- 641