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# Distribution and degradation trend of micropollutants in a surface flow treatment wetland revealed by 3D numerical modelling combined with LC-MS/MS

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## **Abstract**

Conventional wastewater treatment plants are not designed to treat micropollutants; thus, for 20 years, several complementary treatment systems, such as surface flow wetlands have been used to address this issue. Previous studies demonstrate that higher residence time and low global velocities promote nutrient removal rates or micropollutant photodegradation. Nevertheless, these studies were restricted to the system limits (inlet/outlet). Therefore, detailed knowledge of water flow is crucial for identifying areas that promote degradation and optimise surface flow wetlands. The present study combines 3D water flow numerical modelling and liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS/MS). Using this numerical model, validated by tracer experimental data, several velocity areas were distinguished in the wetland. Four areas were selected to investigate the waterflow influence and led to the following results: on the one hand, the number and concentration of micropollutants are independent of the waterflow, which could be due to several assumptions, such as the chronic exposure associated with a low Reynolds number; on the other hand, the potential degradation products (metabolites) were also assessed in the sludge to investigate the micropollutant biodegradation processes occurring in the wetland; micropollutant metabolites or degradation products were detected in higher proportions (both number and concentration) in lower flow rate areas. The relation to higher levels of plant and microorganism metabolites suggests higher biological activity that promotes degradation.

## **Keywords**

Micropollutants, mass spectrometry, Computational Fluid Dynamics, water flow modelling, sludge, surface flow wetland.

## 1 1. Introduction

2 In the past decades, water quality and micropollutant issues have become major concerns  
3 in the water treatment field. Recent progress has confirmed this trend with the  
4 establishment of the Watch list in European laws (Decision 2015/495/EU of 20 March  
5 2015) (Barbosa et al., 2016; Mailler et al., 2017). Micropollutants found in the  
6 environment have several origins, but urban wastewater is one of the most cited  
7 (Kasprzyk-Hordern et al., 2009; Luo et al., 2014; Petrie et al., 2015; Phillips et al., 2012;  
8 Verlicchi et al., 2012). Indeed, a wide variety of our daily activities generate  
9 micropollutants, which end up in wastewater (washing, cooking, or drug consumption)  
10 and, subsequently, in wastewater treatment plants (WWTPs). These wastewater treatment  
11 systems have not been designed to treat micropollutants. Nevertheless, in the literature,  
12 waste water treatment systems and, particularly constructed wetlands (CW), show their  
13 efficiency in the treatment of some micropollutants (Hijosa-Valsero et al., 2010;  
14 Matamoros and Bayona, 2006; Vymazal et al., 2017). In this way, part of the  
15 micropollutants can be caught by the different compartments of these systems, whereas  
16 others are released into the environment. Additionally, WWTPs face sustainability issues  
17 (reuse of water-derived resources and reduction of their environmental footprint) (Wang  
18 et al., 2015). Therefore, constructed wetlands and natural-based treatment systems seem  
19 to be well-suited to these new challenges. In Europe, complementary treatment systems  
20 have been installed for 20 years and can improve micropollutant removal and reduce the  
21 impact of direct release into the environment. WWTPs could disturb the environmental  
22 balance. For example, Tong et al. mention the impact of the nitrogen to phosphorus ratio

23 in lakes found near a WWTP outlet (Tong et al., 2020). Surface flow treatment wetlands  
24 (SFTW) are particularly popular in rural communities (Mara et al., 1992) and offer  
25 several benefits, such as complementary treatment for micropollutants—described in one  
26 of our previous studies (Nuel et al., 2018). Furthermore, the literature mentions  
27 hydraulics, specifically residence time, as one of the major factors that could influence  
28 micropollutant removal (Boonnorat et al., 2016; Ejhed et al., 2018; Esperanza et al.,  
29 2007; Gros et al., 2010). The increase in residence time can promote different  
30 mechanisms that are useful for micropollutant management. For example, longer  
31 residence time improves the biodegradation (Koch et al., 1999; Siegrist et al., 1995) of  
32 conventional pollutants, such as nutrients. Concerning micropollutants, photodegradation  
33 or sorption in sludge could be promoted by higher residence time in this kind of system  
34 (Rühmland et al., 2015), thus reducing their release into the environment. Nonetheless,  
35 most studies focus on the boundary conditions of the system (inlet/outlet) to characterise  
36 removal efficiency and compare global hydraulic parameters, such as residence time  
37 distribution. However, these global parameters could also hide the influence of specific  
38 areas inside water treatment systems. Therefore, a deep knowledge of hydraulics is  
39 crucial to understand the potential influence of water flow velocity on the distribution and  
40 degradation of micropollutants and would be useful to optimise the processes.  
41 Nonetheless, few studies consider different regions inside a system to compare those that  
42 could influence distribution or promote micropollutant removal (Gauillier et al., 2020).  
43 Among these studies, Mali et al. (2018) used a 2D hydrodynamics model combined with  
44 a passive scalar transport equation to determine metal distribution in a port. Their  
45 simulations show the influence of water flow velocity in the studied system. Another

46 study conducted by Gaullier et al. (2020) highlighted the heterogeneous distribution of  
47 pesticides in constructed wetlands using tracer experiments in different water flow  
48 velocity areas. Nevertheless, this study did not consider the degradation process, and  
49 therefore, the fate of these pesticides was not fully investigated. As such, the aim of this  
50 study was to obtain detailed knowledge of water flow velocities in a surface flow wetland  
51 and to determine if these velocities could influence micropollutant distribution and  
52 degradation. Therefore, we propose the first 3D surface wetland model based on real  
53 study site geometry combined with large-scale micropollutant screening. This transient  
54 water flow model was validated by a tracer experiment (comparison between  
55 experimental tracer experiment and simulation using a passive scalar transport equation)  
56 and built to define sludge collection areas. As sludge is a static compartment,  
57 micropollutants are sorbed according to their properties and affinity with solids and can  
58 provide a general overview of the chronic micropollutant load. In this way,  
59 micropollutant analysis was linked to water flow process modelling to understand the  
60 potential influence of the water flow velocity field on the spatial distribution and  
61 degradation of micropollutants.

62

## 63 **2. Materials and methods**

64

### 65 **2.1. Study site**

66 The study site is an SFTW located in Lutter (47°27'47.7" N, 7°22'29.9" E; Grand Est,  
67 France) and implemented in 2009. The SFTW is a shallow-water pond with an

68 impervious layer of clay to counter the high permeability of the natural soil. The SFTW  
69 has a surface of 750 m<sup>2</sup>, maximal width of approximately 13 m, maximal length of  
70 approximately 40 m, and a heterogeneous depth. The SFTW has not yet been cleaned,  
71 and mud has been accumulating since 2009. This SFTW was constructed at the outlet of a  
72 two-stage vertical flow constructed wetland (VFCW) that collects wastewater (1000  
73 population equivalent) before releasing it to the SFTW and, finally, into the river. All  
74 details concerning SFTW can be found in Laurent et al. (2015).

75

## 76 **2.2. Hydrodynamics numerical model**

### 77 **2.2.1. Water flow and passive scalar transport governing equations**

78 Computational fluid dynamics (CFD) simulations were performed on OpenFOAM 4.0,  
79 using the unsteady solver pimpleFoam, which was chosen for its ability to solve the  
80 Navier–Stokes equations in unsteady mode and consider incompressible and turbulent  
81 conditions for the flow resolution. Such turbulent conditions were observed in the SFTW  
82 inlet. The corresponding continuity [1] and momentum equations [2] are given as:

$$83 \quad \nabla \cdot u = 0 \quad [1]$$

$$84 \quad \frac{\partial u}{\partial t} + u \nabla u = -\nabla p + \nu \Delta u \quad [2]$$

85 where  $u$  is the velocity,  $p$  the pressure,  $\nu$  the kinematic viscosity, and  $t$  the time. Because  
86 of calculation costs constraints, only water flow was considered in this study. A tracker

87 simulation was performed to validate the model, considering the tracker as a passive  
88 scalar. The equation governing the passive scalar transport [3] is given as:

89 
$$\frac{\partial C}{\partial t} + \nabla \cdot (uC) - \nabla^2(D_m C) = q_m \quad [3]$$

90 where  $C$  is the tracker concentration,  $u$  is the velocity,  $t$  is the time,  $D_m$  is the tracker  
91 diffusion coefficient (sulforhodamine  $D_m = 3.6 \cdot 10^{-6} \text{ m}^2 \cdot \text{s}^{-1}$ ), and  $q_m$  is the pollutant source  
92 term. A Reynolds-Averaged Navier-Stokes (RANS) method—with a turbulence closure  
93 scheme—was used to solve these equations. The standard  $k$ - $\epsilon$  model was selected for the  
94 turbulence, as it is well-adapted for large flow fields and has been used in several water  
95 flow simulations in ponds (Alvarado et al., 2013; Ouedraogo et al., 2016). The  
96 simulations were performed using second-order schemes, and the results were extracted  
97 after convergence. All simulation results were obtained with residuals lower than  $10^{-7}$ .

### 98 **2.2.2. Computational domains and boundary conditions**

99 The CFD model geometry was built using data collected in the field. Indeed, all the  
100 surface points defining the limits of the computational domain were obtained using a  
101 GPS system. The domain was then split into 1-square-meter subdomains, and the depth  
102 was measured manually using a bathymetry approach. All the data were used to build a  
103 3D geometry of the SFTW. Figure S1 shows an overview of the study site used for the  
104 3D model.

105 Regarding the boundary conditions, the following hypothesis was applied to the  
106 simulations. First, an inlet flow rate was defined for each simulation. SFTW flow rate



107 monitoring was performed using ultrasonic probes (IJINUS, MELLAC, France) and  
 108 built-in exponential section venturis (ISMA, Forbach, France), as described by Nuel et al.  
 109 (Nuel et al., 2017). Then, the average flow rate was calculated and used for each  
 110 simulation. This hypothesis can be applied as the difference between the maximum and  
 111 minimum SFTW flow rate measurements was not significant, according to the results  
 112 obtained by Nuel et al. (Nuel et al., 2017). The outlet pressure was considered at the  
 113 outlet, whereas no slip conditions were considered for the bank and the ground of the  
 114 SFTW. Finally, symmetry conditions were used for the water surface. For the tracker  
 115 experiment, an inlet mass flow rate was defined, with a mass flow rate of approximately  
 116 100 mg/s during the first 300 s of the simulation.

117 Subsequently, the geometry was meshed. The mesh size was chosen to fulfil a  $Y^+$   
 118 criterion between 30 and 300 (in accordance with criteria for the standard k- $\epsilon$  model),  
 119 according to the recommendations found in Versteeg et al. (Versteeg and Malalasekera,  
 120 2007). This  $Y^+$  criterion was calculated using Equation [4]:

$$\Delta s = \frac{\left(\frac{2}{0.026}\right)^{\frac{1}{2}} Y^+ \mu^{\frac{13}{14}}}{(\rho u)^{\frac{13}{14}} L^{-\frac{1}{14}}} \quad [4]$$

121 where  $u$  is the averaged velocity (m/s),  $\rho$  is the water density ( $\text{kg}\cdot\text{m}^{-3}$ ),  $\mu$  is the dynamic  
 122 viscosity ( $\text{kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$ ),  $L$  is the average water depth (m), and  $\Delta s$  is the mesh size in cm.  
 123 Using this criterion, the recommended mesh size was between 1.1 and 11 cm. To reduce  
 124 the calculation cost, the selected mesh size was 10 cm.

125 This choice was checked during the model validation step. The model resulting from  
126 these choices has 1.5 million grid cells.

### 127 **2.2.3. Model validation**

128 The numerical model results were compared with the tracer experimental results  
129 obtained in the field to ensure model and meshing validity. Tracer campaigns were  
130 performed with sulforhodamine B (SRB, C<sub>27</sub>H<sub>29</sub>N<sub>2</sub>NaO<sub>7</sub>S<sub>2</sub>) as a fluorescent dye to  
131 estimate the SFTW residence time, as described in detail in Laurent et al. (Laurent et al.,  
132 2015). Briefly, an instantaneous pulse of tracer was injected at the inlet, and the tracer  
133 concentrations were monitored by a fluorometer (GGUN-FL30, Albilis, Switzerland)  
134 connected to a peristaltic pump operating continuously at 1 L · s<sup>-1</sup>. Fluorometer readings  
135 were calibrated on site by water samples collected at the same location and spiked with  
136 known tracer amounts.

137 These results were then compared to numerical simulations using the passive scalar  
138 simulation described in Section 2.2.1. Alvarado et al. (Alvarado et al., 2013) and Coggins  
139 et al. (Coggins et al., 2017) suggest the use of residence time to validate an SFTW water  
140 flow model. Figure S2 underlines the proper fit between the simulated and experimental  
141 curves. Thus, the model can reproduce the outlet signal. Based on the results, the model  
142 was validated for the rest of the study.

143

## 144 **2.3. Chemicals**

145 Acetic acid and formic acid were acquired from Sigma Aldrich (St. Louis, MO, USA).  
146 The extraction solvents (acetonitrile, methanol, and isopropanol) were obtained from  
147 Fisher Chemicals (New Hampshire, USA). Ammonium formate was purchased from  
148 Fluka Analytical (Missouri, USA), and NaOH was obtained from Agilent Technologies  
149 (California, USA). Deionised water was obtained from a Direct-Q UV (Millipore) station.  
150 Finally, the internal standards used, namely bezafibrate-d4, diclofenac-d4, gemfibrozil-  
151 d6, N-desmethyl sildenafil-d8, sildenafil-d3, sulfamethoxazole-d4, were obtained from  
152 Toronto Research Chemical (Ontario, Canada), whereas the acetaminophen-d4 standard  
153 was purchased from Sigma Aldrich. The labelled internal standards were used to assess  
154 the repeatability of the extraction process and to determine the limits of detection and  
155 quantification (Villette et al., 2019a). The commercial standards used for compound  
156 quantification (irbesartan, oxadiazon, tramadol, etofenprox, celiprolol, desvenlafaxine,  
157 diflufenican, permethrin, propafenone, isoconazole, venlafaxine, fipronilulfone,  
158 acebutolol, amiodarone, and climbazole) were purchased from Sigma Aldrich.

159

160

## 161 **2.5. Micropollutants extraction**

162 Micropollutants were analysed in the sludge samples. Water flow modelling defines the  
163 sludge sampling strategy described in Section 3.1. In each defined area, a composite of  
164 surface sludge (the first ten cm) was collected (in each season), as this layer was in direct  
165 contact with the wastewater. The samples were stored at 4 °C before analysis. All

166 analyses were performed using 3 biological replicates. Micropollutants were extracted as  
167 described by Villette et al. (2019a). Briefly, 10 g of sludge was weighed, and double  
168 extraction was performed. The first overnight extraction was performed using 40 mL of  
169 acetonitrile:water (90:10) with 1% acetic acid at 4 °C under shaking with a magnetic  
170 stirrer. The samples were centrifuged for 15 min at 5500 rpm, and the supernatant was  
171 collected. Then, a second extraction was performed on the pellet using 20 mL of  
172 isopropanol:acetonitrile (90:10) for 15 min at 4 °C under shaking. The samples were  
173 centrifuged for 15 min at 5500 rpm, and the supernatant was recovered and freeze-dried.  
174 Finally, the samples were solubilised in 1 mL of acetonitrile:isopropanol:water (50:45:5).  
175 Parallel blank extraction was performed in each season.

## 176 **2.6. Micropollutant analysis**

177 The samples were analysed in liquid chromatography (LC) coupled to high-resolution  
178 mass spectrometry (HRMS). The method used (Target Screener method (Bruker)) was  
179 mentioned in Villette et al., 2019a and Bergé et al., 2018. This method allows the targeted  
180 identification of drugs and pesticides but could also be investigated for non-targeted  
181 analysis. A Dionex Ultimate 3000 (Thermo) coupled to a Q-TOF Impact II (Bruker) was  
182 briefly used. The method was operated with two solvents: solvent A: H<sub>2</sub>O:MeOH (90:10  
183 v:v) with 0.01% formic acid and 314 mg.L<sup>-1</sup> ammonium formate, and solvent B: MeOH  
184 with 0.01% formic acid and 314 mg.L<sup>-1</sup> ammonium formate. The compounds were  
185 separated using a C18 column (Acclaim™ RSLC 120 C18, 2.2 µm 120A 2.1x100 mm,  
186 Dionex bonded silica products) equipped with a C18 precolumn (Acquity UPLC® C18,  
187 1.7 µm, 2.1 × 5 mm). The compounds were analysed using the spectrometer in positive

188 ion mode with a spectra rate of 2 Hz, on a mass range from 30 to 1000 Da. Fragments  
189 were obtained using broad-band collision-induced dissociation (bbCID) with an MS/MS  
190 collision energy set at 30 eV. An analytical quality check was performed using a mix of  
191 pesticides to assess the retention time (refs 31972 and 31978 Restek). The detailed  
192 procedure and operational parameters can be found in Villette et al. 2019a.

## 193 **2.7. Data processing**

194 The annotations for the LC-HRMS/MS data were performed using TASQ 1.4 (Bruker  
195 Daltonics), which contains a database of 2204 drugs and injected pesticides and has been  
196 used to annotate ions in a targeted way based on the retention time, m/z value, isotopic  
197 pattern of the parent ion (mSigma), and qualifier ions (daughter ion). Using this database,  
198 which contains micropollutant commercial injection standards, all identification reached  
199 level 1 according to the Schymanski classification. The selection criteria were a signal-to-  
200 noise ratio higher than 3, a retention time variation lower than 0.3 min, an exact mass  
201 variation lower than 3 ppm, and matching fragment ions when available. To obtain the  
202 most representative view of the contamination, only micropollutants found in all seasons  
203 and in 3 biological replicates with available commercial standards were quantified. The  
204 mean concentration was determined using all the replicates, and the standard deviation  
205 represents the variation found in the different seasons for each replicate.

206 Additionally, predicted catabolites and conjugates (metabolites) of the micropollutants  
207 were annotated using *in silico* predictions performed in Metabolite Predict 2.0 (Bruker,  
208 Germany) (Pelander et al., 2009). This annotation process has already been described in

209 Villette et al. (2019b). Briefly, 79 biotransformation rules were applied to the structure of  
210 the parent drugs to generate metabolites over two generations. The software then  
211 generated a list of raw formulae containing potential metabolites but also retrieved the  
212 enzymes generating the metabolites. Finally, raw formula lists were imported into  
213 Metaboscope 4.0. for annotation to perform suspect screening of the metabolites. The  
214 data are compared with raw formulae generated using SmartFormula, as mentioned in  
215 Villette et al., 2019b. The metabolites mentioned in this study were only those found in  
216 all seasons and in 3 biological replicates.

217

218 Finally, the data were analysed using a non-targeted method following the processing  
219 mentioned in Villette et al. (2019a). The annotations were performed using a criterion of  
220 mass deviation lower than 3 ppm and mSigma value under 30 to assess the good fit of the  
221 isotope pattern. Raw formula annotations were then generated using C, H, N, O, P, S, Cl,  
222 I, Br, and F elements. Then, tentative identification (level 3 of the Schymanski  
223 classification (Schymanski et al., 2015) was performed using analyte lists created from  
224 the toxic exposome database (<http://www.t3db.ca/>), FooDB (<http://foodb.ca/>), EU  
225 Reference Laboratories for Residues of Pesticides (<http://www.eurl-pesticides.eu>), Phenol  
226 Explorer (<http://phenol-explorer.eu/>), Scientific Working Group for the Analysis of  
227 Seized Drugs (<http://www.swgdrug.org/>), Norman Network ([https://www.norman-  
228 network.net/](https://www.norman-<br/>228 network.net/)), PlantCyc (<https://www.plantcyc.org/>), KNApSAcK  
229 (<http://kanaya.naist.jp/KNAPSAcK/>), and SwissLipids (<http://www.swisslipids.org/>).  
230 Additionally, the metabolites were analysed using a statistical enrichment approach that  
231 is based on chemical similarity with the online ChemRICH tool

232 (<http://chemrich.fiehnlab.ucdavis.edu/>) (Barupal and Fiehn, 2017). All chemical  
233 identifiers (SMILES, PubChem ID, and InChIKey) were manually collected using the  
234 PubChem Identifier Exchanger tool  
235 (<https://pubchem.ncbi.nlm.nih.gov/idexchange/idexchange.cgi>). The identifiers were used  
236 to evaluate the structural similarity between the compounds based on chemical  
237 ontologies.

238 Additionally, the compounds were described using metabolic pathways; these networks  
239 were created using the MetaMapp online tool  
240 (<http://metamapp.fiehnlab.ucdavis.edu/ocpu/library/MetaMapp2020/www/>) (Barupal et  
241 al., 2012). The chemical identifiers were kept from ChemRICH analysis, and Kegg  
242 identifiers were manually searched in the Kegg database (<https://www.genome.jp/kegg/>),  
243 PubChem ID, SMILES, and MetaMapp. The Cytoscape 3.8.0 software was used to draw  
244 the different networks and biological pathways.

## 245 **2.8. Statistical analysis**

246 All samples were replicated three times. In a non-target way, each area was analysed  
247 separately; the samples were clustered in Metaboscape 4.0 (Bruker Daltonics) according  
248 to the seasons, and all the adduct forms were grouped in a bucket. Briefly, using the value  
249 count of group attributes, only compounds found in 80% of group samples (water flow  
250 areas) were selected. The metabolic profile was therefore investigated by comparing area  
251 by area based on a Wilcoxon rank sum test (non-parametric test). Results were  
252 considered significantly different using the fold change differences  $\geq 2$  or  $\leq 2$  and a *p*-  
253 *value*  $< 0.05$ . The fold change (associated with the different couples) and the associated

254 *p-values* were recovered for use in ChemRICH. The complete dataset (statistically  
255 differential and non-differential values) was submitted to ChemRICH, with the fold  
256 change converted to an average ratio. ChemRICH thresholds were *p-value*  $\leq 0.05$  and  
257 fold change  $\geq 2$  or  $\leq -2$  to consider that a compound is significantly up- or down-  
258 regulated in a specific condition (here, the pond) and to obtain the chemical enrichment  
259 analysis results.

260

### 261 **3. Results**

#### 262 **3.1. Water flow process modelling and areas selection**

263 The SFTW was affected by weather conditions; thus, different inlet flow rates were  
264 measured. The distribution of these inlet flow rates measured during eight campaigns  
265 over a period of two years is shown in Figure S3.

266

267

268

269 Among these flow rates, one inlet flow rate condition per season (Figure 1) was  
270 simulated to underline the water flow velocity field diversity that could be observed on  
271 the SFTW. Simulations of the three slowest flow rates (winter with  $7.2 \text{ m}^3 \cdot \text{h}^{-1}$ , summer  
272 with  $5.1 \text{ m}^3 \cdot \text{h}^{-1}$ , and autumn with  $4.2 \text{ m}^3 \cdot \text{h}^{-1}$ ) induced similar hydraulic behaviour, and a  
273 preferential flow between the inlet and outlet was observed. The increase in the inlet flow  
274 rate can generate vortices (simulation A in Figure 1), rendering the inlet-outlet link  
275 unclear. Nevertheless, Figure S3 highlights the extreme nature of this phenomenon.



276 Therefore, the spring simulation (simulation A in Figure 1) in which vortices appear, was  
277 excluded from the rest of the study. The similar water flow behaviour observed in the  
278 three other simulations provided a global overview of the SFTW hydraulic behaviour.  
279 Even if a slow flow rate was created at the SFTW inlet, variations could be observed, and  
280 the water flow velocity field seems to be particularly low near the banks in comparison to  
281 other SFTW areas. Relatively higher water flow can be observed near the inlet and outlet,  
282 whereas a low inlet flow rate is always detected in the SFTW; thus, areas were clustered  
283 according to perpetual (inlet, outlet, and preferential flow areas) or rare (areas near the  
284 banks) water flow.

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288 According to the three lowest flow rate simulations (Figure 1), a sampling strategy was  
289 defined to collect sludge samples in the different flow rate areas. Four areas were  
290 selected, as depicted in Figure 2. The principal points of the water treatment system with  
291 relatively higher and perpetual flows (the inlet (area a) and outlet (area d), respectively)  
292 were selected. To overcome these boundary conditions, two areas were chosen  
293 considering the water flow heterogeneity inside the SFTW. Therefore, sludge was  
294 collected in an area with an intermediate higher and perpetual flow rate (defined in Figure  
295 2 as area b) and in areas near the banks with very low flow rate (area c). These areas were  
296 used for different seasonal sampling campaigns to analyse micropollutants and to  
297 understand the influence of flow rate on the presence of micropollutants in the sludge.

298           **3.2. Distribution of identified micropollutants according to the water flow**  
299 **areas.**

300 Micropollutant analysis was performed for each season in the areas of interest; results are  
301 given in Figure S4 and Dataset 1. Micropollutant distributions fluctuate by season and are  
302 apparently unrelated to water flow.

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305

306 To overcome the seasonal effect and obtain a global overview, only the compounds found  
307 in all seasons were considered; the results are shown in Figure 3. This general analysis  
308 indicates that the number of micropollutants found in the different sampling areas seems  
309 to be similar. Indeed, seven micropollutants were found in areas b and d, six in area c,  
310 and four in area a. Additionally, most of the compounds were found in at least two  
311 sampling areas, even if some micropollutants were only detected in a single area, and  
312 more than half of the micropollutants detected (100% in area a, 71% in area b and d, and  
313 67% in area c) were found in at least two areas. Most micropollutants had concentrations  
314 between 40–400  $\mu\text{g.kg}^{-1}$  of sludge, and little variation in concentration was noticed  
315 between the different detection areas. For example, irbesartan was detected at  
316 concentrations between 76–125  $\mu\text{g.kg}^{-1}$ . Only celiprolol was found at a higher  
317 concentration in area c (491  $\mu\text{g.kg}^{-1}$  in area a and 1187  $\mu\text{g.kg}^{-1}$  in area c).

318           **3.3. Distribution of micropollutants metabolites in the different water flow**  
319 **areas**

320 Studies show that the analysis of parent compounds alone underestimates the amounts of  
321 micropollutants found in the environment. Metabolites (potential conjugates or  
322 degradation products) could be found in higher quantities than parent compounds (Yin et  
323 al., 2017). Therefore, micropollutant metabolites (predicted derivatives) were also  
324 analysed for each season in the areas of interest; the results are shown in Figure S4 and  
325 Dataset 2. A similar trend can be found in the different seasons with a higher number of  
326 metabolites in relatively low water flow areas (b, c).

327  
328

329 This general trend is highlighted by the compounds found in all seasons in Figure 4, and  
330 the global overview indicates that more compounds are identified in lower flow rate areas  
331 (14 in area b and 15 in area c) than in higher flow rate areas (4 in area a and 6 in area d).  
332 Additionally, very few compounds are common to different areas, and a wide variety of  
333 metabolite intensities were detected.

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338 Several drugs and their metabolites were studied (Figure 5), and higher amounts of  
339 tramadol and venlafaxine were found. Higher diversity in area c and irbesartan  
340 metabolites in area b were detected, suggesting that the lower flow rate influences  
341 metabolization. However, the number of metabolites detected is not necessarily related to  
342 the amount of parent compounds; the number of metabolites is positively correlated for  
343 tramadol (higher numbers of metabolites when tramadol is not detected), whereas the  
344 opposite phenomenon was observed for venlafaxine (higher numbers of metabolites when  
345 the venlafaxine concentration is approximately  $123 \mu\text{g}\cdot\text{kg}^{-1}$  sludge).

#### 346 **4. Discussion**

##### 347 **4.1. Influence of boundary conditions**

348

349 Figures 1 and 2 highlight the low-velocity field in the SFTW, which could be caused by  
350 several phenomena, such as the low inlet flow rates compared to the pond sizes, the head  
351 losses resulting from the presence of vegetation, the shallow depth, and the rough sides.  
352 The influence of water depth was investigated by Coggins et al., with a focus on the  
353 impact of sludge accumulation on hydraulic performance (Coggins et al., 2017). Thus,  
354 the areas of SFTW that contain the most accumulated sludge and the areas near the banks  
355 have the lowest flow velocity field and are characterised by a very high hydraulic  
356 residence time and, consequently, a low solid transport phenomenon. However, water

357 quality with higher nutrient removal (nitrogen and phosphorus) can be improved by  
358 increasing residence time (Akratos and Tsihrintzis, 2007; Huang, 2000). A higher water  
359 flow velocity field was detected at the boundary points of the system (inlet and outlet  
360 area) due to the boundary conditions (constrictions), leading to a high rate of sludge  
361 renewal.

362

#### 363 **4.2. No correlation between parent compounds distribution and water flow** 364 **velocities**

365 The micropollutant analysis performed in different areas did not highlight significant  
366 differences in their number and concentration, except for celiprolol. Indeed, almost the  
367 same number of micropollutants and similar concentrations were found in these areas.  
368 However, according to the literature, the sedimentation and sequestration of compounds  
369 in the solid phase should be promoted in lower water flow velocity fields (Montiel-León  
370 et al., 2019). These comments are supported by studies that have investigated the  
371 influence of water flow velocities on contaminant concentrations and sequestration  
372 (Gaullier et al., 2020; Mali et al., 2018). Gaullier et al. (2020) suggest that higher  
373 pesticide storage in lower velocity areas is related to transport types (convection is  
374 promoted in high velocity areas), and Mali et al. found higher metal deposition in a  
375 weaker flow in a port (Mali et al., 2018); nevertheless, the higher velocities mentioned in  
376 their study impact flow patterns and could partly explain the differences.

377 Therefore, our study used deep knowledge of water flow in treatment systems to  
378 determine that water flow velocity fields are not the key parameters governing the  
379 distribution of micropollutants. The difference in water velocities is unlikely to

380 significantly influence a specific micropollutant distribution. The Reynolds number in the  
381 SFTW was low, and the flow observed in the SFTW was generally laminar or low  
382 turbulent (except at the inlet and outlet), reducing the velocity difference in these areas.  
383 This trend was confirmed by the analysis of the areas of xenobiotics putatively identified  
384 in all the sampling areas shown in Figure S6. This broader view highlights that most of  
385 the micropollutants are found in similar proportions in different areas (fold change < 2  
386 (absolute value)). Additionally, the study of physicochemical properties (log Kow,  
387 solubility, pka), as mentioned in Li et al. (2019), does not highlight any correlation  
388 between micropollutant detection and these properties.

389 The impact of water flow velocity could probably be highlighted by other assumptions,  
390 such as solid transport or higher inlet flow rate, probably helps distinguish areas based on  
391 their micropollutant composition. If velocity differences between SFTW areas are  
392 increased, sequestration could be affected. However, all the results found for these  
393 micropollutants should be tempered with the distribution of all the putative identifications  
394 from non-targeted analyses (Figure S7). The general trend emphasizes that the low flow  
395 rate area (area c) seems to accumulate slightly more xenobiotics and plant metabolites  
396 than other areas. These results are apparently more coherent with those found in the  
397 literature.

398 Another assumption that may explain the lack of differences (in concentration or area)  
399 could be related to parent compound degradation, as higher sedimentation probably  
400 occurs in the lower flow rate areas. However, this sedimentation should be hidden by  
401 degradation, which has also been enhanced in these areas.

402

### 403 **4.3. *In situ* low water flow areas promote micropollutant degradation**

404 Parent compounds have been found in minor amounts compared to their metabolites (Yin  
405 et al., 2017). This degradation process could not be neglected in the SFTW, as  
406 micropollutant biodegradation with conjugations and deconjugations has been described  
407 in this type of system (Tiwari et al., 2017). In our study, the distribution of micropollutant  
408 metabolites appears to be primarily influenced by water flow, and more  
409 biotransformation products are found in areas with scarce water flow (14 in area b and 15  
410 in area c versus 4 and 6 in areas a and d, respectively). However, these areas are not  
411 located in the principal water flow channel and could be subject to intermittent flow.  
412 Rožman et al. highlight that a system with intermittent water flow provides a higher  
413 biodegradation capacity than one with permanent water flow, due to the biofilm  
414 development (Rožman et al., 2018). These conclusions support our observations.  
415 Additionally, areas with higher residence time can provide conditions stimulating  
416 degradation, such as increased potential contact time with microorganisms. The influence  
417 of prolonged contact time in a bioreactor to improve micropollutant transformation has  
418 been demonstrated (Asif et al., 2018; Boonnorat et al., 2016).

419 Similarly, the results shown in Figure 5 suggest that the higher numbers of micropollutant  
420 metabolites in the lower flow rate areas (b and c) is not necessarily related to the number  
421 of parent compounds, as phenomena, such as metabolite transport, could occur. A  
422 broader analysis of the data shows that the metabolite distribution apparently follows a  
423 general trend. A closer look at the elemental composition reveals that compounds with  
424 less than 15 or 10 carbons showed the same distribution as that described for

425 micropollutant metabolites (Figure S8 and dataset S3). The lower flow rate areas (b or c)  
426 seem to stimulate compound deposition and transformation, and the conditions promoting  
427 biodegradation were indicated by the non-target analysis and chemical class investigation  
428 using ChemRICH (Barupal and Fiehn, 2017) (Figure S9). The results highlight the  
429 specific detection of alkaloids and monoterpenes in the lower velocity areas; such  
430 observations are not surprising, as plants grow near wetland banks. Additionally,  
431 phosphatidylethanolamines, the primary component of the bacterial membrane, were also  
432 detected. Therefore, the compound annotations seem to indicate higher biological  
433 activities that combine plants and microorganisms. Furthermore, investigation of the  
434 networks and biological pathways detected in the different areas (Figure S10) also  
435 suggests a higher biological activity in lower velocity areas. Complex relationships were  
436 primarily observed in the lower velocity areas (area c), and the network data showed that  
437 succinate was mainly found in the low-flow-rate area. On the one hand, Nguyen et al.  
438 demonstrated that bacteria fed with this succinate could improve energetic efficiency,  
439 leading to a higher drug (sulfomethoxazole) removal rate (Nguyen et al., 2017). On the  
440 other hand, this activity was also observed with the detection of p-cymene detection,  
441 which is a biogas marker and component of anaerobic digestion. (Moreno et al., 2014)

442 Finally, a general biodegradation process seems to occur in SFTW. Most of the  
443 metabolites found (59%) seem to be generated according to a biodegradation process  
444 using cytochrome P450 hydroxylation or epoxidation followed by different transferase,  
445 dehydrogenase, hydrolase, and esterase activities (Figure S11). Cytochrome P450 plays a  
446 key role in Phase I metabolism for several herbicides, can degrade a wide variety of



447 micropollutants due to their low specificity (Cañameras et al., 2015), and could explain  
448 the general process described here. This study was restricted to biodegradation, but other  
449 abiotic processes, such as photodegradation or environmental conditions (pH, redox),  
450 could also be considered to understand the fate of micropollutants in the SFTW (De  
451 Laurentiis et al., 2012; Ávila et al., 2013; Lee et al., 2014; Rühmland et al., 2015).

## 452 **5. Conclusion**

453

454 This study investigated micropollutant distribution and degradation trends in SFTW  
455 wetlands. The investigation was conducted by combining the following:

456 - a deep knowledge of hydraulic behaviour in the SFTW obtained through a 3D model  
457 based on real study site geometry

458 - Large-scale screening of micropollutants and their metabolites

459 Based on this new approach, the results underlined the following micropollutant  
460 behaviours in the SFTW wetland:

- 461 - A homogeneous distribution of parent compounds throughout the SFTW wetland
- 462 - Higher amounts of micropollutant metabolites in lower flow rate areas than in  
463 areas with faster flow rates.

464 Therefore, the low flow rate conditions seem to promote degradation, and the results  
465 suggest that:

- 466 - the velocity differences have an impact on metabolite distribution

- 467 - environmental conditions promoting micropollutant degradation are found in low  
468 flow rate conditions
- 469 - higher biological activity was detected due to the higher number of  
470 microorganisms or plant metabolites and the analysis of small molecules  
471 (molecular formula comprised of less than 15 or 10 carbons)

472 Future research needs should be considered to strengthen micropollutant distribution and  
473 degradation trends in water treatment systems. For example, the results obtained in this  
474 study highlighted the independence of micropollutant distribution from the velocity field  
475 but were obtained on our models that did not considered, for example, transport of solids  
476 or abiotic transformations. Therefore, these assumptions could be considered in future  
477 research. Additionally, the results were only obtained for SFTW wetlands. To strengthen  
478 the conclusions observed in this kind of system, the results should be compared with  
479 those of different systems operating in different geographical areas with different flow  
480 rates.

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486

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491

## 492 **Author Contributions**

493 Loïc Maurer performed the CFD modelling, sample preparation, data analysis, prepared  
494 the figure, designed the research, and wrote the manuscript. Claire Villette performed  
495 LC-Q-TOF-HRMS/MS data acquisition and discussed the manuscript. Nicolas  
496 Reiminger, Xavier Jurado, and Julien Laurent participated in the CFD modelling and  
497 discussed the manuscript. Adrien Wanko discussed the manuscript. Maximilien Nuel  
498 monitored the water flow on the study site. Robert Mosé discussed the manuscript.  
499 Dimitri Heintz discussed the manuscript and designed the research.

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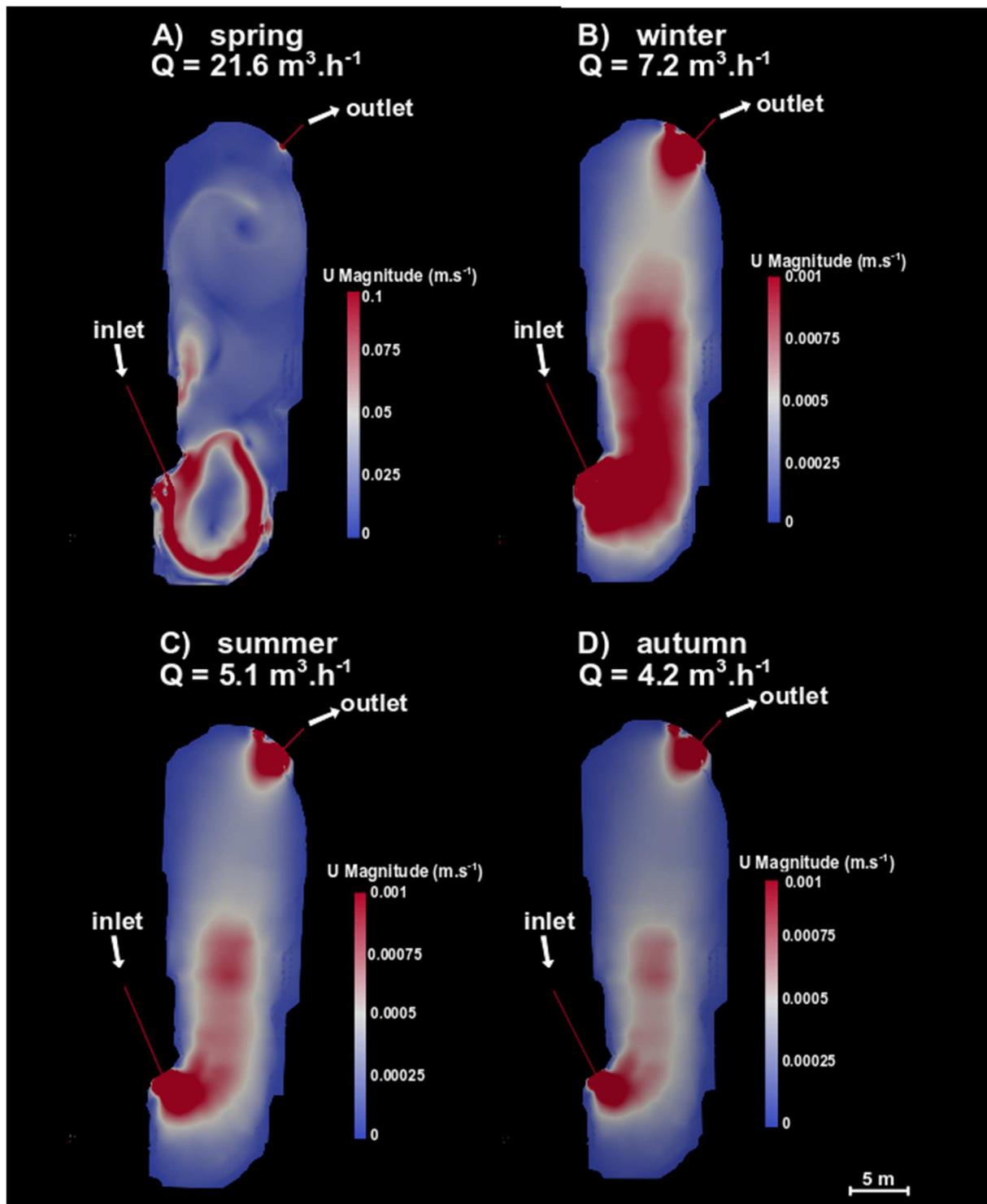
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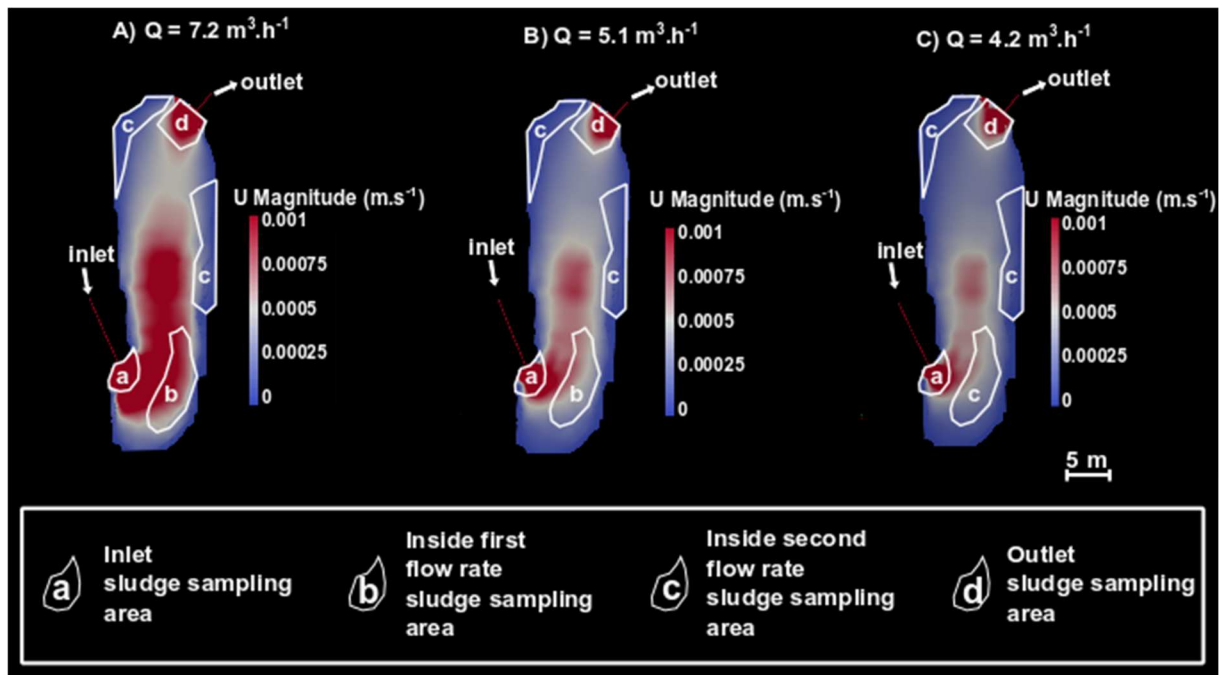
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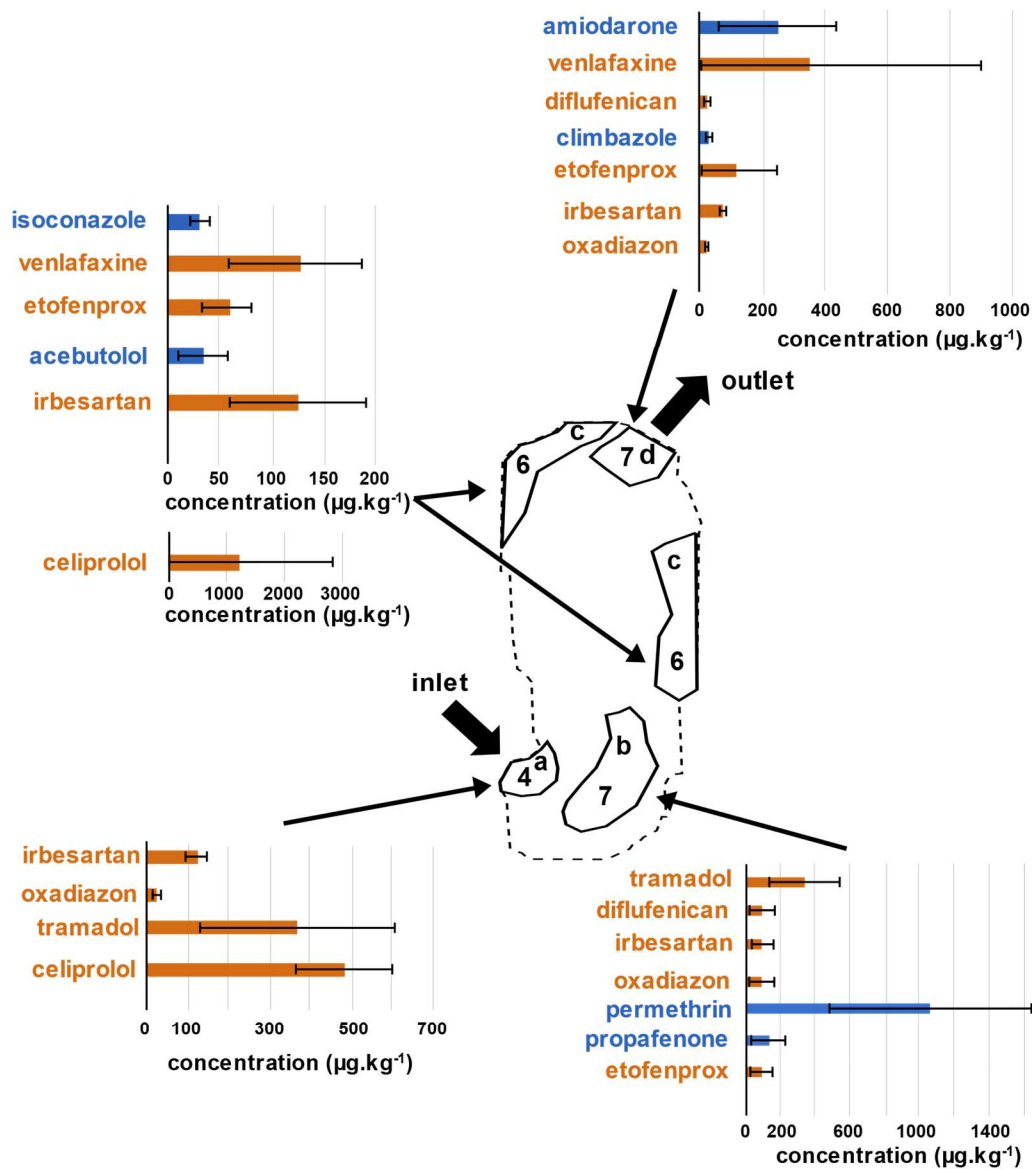


**Figure 1.** SFTW water flow in the different inlet flow rate conditions observed for each season. The water flow was simulated using the different conditions measured at the study site. The higher water flow monitored was approximately 21.6 m<sup>3</sup>.h<sup>-1</sup> and was simulated in case A (spring). The conditions the most representative of average flow rate were observed in cases B (winter with 7.2 m<sup>3</sup>.h<sup>-1</sup>), C (summer with 5.1 m<sup>3</sup>.h<sup>-1</sup>), and D (autumn with 4.2 m<sup>3</sup>.h<sup>-1</sup>).



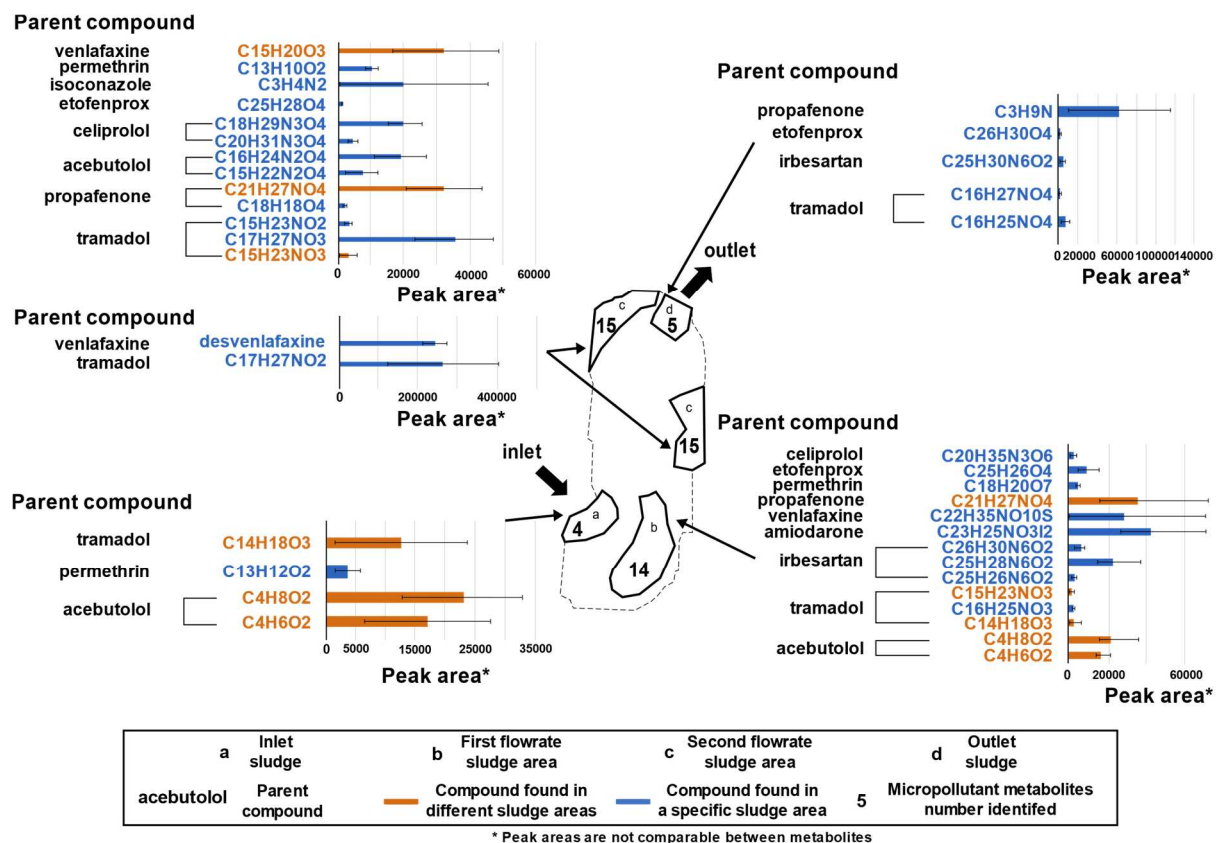


**Figure 2.** Water flow areas defining the sludge sampling strategy in the SFTW. Four areas based on similar water flow behaviour were selected to collect sludge. The sludge areas were chosen to represent faster and slower water flow areas inside and at the system limits. Thus sludge was sampled at inlet and outlet areas (a and d, respectively), in relatively lower flow areas (area c), and relatively higher flow areas (area b) inside the SFTW. A) Sampling strategy applied with an inlet flow rate of  $7.2 \text{ m}^3 \cdot \text{h}^{-1}$ . B) Sampling strategy applied with an inlet flow rate of  $5.1 \text{ m}^3 \cdot \text{h}^{-1}$ . C) Sampling strategy applied with an inlet flow rate of  $4.2 \text{ m}^3 \cdot \text{h}^{-1}$ . The spring simulation was not considered, as it was not representative of the average conditions.

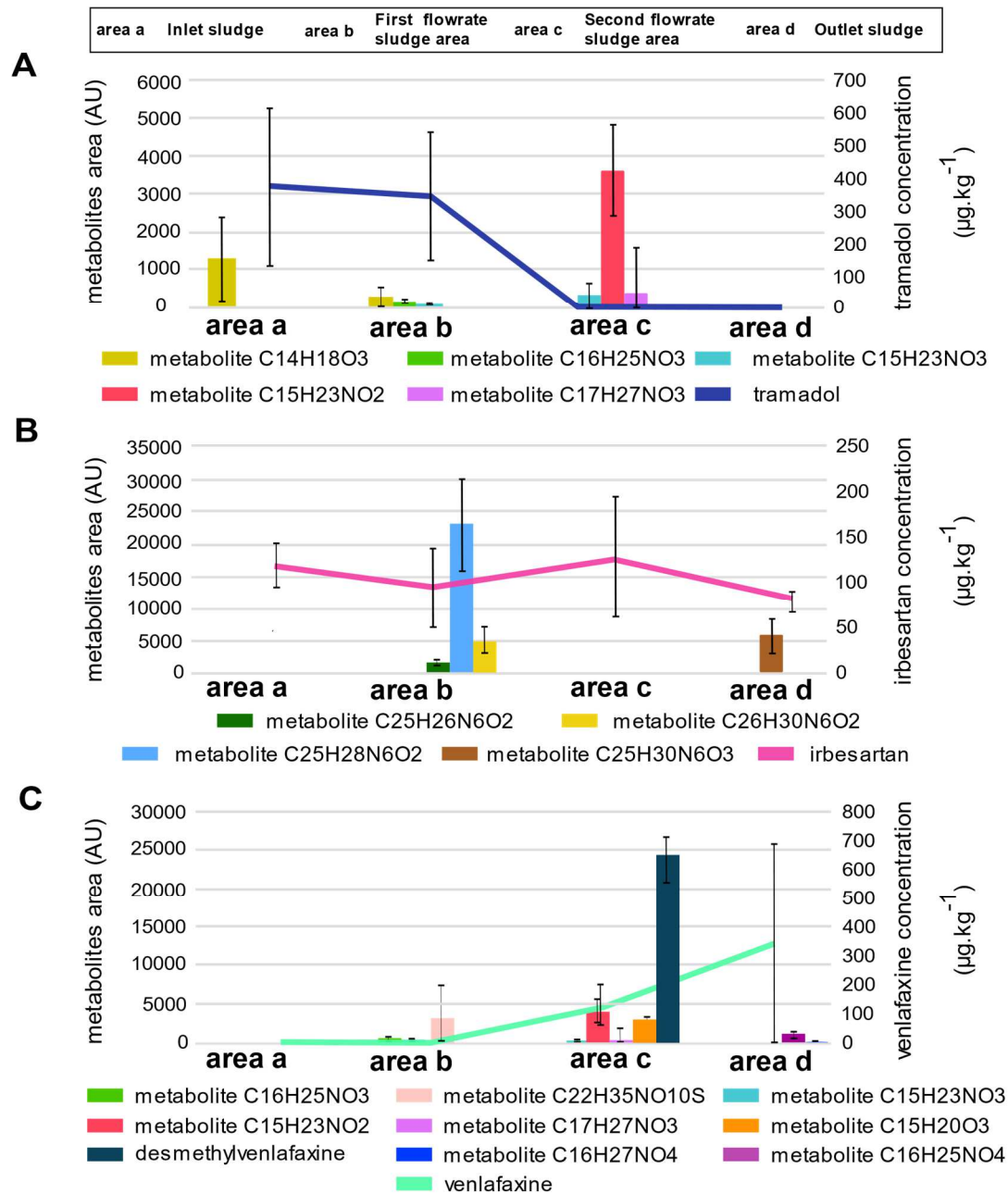


a Inlet sludge	b First flowrate sludge area	c Second flowrate sludge area	d Outlet sludge
Compound found in different sludge areas		Compound found in a specific sludge areas	
			7 Micropollutants number identified

**Figure 3.** Distribution and quantification of micropollutants found for all seasons in the different flow rate areas of the SFTW. Compounds quantified were found in triplicates in each season. The concentration displayed shows the average concentration of all the samples; the standard deviation shows the concentration variability throughout the seasons. Compounds found in different sludge areas (orange) were distinguished from those found specifically in a single area (blue).



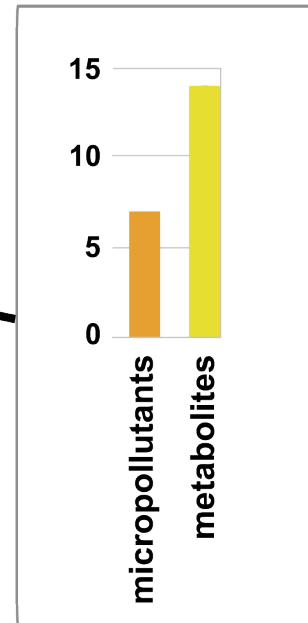
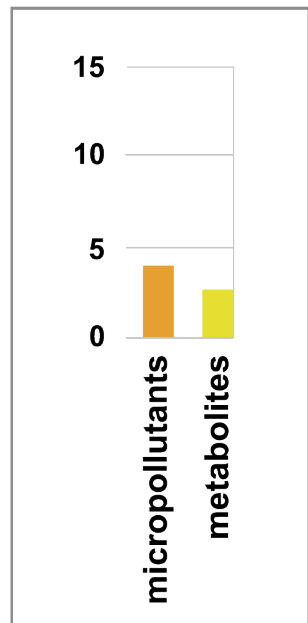
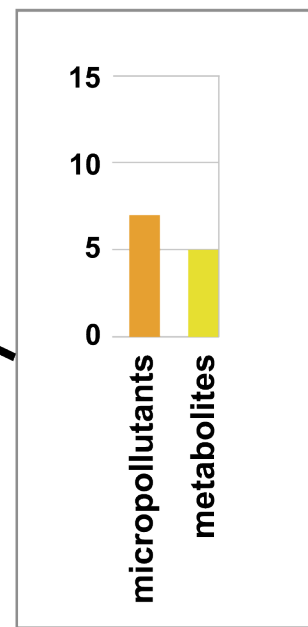
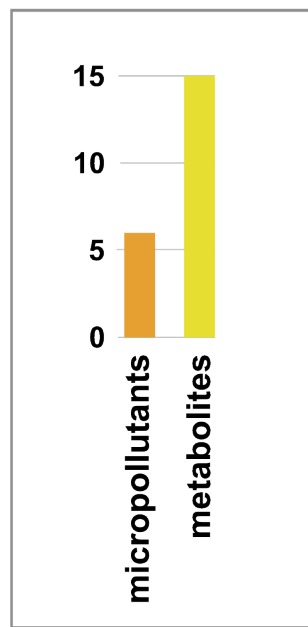
**Figure 4.** Distribution and intensity of micropollutants metabolites (catabolites and conjugated) found in each season in the different flow rate areas of the SFTW. The intensity displayed shows the average intensity of all the samples, and the standard deviation shows the intensity variability throughout the seasons. All the samples were analysed in triplicates for each season, and the standard deviation represents the intensity variation found through the seasons. Metabolites found in different sludge areas (orange) were distinguished from those found specifically in a single area (blue).



**Figure 5.** Distribution of tramadol, irbesartan, venlafaxine, and their metabolites in the different flow rate areas of the SFTW. A) Tramadol and its metabolites found in the different areas. B) Irbesartan and its metabolites found in the different areas. C) Venlafaxine and its metabolites found in the different areas. The concentration of parent compounds, found at least in two areas, is not correlated with the water flow areas. The metabolites of these compounds were primarily (higher intensity and diversity) found in the lower areas (b and c). All analyses were performed in triplicates for each season, and the standard deviation represents the concentration/intensity variation found through the seasons.

# Micropollutants & metabolites analysis

outlet



inlet

Water flow  
numerical  
modelling



low  
velocity



intermediate  
velocity



high  
velocity