



**HAL**  
open science

## Occurrence and removal of emerging pollutants in urban sewage treatment plants using LC-QToF-MS suspect screening and quantification

Laure Wiest, Antoine Gosset, Aurélie Fildier, Christine Libert, Matthieu Hervé, Elisabeth Sibeud, Barbara Giroud, Emmanuelle Vulliet, Thérèse Bastide, Philippe Polomé, et al.

### ► To cite this version:

Laure Wiest, Antoine Gosset, Aurélie Fildier, Christine Libert, Matthieu Hervé, et al.. Occurrence and removal of emerging pollutants in urban sewage treatment plants using LC-QToF-MS suspect screening and quantification. *Science of the Total Environment*, 2021, 774, pp.145779. 10.1016/j.scitotenv.2021.145779 . hal-03162034

**HAL Id: hal-03162034**

**<https://univ-lyon1.hal.science/hal-03162034>**

Submitted on 29 Sep 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Occurrence and removal of emerging pollutants in urban sewage treatment plants using LC-**  
2 **QToF-MS suspect screening and quantification.**

3 Laure Wiest<sup>1</sup>, Antoine Gosset<sup>2,3,4\*</sup>, Aurélie Fildier<sup>1</sup>, Christine Libert<sup>5</sup>, Matthieu Hervé<sup>5</sup>, Elisabeth  
4 Sibeud<sup>5</sup>, Barbara Giroud<sup>1</sup>, Emmanuelle Vulliet<sup>1</sup>, Thérèse Bastide<sup>1</sup>, Philippe Polomé<sup>3</sup>, Yves Perrodin<sup>2</sup>

5 <sup>1</sup> Univ Lyon, CNRS, Université Claude Bernard Lyon 1, Institut des Sciences Analytiques, UMR  
6 5280, 5 Rue de la Doua, F-69100 Villeurbanne, France

7 <sup>2</sup> Université de Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR5023 LEHNA, F-  
8 69518, Vaulx-en-Velin, France

9 <sup>3</sup> Université de Lyon & Université Lyon 2, Lyon, F-69007, France ; CNRS, UMR 5824 GATE Lyon  
10 Saint-Etienne, Ecully, F-69130, France

11 <sup>4</sup> Ecole Urbaine de Lyon, Institut Convergences, Commissariat général aux investissements d'avenir,  
12 Bât. Atrium, 43 Boulevard du 11 Novembre 1918, F-69616 Villeurbanne, France

13 <sup>5</sup> Greater Lyon Urban Community, Water and Urban Planning Department, 69003 Lyon, France

14 \*Corresponding author: [antoinegosset@hotmail.com](mailto:antoinegosset@hotmail.com)

15

16 Abstract

17 Urban wastewaters (WW) are a major vector of many emerging pollutants (EPs) to aquatic  
18 ecosystems, as urban wastewater treatment plants (WWTPs) are not designed to abate them. New  
19 methods are now critically necessary for a more comprehensive analysis of WW samples and for the  
20 assessment of the WWTP efficiency in EP removal. To this end, the present study aims to develop a  
21 methodology to identify and quantify EPs, especially pharmaceutical residues and pesticides, in the  
22 raw and treated wastewater of 10 heterogeneous WWTPs in a highly urbanized territory in France  
23 over three sampling campaigns, through the following steps: (1) development and implementation  
24 of a suspect screening of EPs in WW samples, based on a solid phase extraction followed by an LC-  
25 QToF-MS analysis; (2) confirmation of their identification by reinjection of WW samples spiked  
26 with authentic analytical standards; (3) quantification of previously identified compounds by targeted  
27 LC-QToF-MS analysis in raw and treated effluents and assessment of their removal efficiency by  
28 WWTPs. Forty-one EPs, including 37 pharmaceutical residues (such as anti-depressive, anti-  
29 hypertensive, or antipsychotic drugs) and 4 pesticides, were identified by suspect screening. Some  
30 of them (e.g. milnacipran) are reported for the first time in urban WWTPs in this study. High  
31 variability in detection frequency and concentrations were observed in function of the EP and  
32 WWTP. Nevertheless, median removal rates were considered negative or low for more than 50 % of

33 the EPs (respectively 4 and 17), leading to a quantification of significant concentrations in treated  
34 WW. Their release into receiving streams may thus lead to ecotoxicological risks that should be  
35 evaluated in order to prevent any degradation of the exposed ecosystems.

### 36 Key-words

37 Emerging pollutants; Wastewater; Chemical analysis; Suspect-screening; Pharmaceuticals;  
38 Removal efficiency

39

## 40 1. Introduction

41 Emerging pollutants (EPs), such as pharmaceutical residues, have now been widely observed in  
42 various aquatic compartments (e.g. streams, lakes, groundwaters) (Gavrilescu et al., 2015; López-  
43 Pacheco et al., 2019; Pinasseau et al., 2019; Vulliet and Cren-Olivé, 2011). Most of them are a great  
44 threat to communities of aquatic organisms, as they can cause significant acute and chronic ecotoxic  
45 effects at low concentrations (Orias and Perrodin, 2013; Gosset et al., 2017). Urban wastewaters  
46 (WW) are a major vector of many EPs to aquatic ecosystems, as urban wastewater treatment plants  
47 (WWTPs) are not designed to abate them (Deblonde et al., 2011). Ecotoxicological risks associated  
48 with their dispersion in receiving surface water have thus already been assessed and proved  
49 worldwide (Martín et al., 2012; Mohan and Balakrishnan, 2019; Verlicchi et al., 2012; Yan et al.,  
50 2014).

51 Historically, WWTPs have been designed to abate nutrients (nitrogen, phosphorus), particles,  
52 and carbonaceous substances (Luo et al., 2014). Removal of EPs through conventional treatment  
53 processes (for example, a pre-treatment step, followed by primary decantation and a biological  
54 degradation by conventional activated sludge or biofiltration) remains limited (Besha et al., 2017;  
55 Kümmerer et al., 2019; Palli et al., 2019). Advanced tertiary treatments (e.g., ozonation, activated  
56 carbon adsorption) have been developed to improve water quality by increasing micropollutant  
57 removal efficiency (Guillossou et al., 2019; Östman et al., 2019) although some may generate more  
58 ecotoxic by-products (Bertanza et al., 2013; Wigh et al., 2016).

59 Thus, a thorough assessment of pollutant removal by WWTPs is necessary to optimize treatment  
60 and to avoid the release of ecotoxic compounds into aquatic ecosystems (Luo et al., 2014). Most

61 studies are limited to a single WWTP and a small set of compounds, for example, based on the  
62 European priority list (described in the European Water Framework Directive, WFD, 2000), or  
63 according to their consumption and PBT (Persistence, Bioaccumulation, Toxicity) criteria (Krauss  
64 et al., 2019; Verlicchi et al., 2012; Wiest et al., 2018). Furthermore, as chemical concentrations and  
65 loads in influents and effluents are largely influenced by sewershed specificities (Krauss et al., 2019)  
66 and WWTP treatments, it is also necessary to study a set of WWTPs with different processes to  
67 obtain a more exhaustive view of micropollutant removal.

68 Moreover, tens of thousands of chemicals are registered for commercial use in Europe.  
69 Monitoring of small sets of micropollutants can overlook highly ecotoxic substances and lead to a  
70 bias in the final risk assessment for aquatic organisms. Analytical methods for EPs that are more  
71 comprehensive than the routinely used targeted methods are needed (Hug et al., 2014). In this  
72 context, high-resolution mass spectrometry is a promising tool that makes it possible to progress  
73 from the screening of one hundred to several thousand analytes (Brack et al., 2019). It is increasingly  
74 used to carry out the so-called "suspect screening" chemical analysis (Pinasseau et al., 2019;  
75 Ccanccapa et al. 2019; Wang et al., 2019). This kind of screening is based on the comparison of  
76 molecular characteristics of unknown components detected in the sample with databases of suspect  
77 compounds. The correspondence between these characteristics allows the identification of the  
78 compounds present. This approach has been developed and applied to identify different families of  
79 EPs such as pharmaceuticals, pesticides, surfactants, industrial chemicals (e.g. chemical synthesis  
80 intermediates, additives) and their degradation products/metabolites in wastewaters (Deeb et al.,  
81 2017; Gros et al., 2017; Hug et al., 2014; Singer et al., 2016; Wang et al., 2019). This strategy was  
82 also applied to evaluate the efficiency of WWTP treatments to remove micropollutants, but only  
83 based on the comparison of relative peak areas between the WWTP treated and untreated effluents  
84 (Deeb et al., 2017; Wang et al., 2019). The qualitative data did not allow the calculation of precise  
85 removals, given the differences in matrix complexity of the two types of water. The combination of  
86 suspect screening with confirmation and quantification of identified compounds using analytical  
87 standards could give a more exhaustive and comprehensive WWTP efficiency assessment, and load  
88 emission evaluation in receiving waters. To our knowledge, up to now, this strategy has only been

89 used in the work published by Gros et al (2017) on grab samples of four WWTPs in Sweden,  
90 analyzing 1300 pesticides, pharmaceuticals, and personal care products (PPCPs), fire retardants  
91 (FRs) and polyfluoroalkyl substances (PFASs).

92 Consequently, the main objectives of the present work were to: (1) develop and apply a  
93 quantitative suspect screening of pharmaceutical residues and pesticides (~2000 substances) in urban  
94 wastewater from ten WWTPs, based on LC-QToF-MS; (2) study the advantages and limits of the  
95 suspect screening approach with regard to previous monitoring based on targeted analyses (3) thanks  
96 to these data, determine the occurrence in raw and treated WW of the identified compounds,  
97 including low studied substances; (4) and finally assess their removal efficiency.

## 98 2. Materials and Methods

### 99 2.1. Standards and chemicals

100 Chemical standards (targeted compounds and labelled internal standards) used for  
101 confirmation and quantification are given in Table S1. Their purity was up to 99 %. Ultra-pure water  
102 (Milli-Q) was supplied from Fischer and methanol from BioSolve (Dieuse, France). Individual stocks  
103 of standard solutions were prepared at 800 mg/L in methanol, and internal standards at 250 mg/L.  
104 Standard solutions were stored in the dark at  $-18\text{ }^{\circ}\text{C}$ . Working solutions were prepared in ultra-pure  
105 water, stored at  $4\text{ }^{\circ}\text{C}$  and renewed monthly.

### 106 2.2. Studied sites and sampling procedure

107 In the present work, selected WWTPs are located in the highly urbanized Lyon (France) city  
108 area and sub-urban municipalities, called “Grand Lyon”. In a previous study, Gosset et al. (2020)  
109 identified 33 WWTPs releasing effluents in freshwater creeks in this region. In this study, 10 were  
110 selected based on 5 main criteria: variability of pollution sources, large range of flow rates of WWTP  
111 influent/effluent and receiving watercourse (Brus an Perrodin, 2017), diversity of WWTP treatments,  
112 work on a highly anthropized surrounding region (leading to a potentially high-risk context), and  
113 finally accessibility and equipment for sampling and monitoring. Their location and main

114 characteristics (population connected, incoming annual flow rate, treatments) are provided in Figure  
115 1 and Table 1, respectively. WWTP daily average incoming flow rates vary from 157 to 215092  
116 m<sup>3</sup>/day, with a fairly good correlation with the incoming pollution load (3025 to 622800 population  
117 equivalent (PE)). Only WWTP 3 collects exclusively WW from an industrial area. All the others  
118 receive a variable proportion of industrial effluents, between 0.11 and 32.11 %. Regarding WWTP  
119 7, 8, 9 and 10, hospital effluents are also connected to the municipal network. All WWTPs are  
120 equipped with three pretreatment systems: a screening, a grit chamber and a grease remover. Three  
121 main primary treatments (coagulation-flocculation, sedimentation tank, buffer tank) and three  
122 secondary treatments (activated sludges, biofilter, radial flow fluidized filter) are employed, in  
123 function of the WWTP. Finally, an advanced tertiary treatment based on a biofiltration (Biostyr®) is  
124 implemented in WWTP 10, to reduce suspended solids, carbon and nitrogen pollution.

125 Influent and effluent WW were sampled in May, October/November and December 2019,  
126 to take into account the temporal variability of micropollutant discharges and climatic changes during  
127 the year. These three sampling periods are referred to as C1, C2 and C3 throughout the article. Two  
128 of them were carried out in dry weather conditions (C1 and C3), and one (C2) was performed in low  
129 wet weather conditions ( $\leq 2.3$  mm rainfall). As it was not possible to carry out all samplings on the  
130 same day, they were carried out over a period of less than 10 days. Influent samples were collected at the  
131 inlet of the treatment plant, at the pretreatment inlet or outlet, depending on WWTP (see Table 1).  
132 Effluents were obtained after secondary treatment, except WWTP 10 for which there is a tertiary  
133 treatment. Composite 24-h samples (starting and ending at 8 a.m.) according to the flow rate were  
134 collected with the same sampling strategy for all WWTPs to ensure representative sampling. WW  
135 was sampled using refrigerated automatic samplers with high-density polyethylene containers. Just  
136 after sampling, 1 L of each influent/effluent was directly transferred in a 1-L brown glass bottle for  
137 EP analyses. Three 250-mL brown glass bottles were also filled for conventional physico-chemical  
138 analyses. Then, they were transported to the laboratory in an icebox (dark conditions) and treated for  
139 chemical analyses within the 8 following hours.

140  
141 2.3. Conventional physico-chemical analyses

142 Conventional physico-chemical parameters (such as pH, conductivity, Total Suspended Solids  
143 (TSS), Chemical Oxygen Demand (COD)) and anion/cation concentrations ( $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  
144  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ ) were quantified according to European standards, as described  
145 by Perrodin et al. (2016). Anion/cation analyses were performed after filtering the samples through  
146  $0.45\ \mu\text{m}$  (dissolved fraction) while the other parameters were measured on the whole effluents.

## 147 2.4. Extraction and analysis of EPs

### 148 2.4.1. Sample preparation and extraction

149 Sample preparation and extraction were conducted based on the method described in Wiest  
150 et al. (2018), used for targeted analysis. After a filtration on  $0.7\ \mu\text{m}$  glass fiber filters with a filtration  
151 System (IT30 142 HW) from Millipore (Molsheim, France), 1 mol/L citric acid solution was added,  
152 and 1200/500  $\mu\text{L}$  of a 2 mg/L solution of 20 deuterated internal standards were diluted into 600/250  
153 mL of the sample, for effluent/influent respectively. Samples were passed through an automated  
154 Solid Phase Extraction (SPE) system (AutoTrace™ 280, from Thermo Fisher®, Roissy, France) in  
155 duplicates, using Oasis HLB™ cartridges (6 mL, 150 mg) from Waters® (Guyancourt, France). 250  
156 mL/100 mL of filtered WWTP effluents/influents were loaded. Then, cartridges were rinsed with  
157 ultrapure water, dried with nitrogen and eluted with methanol. Eluates were dried under nitrogen (at  
158  $40\ ^\circ\text{C}$ ) and samples stored at  $-18\ ^\circ\text{C}$ . Just before injection, samples were suspended in 1 mL of 90/10  
159 ultrapure water/methanol.

### 160 2.4.2. LC-QToF-MS suspect screening and quantification

161 The general analytical strategy is presented in Figure 2. It comprises suspect screening,  
162 confirmation and quantification of EPs by Liquid Chromatography coupled with High Resolution  
163 Mass Spectrometry. Samples from May 2019 (C1) were used to carry out the suspect screening  
164 analysis to identify and confirm compounds. Then, confirmed substances were quantified in all WW  
165 samples (C1, C2, and C3).

166 The suspect screening analysis was done on the first extract of C1 following the protocol  
167 derived from Pinasseau et al. (2019). Separation and detection were performed using an Ultimate

168 3000 Ultra High Performance Liquid Chromatography (UHPLC) system from Thermo Scientific®  
169 (MA, USA) coupled with a Quadrupole Time of Flight mass spectrometer from Bruker Daltonics®  
170 Maxis Plus. Analyses were carried out in reverse phase (elution gradient) employing an Acclaim  
171 RSLC 120 C18 column (2.2  $\mu\text{m}$ , 100 x 2.1 mm, Thermo Scientific®), protected with a KrudKatcher  
172 Ultra In-Line Filter guard column from Phenomenex (Torrence, CA, USA) and heated at 30 °C. The  
173 injected volume was 5  $\mu\text{L}$ . Mobile phases consisted of: an aqueous phase (90%/10% ultrapure  
174 water/methanol mixture with 5mM ammonium formate and 0.01% formic acid) and an organic phase  
175 (methanol with 5mM ammonium formate and 0.01% formic acid). More details on gradient elution  
176 and mass spectrometer calibration are available in Pinasseau et al. 2019. For quality assurance and  
177 quality control (QA/QC), several laboratory control samples were performed. The accuracy of the  
178 mass detector was checked at the start of each cycle and recalibrated if the mass error was more than  
179 0.5 ppm. Every 12 injections, a quality control was injected to verify sensitivity and retention times  
180 during data acquisition. A mixture of 10  $\mu\text{l}$  of each extract was prepared and spiked to 500  $\mu\text{g/L}$  with  
181 a standard solution of 53 compounds (Table S2). These compounds were also used to adjust retention  
182 times in the database. Background signals were identified by analysis of blanks (solvent, procedural,  
183 and trip blank). Solvent blanks were also injected to monitor column carryover. All extracts were  
184 analyzed in positive electrospray ionization with the following settings: capillary voltage of 3600 V,  
185 end plate offset of 500 V, nebulizer pressure of 3 bar ( $\text{N}_2$ ), drying gas of 9 L/min ( $\text{N}_2$ ), and drying  
186 temperature of 200 °C. The analysis was performed in broadband Collision Induced Dissociation  
187 acquisition mode over the mass range of 80-1000 Da with a scan rate of 2 Hz at 40eV. With this Data  
188 Independent Acquisition mode (DIA), all compounds are fragmented in the collision cell, without  
189 prior ion selection. Data were acquired with OtofControl 4.1 and Hystar 4.1, Bruker Daltonics®  
190 software and processed using TASQ (Target Analysis for Screening and Quantitation) software  
191 (version 1.4, Bruker Daltonics®).

192 All detected signals, couples of exact mass and retention time ( $m/z$ ;  $t_R$ ), were compared with  
193 two databases: PesticideScreener 2.1 and ToxScreener 2.1 (Bruker Daltonics®). These databases  
194 contain exact masses, retention time, isotope pattern and fragments of 1200 pesticides and 800  
195 pharmaceutical compounds, respectively. To perform the suspect screening, tolerances on



196 identification criteria (exact mass, retention time, isotope pattern and fragments) were determined in  
197 relation to those present in the databases. The filtering strategy of the substances identified by the  
198 software was already described in Pinasseau et al. 2019. Briefly, for each identified substance and  
199 each identification criteria, a score, high [H], medium [M] or low [L], was determined as follow:  
200  $\Delta m/z$  (mDa) <1.5 [H], between 1.6 and 2.5 [M]; > 2.5 [L];  $\Delta t_R$  (min)  $\pm$  0.25 [H],  $\pm$  0.40 [M],  $\pm$  0.50  
201 [L]; mSigma (correlation between the isotope pattern of the expected spectra and the experimental  
202 spectra)  $\leq$  25 [H], between 25 and 60 [M] > 60 [L]. And the last criteria was based on the detection  
203 (S/N >3) of a minimum of 50% of the fragments with tolerance on exact mass precision  $\Delta m/z$  < 20  
204 ppm. Only compounds for which the scores of the three criteria were high ([H]) and 50% of the  
205 fragments were detected were considered for confirmation, leading to a list of 41 compounds.

206 After the supposed identification, to confirm the suspected features, C1 sample extracts were  
207 further spiked with analytical standards of the suspected compounds and were injected with the  
208 unspiked C1 sample extracts on the same apparatus and in the same analytical conditions as for  
209 suspect screening. Compounds were spiked in the final extract of samples at concentrations between  
210 100 and 500  $\mu\text{g/L}$ , depending of the sensitivity of the analyte. Fragmentation in Data Dependent  
211 Acquisition mode (DDA) using a selection of the exact mass of the suspected compounds. Then, the  
212 Extracted Ion Chromatogram (EIC) and the MS/MS spectra of the suspected compounds for spiked  
213 and unspiked sample extracts were compared (see an example in Figure S1). In some cases, initial  
214 spiked concentration was not enough and spiked extracts with higher concentrations were re-injected  
215 until enough sensitivity was obtained to compare spiked and unspiked spectra. The tolerance on the  
216 identification criteria were the same as in the suspect screening. If needed, detected fragments were  
217 further checked using MassBank. Confirmed substances and their monoisotopic masses and retention  
218 times are reported in Table S3.

219 Finally, quantification by internal standard calibration was performed in DIA mode, on the  
220 second extract of C1, and on the C2 and C3 extracts, using 20 internal standards. Method limits of  
221 quantification (LOQ) for influent and effluent WW, as well as internal standards used for  
222 quantification, are summarized in Table S4. LOQ was the lowest concentration for which all the  
223 identification criteria were respected and the accuracy was higher than 80%. Lower LOQ were

224 obtained regarding WWTP 3, because its WW was less complex, leading to lower matrix effects.  
225 LOQ for this WWTP are also reported in Table S4.

### 226 2.4.3 LC-MS/MS confirmation

227 Due to the lack of sensitivity for some fragments, eight drugs were difficult to confirm by  
228 LC-QToF MS, especially in influent WW: amantadine, gabapentin, sulfamethoxazole, ketoprofen,  
229 oxazepam, fluconazole, celiprolol and rosuvastatin (see section 3.2). For these substances, LC-  
230 MS/MS analysis was performed only for confirmation, using the following procedure.  
231 Chromatographic separation was carried out with an H-Class liquid chromatograph system  
232 (Waters®, Milford, MA, USA), using a Kinetex C18 column (50 mm×2.1 mm i.d., 2.6 μm), protected  
233 with a KrudKatcher Ultra In-Line Filter guard column from Phenomenex® (Torrence, CA, USA).  
234 The column oven temperature was set at 40 °C. Mobile phases consist in water with 0.1 % formic  
235 acid and methanol. Flow rate was 0.45 mL/min and the sample volume injected was 2 μL.

236 The LC instrument was coupled to a Xevo TQ-S (Waters®) triple quadrupole mass  
237 spectrometer equipped with an electrospray ion source operated in positive mode. Analysis of the  
238 target compounds was achieved in Multiple Reaction Monitoring (MRM) mode. Two or three MRM  
239 transitions were monitored for each targeted compound. Retention time and MRM ratio were used  
240 to confirm the identification of the substance in the samples (Table S5). All data were acquired and  
241 processed using Masslynx 4.1 software.

### 242 2.5. Pollutant mass loads and removal assessment

243 Daily pharmaceutical and pesticide mass loads were calculated using their concentrations in  
244 the influent or effluent, and the associated wastewater flow rate at the inlet or outlet, employing the  
245 following equation (1):

$$246 \quad DML_x = \frac{Conc_x \times (FR \times 1000)}{10^9}$$

247 (1)

248 Where  $DML_x$  is the daily mass load of the pollutant  $x$  in the influent or effluent, in g/day;  $Conc_x$  is  
249 the concentration of the pollutant  $x$  in the raw or treated WW, in ng/L; and  $FR$  is the flow rate at the  
250 inlet or outlet in  $m^3/day$ .

251 Finally, the removal rate for each WWTP, pollutant and period was obtained by the  
252 equation (2):

$$253 \quad RR = \left(1 - \frac{DML_{effluent}}{DML_{influent}}\right) \times 100$$

254 (2)

255 Where  $RR$  is the removal rate in %;  $DML_{effluent}$ , the daily mass load in the effluent, in g/day;  
256  $DML_{influent}$ , the daily mass load in the influent, in g/day. Calculating  $RR$  when EPs are not detected  
257 or quantified can lead to errors. Consequently, in case of EPs below their respective LOQ (<LOQ)  
258 or non-detection (n.d.) into effluent samples, their concentrations were respectively fixed at LOQ/2  
259 and 0 ng/L in order to calculate removal efficiencies. On the contrary, when one of these two cases  
260 appeared in the influent samples, the removal calculation was not carried out. Finally, when EPs were  
261 below their LOQ in both WWTP's inlet and outlet samples, they were not calculated.

### 262 3. Results and discussion

#### 263 3.1. Conventional physico-chemical parameters

264 In order to ensure the correct performance of the WWTPs, and so that the results of the study  
265 can be compared with the scientific literature, several conventional physico-chemical parameters  
266 were monitored during the three campaigns. Their minimum, median, mean, maximum values and  
267 frequency of detection for all 30 samples are reported in Table 2. A similar range of values was  
268 observed for the different influent and effluent parameters in various studies performed on French  
269 WWTPs (Deycard et al., 2014; Fulazzaky et al., 2015; Gasperi et al., 2012; Pasquini et al., 2014;  
270 Wiest et al., 2018). Total suspended solids (Mean = 270.80 mg/L) and chemical oxygen demand  
271 (Mean = 588.37 mg/L) are relatively high and typical of highly urbanized territories. Effluent  
272 parameters, and in particular TSS and COD, of the 10 WWTPs respect the stricter regulations of the

273 French Order (N°223 09/23/2017) on collective sewerage systems: values respectively below 35  
274 mg/L and 125 mg O<sub>2</sub>/L (Pasquini et al., 2014). Moreover, the mean removal rate by WWTPs for  
275 these two parameters is respectively 94.47 % and 92.37 %, while this Order imposes an efficiency  
276 of up to 90 % and 75 % for the largest WWTPs (BOD<sub>5</sub> > 1,2 kg/day). These values show the normal  
277 and efficient functioning of the studied WWTPs during the three sampling campaigns. Accordingly,  
278 the removal rates for the various detected EPs can be considered as typical and representative of the  
279 10 WWTP's efficiency (Pasquini et al., 2014).

### 280 3.2. Advantages and limits of the quantitative suspect-screening approach

281 LC-QToF-MS analyses performed in this study are summarized in Figure 2. In order to be  
282 sure to publish reliable data and to avoid any false positive, we chose to study only EPs that were  
283 confirmed by comparison with their corresponding analytical standard, that is with a confidence of  
284 level 1 according to Schymanski et al. (2014). Thanks to this strategy, 41 EPs were ultimately  
285 confirmed and quantified by internal standard calibration (see Tables S2 and S3). The same  
286 identification criteria were used for suspect screening and quantification. During the quantification  
287 step, strictly following these criteria, eight **compounds** (amantadine, gabapentin, sulfamethoxazole,  
288 ketoprofen, oxazepam, fluconazole, celirolol and rosuvastatin) were not successfully identified in  
289 raw WW, whereas they were identified in the corresponding treated WW. The unfulfilled criteria  
290 was the detection of fragments, due to a high level of noise in chromatograms of raw WW. An  
291 example of the obtained chromatograms for fluconazole is presented in Figure S2. For these  
292 substances, an additional LC-MS/MS analysis (see Section 2.4.3) of the sample extract was  
293 performed, which allowed the confirmation of the presence of these compounds in raw WW.

294 Among the 41 EPs identified in the raw and treated WW samples, 37 pharmaceuticals and 4  
295 pesticides (DEET, diuron, fluopyram and terbutryn) were observed. To illustrate the usefulness of  
296 suspect screening to detect **substances** that have been poorly/not studied in the literature, Figure 3  
297 represents the number of scientific studies with a topic in relation to each EP detected here and  
298 wastewater or specifically urban/municipal wastewater (Web of Knowledge search, last access: 04-  
299 01-2020). Among the EPs, some of them, such as diclofenac (a nonsteroidal anti-inflammatory drug),

300 carbamazepine (anticonvulsant), sulfamethoxazole or trimethoprim (antibiotics) have already been  
301 widely reported and quantified in influents and effluents, and reviewed for many WWTPs worldwide  
302 (Couto et al., 2019; Deblonde et al., 2011; Luo et al., 2014). The name of each of them appears in  
303 more than 700 studies (Figure 3). On the contrary, this graph clearly highlights a low number of  
304 studies (<10) for 9 of them considering all types of wastewaters: trospium (overactive bladder  
305 treatment), milnacipran (serotonin–norepinephrine reuptake inhibitor), tiapride (neuroleptic),  
306 fluopyram (fungicide), flecainide (antiarrhythmic agent), methocarbamol (muscle relaxant),  
307 celiprolol (beta blocker), disopyramide (antiarrhythmic drug) and sitagliptin (antidiabetic drug). For  
308 specific municipal/urban wastewater, 19 of the EPs (46%) can be considered as poorly  
309 investigated/evoked. Finally, no previous studies were found for milnacipran. This shows that  
310 suspect screening not only allows to detect non-targeted substances, as in Singer et al. (2016), but  
311 also to identify not yet unsuspected or investigated ones in wastewater. Hug et al. (2014) reached to  
312 a similar conclusion, detecting six EPs never reported as pollutants previously, by suspect-screening  
313 in urban wastewater effluents. Additionally, some recent studies have also drawn the same findings  
314 for other urban discharges, such as stormwater or highway runoffs (Du et al., 2017; Pinasseau et al.,  
315 2019). As a result, it is clear that the scientific community is still far from having identified all the  
316 pollution linked to wastewater and therefore the associated environmental risks. The suspect-  
317 screening approach developed in this work is an efficient tool in the attempt to fill this knowledge  
318 gap.

### 319 3.3. Occurrence and concentrations of emerging pollutants in raw wastewaters

320 In order to study the contamination of raw wastewater by the 41 EPs, to compare it with  
321 other studies/countries and to discuss their potential sources, their concentrations and occurrences  
322 were monitored in influents of all treatment plants over the three sampling periods. The box plot and  
323 data synthesis of EP concentrations are presented in Figure 4 and Supplementary Table S6. Among  
324 the 37 pharmaceutical residues identified, 12 (atenolol, benzoylecgonine, carbamazepine, cetirizine,  
325 codeine, fexofenadine, flecainide, irbesartan, sitagliptin, trimethoprim, valsartan, venlafaxine) were  
326 detected in all samples, 29 had a detection frequency greater than or equal to 90% and 36 exceeded

327 70%. There is therefore a widespread contamination of the influent samples by pharmaceutical  
328 residues. Only one analyte, the antidepressant milnacipran, was poorly detected (36.67%). The  
329 highest median concentrations were found for gabapentin (5068.81 ng/L), valsartan (2823.46 ng/L),  
330 sitagliptin (2298.62 ng/L) and naproxen (1941.76 ng/L). On the contrary lower ones were determined  
331 for cetirizine (113.82 ng/L), trospium (92.99 ng/L), disopyramide (46.42 ng/L) and clopidogrel  
332 (27.68 ng/L). In 2012, Verlicchi et al. carried out a review mostly of European studies about the  
333 contamination of wastewater by 118 pharmaceuticals, and in particular 13 detected here. The  
334 concentration ranges for atenolol, bisoprolol, carbamazepine, celiprolol, codeine, diclofenac,  
335 gabapentin, ketoprofen, naproxen, sulfamethoxazole, trimethoprim and valsartan were similar to  
336 ours, although there were some variations in the average concentrations (e.g. lower in the present  
337 case for trimethoprim and carbamazepine; higher for celiprolol). More recently, three studies (Burns  
338 et al., 2018; Gurke et al., 2015; Saussereau et al., 2013) provided complementary data about  
339 contamination of English, German, and French WWTP influents by cetirizine, citalopram,  
340 disopyramide, fexofenadine, flecainide, irbesartan, lidocaine, oxazepam, sitagliptin, telmisartan,  
341 venlafaxine and verapamil. The same range of values held for all EPs, except for valsartan (mean  
342 conc.: 29685 ng/L; Gurk et al., 2015), oxazepam and sitagliptin (mean conc.: 22.7-37.2 ng/L and  
343 187-742 ng/L respectively; Burns et al., 2018). Average valsartan concentrations are 10 times lower  
344 in our study, while they are approximately 100 and 10 times higher for oxazepam and sitagliptin,  
345 respectively. Benzoylcegonine was the only detected metabolite of an illicit drug, cocaine (but also  
346 used in a drug for muscle pain). Nefau et al. (2013) performed a complete wastewater contamination  
347 study for 25 WWTPs across France, and observed a detection frequency above 80 % (100 % here),  
348 with concentrations varying between 21 and 3050 ng/L, which is in accordance with the present study  
349 (48.8 - 2140.63 ng/L). Finally, as already expressed in part 3.2, no comparison with literature was  
350 possible for some EPs, such as methocarbamol, milnacipran, tiapride and trospium, as to our  
351 knowledge no data on urban raw WW were reported in any study before the present one.

352         Among the detected pharmaceutical residues, only 5 of them (atorvastatin (med. conc.: 207  
353 ng/L), bisoprolol (389.28 ng/L), codeine (617 ng/L), diclofenac (1115.7 ng/L) and lidocaine (364.61  
354 ng/L)) are in the list of the 30 most sold (in quantity) in French cities (ANSM, 2014). Moreover,

355 Figure 4 shows that these compounds do not present concentrations among the highest of the 37  
356 pharmaceutical residues. Several factors may explain this statement:

357 - The difference in the dosage of active substances according to drug formulations: some  
358 drugs are less prescribed but contain higher doses of active substance, such as gabapentin (100 to  
359 800 mg tablets), valsartan (40 to 160 mg) or naproxen (550 mg), compared to bisoprolol (1.25 to 10  
360 mg) and atorvastatin (10 to 80 mg) (Vidal, 2020).

361 - The metabolization of drugs in the human body prior to their excretion, creating metabolites  
362 that are not in the suspect screening database (Miège et al., 2006).

363 - The differences in capability of some pharmaceuticals to be adsorbed on wastewater  
364 particles (sewage sludges/biosolids) (Archer et al., 2017).

365 - The differences in the biodegradation of pharmaceutical compounds in the sewers before  
366 the WWTP inlets. Laquaz et al. (2020) observed for example on a 2.7 km long sewer some decreases  
367 (or increases depending on the sampling campaign) of diclofenac, atenolol and ketoprofen  
368 concentrations between upstream and downstream sites. In the present case, more than 3200 km of  
369 sewer pipes convey the urban WW to the 10 WWTPs of the territory.

370 - The influence of demographic and socio-economic parameters (e.g. mean age, mean salary,  
371 presence of hospitals or factories) of the 10 WWTP sewersheds. These factors influence drug  
372 consumption, and then the mass load emitted (Choi et al., 2019), the final concentrations in the  
373 various sewers, and finally, the median concentrations presented here.

374 Concerning pesticides, three of them were highly detected into the influents ( $\geq 80\%$ ): diuron  
375 (med. Conc.= 19.46 ng/L), terbutryn (23.99 ng/L) and DEET (295.07 ng/L). These results are in  
376 accordance with European scientific literature. For example, Gasperi et al. (2008) detected diuron in  
377 all their wastewater samples from Paris, France, with a concentration range of 0.03-0.47  $\mu\text{g/L}$   
378 (present case: < LOQ - 1.43  $\mu\text{g/L}$ ). Conversely, they did not detect terbutryn, which is in  
379 contradiction with this study, and can be explained by a LOQ higher (60 ng/L) than the current one.  
380 Moreover, Köck-Schulmeyer et al. (2013) also observed a diuron median concentration of 42.2  
381 ng/L (detection frequency: 88%) in the influent of Spanish WWTPs. The omnipresence of these two  
382 herbicides in urban wastewater can be surprising because they have been banned in French

383 agriculture since 2003 (terbutryn) and 2008 (diuron). Their current source mainly lies in their use as  
384 algicides in surface coatings (paints and renders) of walls and roofs of urban buildings (Bollmann et  
385 al., 2014; Gros et al., 2017; Tlili et al., 2017). Conversely, the high detection frequency (93.3%) of  
386 DEET mosquito repellent is consistent with the literature, as it is the most used mosquitoes repellent  
387 in the world, and one of the most commonly detected organic contaminants in aqueous matrices (e.g.  
388 wastewater and surface water) (Merel and Snyder, 2016). Moreover, associated concentrations in  
389 influents are in the wide range of values observed in Europe and Worldwide (Dos Santos et al., 2019;  
390 Tran et al., 2018). Finally, fluopyram was detected in only 6 raw WW samples (WWTPs 3, 8 and 10;  
391 detection freq. = 20 %), with a high median concentration (529.36 ng/L). The presence of fluopyram  
392 in urban wastewater is surprising because of its main use as a fungicide in agriculture. Its detection  
393 could be explained by the presence of cereal crops in the sewershed areas of WWTPs 8 and 10, and  
394 the presence of a pesticide factory in that of WWTP 3 (entirely industrial). Nevertheless, the current  
395 results are consistent with a recent study that detected fluopyram in urban stormwater from an  
396 industrial area (containing several small crops) in the same conurbation (Pinasseau et al., 2019).

#### 397 3.4 Overall removal assessment of emerging pollutants by WWTP treatments

398 To assess the removal of EPs and the potential correlation with their structures and physico-  
399 chemical properties, removal rates (RR) of the 41 EPs were calculated, as detailed in section 2.5.  
400 Figure 5 represents the removal efficiency of each identified pollutant for all WWTP and sampling  
401 campaigns. All the associated raw data are provided in Supplementary data. The overall removal  
402 corresponds to the loss of EP parent compounds from the aqueous phase of WWs (Luo et al., 2014).  
403 A strong variation was observed between the 41 compounds (from -96.7 % median removal rate  
404 (MRR) for clopidogrel to 92.9 % for benzoylecgonine), including between substances of the same  
405 therapeutic family (-9.5 % and 59.2 % for irbesartan and valsartan, respectively – 2 antihypertensive  
406 drugs), as already reported (Campo et al., 2013; Gurke et al., 2015; Tran et al., 2018). Four different  
407 MRR trends, following the classification of Tsui et al. (2014), were observed depending on the EP  
408 (Table 3):



409 (1) An important/high median removal rate (MRR > 70 %), for 7 EPs: benzoylecgonine,  
410 telmisartan, naproxen, gabapentin, acebutolol, ketoprofen and fexofenadine. Two compounds still  
411 sometimes present low or negative elimination rates (gabapentin and benzoylecgonine). The best  
412 removal rates were obtained for benzoylecgonine (a metabolite of cocaine, which is also used as  
413 analgesic) and telmisartan (MRR: 92.9 and 90.7 %, respectively). Acebutolol, fexofenadine and  
414 telmisartan presented removal rates higher than those reported in the literature, whereas the others  
415 exhibited values in accordance with previous studies on conventional (secondary treatment : mainly  
416 activated sludges or membrane biological reactors) WWTPs (Table 3 - Archer et al., 2017; Burns et  
417 al., 2018; Couto et al., 2019; Deblonde et al., 2011; Golovko et al., 2014; Gurke et al., 2015; Luo et  
418 al., 2014; Repice et al., 2013; Santos et al., 2013; Saussereau et al., 2013; Tran et al., 2018; Yadav et  
419 al., 2019). The main mechanisms for the removal of pollutants are biotransformation/biodegradation,  
420 volatilization, and adsorption on sludge. The volatilization of EPs (in particular pharmaceuticals)  
421 appears limited during WWTPs treatments (Besha et al., 2017; Luo et al., 2014; Verlicchi et al.,  
422 2012). Bacterial bioavailability, potential of biodegradation, and adsorption phenomena are directly  
423 linked to the hydrophobicity/hydrophilicity of EPs (reflected by  $K_{ow}$ ) (Cirja et al., 2008). For  
424 compounds with  $\log K_{ow} < 2.5$ , adsorption is not expected, for those with  $\log K_{ow}$  between 2.5 and 4  
425 moderate sorption is intended, and for EPs with  $\log K_{ow}$  up to 4, a high sorption potential exists  
426 (Rogers, 1996; Cirja et al., 2008; Luo et al., 2014). In our case, all EPs present a  $\log K_{ow}$  below 4  
427 (2.71; 1.794; 1.391; 1.95; 1.651 and 3.529 respectively for benzoylecgonine, naproxen, gabapentin,  
428 acebutolol, ketoprofen, and fexofenadine), except for telmisartan (5.046) (INERIS, 2020;  
429 ChemSpider, 2020). Its high sorption potential, explaining the high removal values, has already been  
430 observed recently by Iranzo et al. (2018), who quantified very high concentrations of telmisartan in  
431 Spanish WWTP sewage sludge (> mg/kg). On the contrary, efficient removal of ketoprofen and  
432 naproxen, for example, has already been observed (Jelic et al., 2011), unrelated to adsorption in  
433 sludge. Nevertheless, caution must be exercised with regard to the high disposal values observed, as  
434 the (bio-)degradation by-products were not quantified in this study (Barbieri et al., 2012). The pH  
435 also plays an important role in removal as it influences the molecular charge, and thus the capacity

436 of an EP to be adsorbed on sludge (Verlicchi et al., 2012). In our case ( $7.40 < \text{pH} < 8.10$ ), acebutolol  
437 is positively charged, which could also partially explain its efficient removal during treatment.

438 (2) A moderate median removal rate ( $30 \% < \text{MRR} < 70 \%$ ), for 13 EPs: rosuvastatin (67.3  
439 %), atenolol, DEET, valsartan, atorvastatin, sulfamethoxazole, verapamil, citalopram, trospium,  
440 trimethoprim, sitagliptin, codeine and celiprolol (30.69 %). Concerning statins, rosuvastatin removal  
441 rates (MRR: 67.3 %; mean removal: 64.5 %) were in accordance with Golovko et al. (2014), which  
442 observed a mean removal rate of 68 % in some urban WWTP from the Czech Republic. Conversely,  
443 atorvastatin was not as efficiently removed (MRR/mean removal of 56.5 and 55.6 %, respectively)  
444 than in previous studies ( $> 66.7 \%$ ) (Archer et al., 2017; Couto et al., 2019; Golovko et al., 2014).  
445 The antihypertensive compounds, atenolol, celiprolol and verapamil were eliminated in the range of  
446 values observed in previous studies (Table 3), but with high variability (See Figure 5).

447 (3) A poor median removal rate ( $0 \% < \text{MRR} < 30 \%$ ), for 17 EPs: bisoprolol (29.08 %),  
448 methocarbamol, amantadine, fluopyram, cetirizine, amisulpride, oxazepam, milnacipran, flecainide,  
449 diclofenac, EDDP, carbamazepine, venlafaxine, terbutryn, disopyramide, lidocaine and fluconazole  
450 (2.5 %). The observed values are also generally in agreement with the literature ( $< 30 \%$ ), as a large  
451 variability in removal rates has been previously observed in conventional WWTPs (Table 3). For  
452 example, carbamazepine (MRR: 15.75%; mean removal: 11.80 %) was eliminated with rates ranging  
453 from -12 % to 94.9 % depending on the country and the WWTP. Most of moderately and poorly  
454 removed EPs have  $\log K_{ow} < 4$ , resulting in a partial elimination due more to bad biodegradation than  
455 to partial sorption in sludges. Thus, the observed removal rates could have two main explanations:  
456 the first is that the wastewater residence time (low Hydraulic Retention Time (HRT) values) was too  
457 weak, which would have not allowed complete biodegradation of the substances by the catabolic  
458 actions of microbes (Gros et al., 2010; Couto et al., 2019). The second lies in the chemical structure  
459 of the EP: esters, nitriles and/or aromatic alcohol functional groups may lead to increase the microbial  
460 biodegradability of EPs when iodide, nitro-, azo-, sulfo-, halogen (e.g. chlorine) and/or aromatic  
461 amine functional groups would decrease their biodegradability (Besha et al., 2017; Cirja et al., 2008;  
462 Zorita et a., 2009). Moreover, linear EPs with short side chains and unsaturated aliphatic structures

463 are more easily biodegraded than long and highly branched side chains EPs, with saturated or  
464 polycyclic structures (Luo et al., 2014). Nevertheless, a relationship between chemical structure and  
465 removal efficiency is often difficult to demonstrate. In the present case, the complexity of well-  
466 removed EPs does not differ drastically from the poorly eliminated ones (e.g., the presence in many  
467 of them of several benzene groups). However, it can be observed that some of the poorly removed  
468 EPs present one or several aromatic amines (e.g. amantadine, amisulpride) or halogen groups (fluor:  
469 flecainide and fluopyram; sulphur: amisulpride and terbutryn; chlorine: amantadine, cetirizine and  
470 oxazepam). The medium/low removal rate of diclofenac previously reported (Deblonde et al., 2011;  
471 Vieno and Sillanpää, 2014) was also imputed to the presence of 2 chlorine groups (Cirja et al., 2008;  
472 Jelic et al., 2011; Kimura et al., 2005).

473 (4) A negative median removal rate (MRR < 0 %), for 4 EPs: clopidogrel (-96.73 %),  
474 tiapride, irbesartan and diuron (-7.55 %). These results are consistent with the literature (Table 3 –  
475 Sassereau et al., 2013; Santos et al., 2013), except for the pesticide diuron for which one only positive  
476 removal has been previously reviewed (26.7–71.9%) (Luo et al., 2014). Negative removal was  
477 observed at least once for 33 of 41 identified compounds in the WW. Moreover, for a third of them,  
478 a higher mass load into the effluents was observed recursively (more than 5 times). For clopidogrel  
479 (an antiaggregant) or tiapride, a positive removal rate was observed only two times. Three main  
480 mechanisms can explain the present results: (a) a release/desorption from fecal particles under  
481 specific abiotic conditions (Archer et al., 2017); (b) a release from particles broken under the  
482 microbial action, as already observed for trimethoprim (Göbel et al., 2007), and finally, (c) a  
483 deconjugation during biological processes of glucuronide or sulfate-conjugated pollutant  
484 metabolites, as already discussed for some of the EPs of this study (e.g. diclofenac, carbamazepine  
485 or venlafaxine) (Archer et al., 2017; Campo et al., 2013; Gurke et al., 2015; Petrie et al., 2015;  
486 Verlicchi et al., 2012; Vieno and Sillanpää, 2014; Zorita et al., 2009). Nevertheless, if these general  
487 mechanisms are known, their implication in the present results remains impossible to assess as  
488 conjugated metabolites, as well as particulate phase and sludge pollutions, were not examined in this  
489 study (Gurke et al., 2015; Petrie et al., 2015). Negative removal rates could also be related to a

490 problem of sampling strategy, in particular the collection of 24-hour samples whereas HRT values  
491 from wastewater treatment plants may be higher. Problems with sample preservation prior to  
492 analysis, or the fact that some samples in this study were collected during low rainfall events, may  
493 also be responsible for these negative rates (Köck-Schulmeyer et al., 2013).

494 Finally, no removal rate comparison was possible for 6 EPs (trospium, fluopyram,  
495 methocarbamol, milnacipran, amisulpride, and tiapride) as no data were reported in any study to our  
496 knowledge. The high variability of removal efficiency observed for EPs can be explained by many  
497 factors: the variation of temperature of operation (higher removal rates are expected in summer  
498 compared to winter), the redox conditions, the pH, the biomass concentration/population, and the  
499 sludge retention time (SRT) and HRT (Gros et al., 2010). In this study, results might not be related  
500 to a difference of pH, as it changed poorly among the WWTPs and sampling periods (Ben et al.,  
501 2018). Thus, it might be primarily due to the diversity of WWTP treatments and the difference of  
502 pollution and efficiencies for the diverse sampling periods (Wiest et al., 2018). In addition, the  
503 detection of EPs at concentrations close to their respective LOQ could have led to variability and  
504 unreliability of results and associated conclusions (Jelic et al., 2011).

### 505 3.5 Occurrence, concentrations and hazard related to emerging pollutants in treated wastewaters

506 To investigate the potential contamination of the receiving watercourses, the concentrations  
507 and occurrence of the 41 emerging pollutants in the effluents for all WWTPs and sampling periods  
508 were studied, and are compiled in Figure 6 and in Supplementary Table S6. Due to their partial  
509 removal, each EP was detected at least once in the effluents. All substances were detected in treated  
510 effluents with high detection frequency (>70%), except for rosuvastatin (66.67 %), gabapentin (46.67  
511 %), milnacipran (36.67 %) and fluopyram (20%, as in raw WW). Eight EPs were detected in all  
512 samples (atenolol, carbamazepine, cetirizine, diuron, irbesartan, sitagliptin, trimethoprim,  
513 venlafaxine). Despite its good median removal rate (86.33), gabapentin exhibited higher  
514 concentration (med.conc.: 3486.31 ng/L), followed by sitagliptin (1598.36 ng/L), valsartan (1574.43  
515 ng/L), and irbesartan (1332.83 ng/L). On the contrary, disopyramide (42.2 ng/L) fluopyram (32.1

516 ng/L), diuron (27.09 ng/L) and terbutryn (17.53 ng/L) presented the lowest ones. Concentrations are  
517 generally in accordance with data reviewed in the literature (e.g. dos Santo et al., 2019; Luo et al.,  
518 2014; Nannou et al., 2020; Tlili et al., 2017; Verlicchi et al., 2012). The significant concentrations of  
519 pharmaceutical residues detected in treated WW can be easily explained by a resistance to treatments  
520 (e.g. irbesartan) (Gros et al., 2010; Wiest et al., 2018) or to very high raw wastewater concentrations  
521 (e.g. gabapentin), for which efficient treatments are not sufficient to decrease significantly the  
522 concentrations emitted into the environment. The widespread contamination of diuron and terbutryn  
523 in current effluent samples is consistent with the study performed by Tlili et al. (2017) according to  
524 which herbicide contamination of effluents from the Swiss WWTPs of the two small rural towns of  
525 Steinach and Herisau was not of agricultural origin but was dominated by these two biocides.  
526 Compared to raw WW, one study was recently reported the presence of trospium and tiapride in  
527 urban effluents of 6 Swiss WWTPs (Singer et al., 2016), with concentrations ranging from less than  
528 10 (LOQ) to 74 ng/L and from 8 to 37 ng/L, respectively. Trospium concentrations were in  
529 accordance with the present results (range: n.d. – 183.91 ng/L med. conc.: 58.16 ng/L), but lower  
530 than those reported here for tiapride (range: n.d. – 1.23 µg/L; med. conc.: 486.53 ng/L). No  
531 comparison with literature was possible for some EPs (e.g. methocarbamol, milnacipran, and  
532 fluopyram) as no data have been reported on treated wastewaters in any study to our knowledge.

533 Ecotoxicological hazard related to treated WW pollution can be discussed by comparing the  
534 median concentration (See Table S6) of EPs in effluents (Measured Environmental Concentration -  
535 MEC) with their environmental threshold values (PNEC - Predicted No Effect Concentration) in  
536 order to calculate related median hazard quotients ( $HQ_{med} = MEC/PNEC$ ) (Gosset et al., 2017). An  
537  $HQ_{med}$  value above 1 implies a significant ecotoxicological hazard for aquatic ecosystems. For  
538 example, PNEC values determined for atorvastatin (0.26 ng/L), atenolol (5 ng/L), citalopram (6.35  
539 ng/L), diclofenac (20 ng/L) and telmisartan (26 ng/L) by Orias and Perrodin (2013) and Zhou et al.  
540 (2019) led us to calculate  $HQ_{med}$  values of 358.42, 73.05, 18.28, 39.27 and 3.42, respectively. These  
541 significant and high-hazard values are in accordance with recent studies (e.g. Ramírez-Morales et al.,  
542 2020) and illustrate that it would be crucial to assess the final ecotoxicological risk associated with

543 the whole set of EPs for the receiving ecosystems in this region, which presents a diversity of  
544 exposure conditions (e.g. dilution) in treated WW. This is the subject of the second article in this  
545 series (Gosset et al., submitted).

#### 546 4. Conclusion and perspectives for further study

547 This study presents the results of a highly comprehensive analytical methodology, which  
548 was successfully developed based on the coupling of a LC-QToF-MS "suspect screening" followed  
549 by a targeted quantification of identified EPs. It was applied to raw and treated wastewater from 10  
550 wastewater treatment plants in a highly urbanized area. Due to the wide variety of profiles (e.g.  
551 sewershed) of the 10 treatment plants, chemical analyses showed wide variability in the  
552 concentration of the 41 confirmed EPs in the raw wastewater, and in their removal during treatment.  
553 Consequently, efforts (e.g. reduction at source, improvement of treatments) should be made  
554 regarding many EPs refractory to WW treatment that are frequently detected in WWTP outfalls (e.g.  
555 clopidogrel or venlafaxine). Their concentrations in discharged effluents (median conc. between  
556 17.53 and 3486.31 ng/L) could potentially pose a risk to receiving watercourses. The number of  
557 valuable data obtained from our study proved the relevance of the suspect screening approach to  
558 evaluate wastewater contamination, providing findings about EPs never studied, to our knowledge,  
559 in urban influent/effluent (e.g. methocarbamol and milnacipran). Using this methodology on other  
560 sources of pollution such as combined sewer overflows, or over several campaigns to assess the  
561 seasonal and annual variation would be of great benefit. In parallel, more extensive databases of  
562 compounds allowing for the detection of additional pharmaceuticals and pesticides are necessary to  
563 improve this strategy. Harmonized guidelines and validated procedures would also be very useful to  
564 promote the use of these tools for future research work.

565

#### 566 Acknowledgment

567 This work was supported by the Lyon Urban School, through a funding grant from the French  
568 National Research Agency (Programme Investissements d'Avenir (ANR-17-CONV-0004)). This

569 study also received a scientifically, technic and financially support of the French Ministry of Ecology  
570 (through the ENTPE), the Greater Lyon and the University Lyon 2.

571 This work was performed within the framework of the EUR H2O'Lyon (ANR-17-EURE-0018) of  
572 Université de Lyon (UdL), within the program "Investissements d'Avenir" operated by the French  
573 National Research Agency (ANR).

#### 574 References

575 ANSM, 2014. Analyse des ventes de médicaments en France en 2013/Analysis of drug sales in  
576 France in 2013. (Last consultation : 10-27-2020). [https://ansm.sante.fr/S-](https://ansm.sante.fr/S-informer/Communiqués-Communiqués-Points-presse/Ventes-de-medicaments-en-France-le-rapport-d-analyse-de-l-annee-2013-Communique#:~:text=Les%20donn%C3%A9es%20pour%202013%20font,euros%20de%20ventes%20aux%20h%C3%B4pitaux)  
577 [informer/Communiqués-Communiqués-Points-presse/Ventes-de-medicaments-en-France-le-](https://ansm.sante.fr/S-informer/Communiqués-Communiqués-Points-presse/Ventes-de-medicaments-en-France-le-rapport-d-analyse-de-l-annee-2013-Communique#:~:text=Les%20donn%C3%A9es%20pour%202013%20font,euros%20de%20ventes%20aux%20h%C3%B4pitaux)  
578 [rapport-d-analyse-de-l-annee-2013-](https://ansm.sante.fr/S-informer/Communiqués-Communiqués-Points-presse/Ventes-de-medicaments-en-France-le-rapport-d-analyse-de-l-annee-2013-Communique#:~:text=Les%20donn%C3%A9es%20pour%202013%20font,euros%20de%20ventes%20aux%20h%C3%B4pitaux)  
579 [Communique#:~:text=Les%20donn%C3%A9es%20pour%202013%20font,euros%20de%20ve-](https://ansm.sante.fr/S-informer/Communiqués-Communiqués-Points-presse/Ventes-de-medicaments-en-France-le-rapport-d-analyse-de-l-annee-2013-Communique#:~:text=Les%20donn%C3%A9es%20pour%202013%20font,euros%20de%20ventes%20aux%20h%C3%B4pitaux)  
580 [ntes%20aux%20h%C3%B4pitaux](https://ansm.sante.fr/S-informer/Communiqués-Communiqués-Points-presse/Ventes-de-medicaments-en-France-le-rapport-d-analyse-de-l-annee-2013-Communique#:~:text=Les%20donn%C3%A9es%20pour%202013%20font,euros%20de%20ventes%20aux%20h%C3%B4pitaux)).

581 Archer, E., Petrie, B., Kasprzyk-Hordern, B., Wolfaardt, G. M., 2017. The fate of pharmaceuticals  
582 and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and  
583 illicit drugs in a WWTW and environmental waters. *Chemosphere*, 174, 437-446.

584 Barbieri, M., Carrera, J., Ayora, C., Sanchez-Vila, X., Licha, T., Nödlér, K., Osorio, V., Pérez, S.,  
585 Köck-Schulmeyer, M., López de Alda, M., Barceló, D., 2012. Formation of diclofenac and  
586 sulfamethoxazole reversible transformation products in aquifer material under denitrifying  
587 conditions: batch experiments. *Sci. Total Environ.* 426, 256-263.

588 Ben, W., Zhu, B., Yuan, X., Zhang, Y., Yang, M., Qiang, Z., 2018. Occurrence, removal and risk of  
589 organic micropollutants in wastewater treatment plants across China: Comparison of wastewater  
590 treatment processes. *Water Res.* 130, 38-46.

591 Bertanza, G., Papa, M., Pedrazzani, R., Repice, C., Mazzoleni, G., Steimberg, N., Feretti, D., Ceretti,  
592 E., Zerbini, I., 2013. EDCs, estrogenicity and genotoxicity reduction in a mixed (domestic+textile)  
593 secondary effluent by means of ozonation: A full-scale experience. *Sci. Total Environ.* 458-460,  
594 160-168.

595 Besha, A. T., Gebreyohannes, A. Y., Tufa, R. A., Bekele, D. N., Curcio, E., Giorno, L., 2017.  
596 Removal of emerging micropollutants by activated sludge process and membrane bioreactors and  
597 the effects of micropollutants on membrane fouling: A review. *J. Environ. Chem. Eng.* 5(3), 2395-  
598 2414.

599 Bollmann, U. E., Tang, C., Eriksson, E., Jönsson, K., Vollertsen, J., Bester, K., 2014. Biocides in  
600 urban wastewater treatment plant influent at dry and wet weather: Concentrations, mass flows and  
601 possible sources. *Water Res.* 60, 64-74.

602 Brack, W., Hollender, J., de Alda, M.L., Mueller, C., Schulze, T., Schymanski, E., Slobodnik, J.,  
603 Krauss, M., 2019. High-resolution mass spectrometry to complement monitoring and track emerging  
604 chemicals and pollution trends in European water resources. *Env. Sci. Eur.* 31.

605 Brus, A., Perrodin, Y., 2017. Identification, assessment and prioritization of ecotoxicological risks  
606 on the scale of a territory: Application to WWTP discharges in a geographical area located in  
607 northeast Lyon, France. *Chemosphere* 189, 340-348.

608 Burns, E. E., Carter, L. J., Kolpin, D. W., Thomas-Oates, J., Boxall, A. B., 2018. Temporal and  
609 spatial variation in pharmaceutical concentrations in an urban river system. *Water Res.* 137, 72-85.

610 Campo, J., Masiá, A., Blasco, C., Picó, Y., 2013. Occurrence and removal efficiency of pesticides in  
611 sewage treatment plants of four Mediterranean River Basins. *J. Hazard. Mater.* 263, 146-157.

612 Ccancapa-Cartagena, A., Pico, Y., Ortiz, X., Reiner, E.J., 2019. Suspect, non-target and target  
613 screening of emerging pollutants using data independent acquisition: Assessment of a Mediterranean  
614 River basin. *Sci. Total Environ.* 687, 355-368.

615 ChemSpider, 2020. Chemical database of the Royal Society of Chemistry.  
616 <http://www.chemspider.com/> (Last consultation : 11-03-2020)

617 Choi, P. M., Tschärke, B., Samanipour, S., Hall, W. D., Gartner, C. E., Mueller, J. F., O'Brien, J. W.  
618 2019. Social, demographic, and economic correlates of food and chemical consumption measured  
619 by wastewater-based epidemiology. *PNAS* 116(43), 21864-21873.

620 Cirja, M., Ivashechkin, P., Schäffer, A., Corvini, P. F., 2008. Factors affecting the removal of organic  
621 micropollutants from wastewater in conventional treatment plants (CTP) and membrane bioreactors  
622 (MBR). *Rev. Environ. Sci. Biotechnol.* 7(1), 61-78.

623 Couto, C. F., Lange, L. C., Amaral, M. C., 2019. Occurrence, fate and removal of pharmaceutically  
624 active compounds (PhACs) in water and wastewater treatment plants—A review. *J. Water*  
625 *Proc. engineering* 32, 100927.

626 Deblonde, T., Cossu-Leguille, C., Hartemann, P., 2011. Emerging pollutants in wastewater: a review  
627 of the literature. *Int. J. Hyg. Environ. Health* 214(6), 442-448.

628 Deeb, A., Stephan, S., Schmitz, O. J., Schmidt, T. C., 2017. Suspect screening of micropollutants  
629 and their transformation products in advanced wastewater treatment. *Sci. Total Environ.* 601, 1247-  
630 1253.



631 Deycard, V. N., Schäfer, J., Blanc, G., Coynel, A., Petit, J. C., Lanceleur, L., Dutruch, L., Bossy, C.,  
632 Ventura, A., 2014. Contributions and potential impacts of seven priority substances (As, Cd, Cu, Cr,  
633 Ni, Pb, and Zn) to a major European Estuary (Gironde Estuary, France) from urban wastewater. *Mar.*  
634 *Chem.* 167, 123-134.

635 dos Santos, M. M., Hoppe-Jones, C., Snyder, S. A., 2019. DEET occurrence in wastewaters:  
636 Seasonal, spatial and diurnal variability-mismatches between consumption data and environmental  
637 detection. *Environ. Int.* 132, 105038.

638 Du, B., Lofton, J. M., Peter, K. T., Gipe, A. D., James, C. A., McIntyre, J. K., Scholz N.L., Baker,  
639 J.E., Kolodziej, E. P., 2017. Development of suspect and non-target screening methods for detection  
640 of organic contaminants in highway runoff and fish tissue with high-resolution time-of-flight mass  
641 spectrometry. *Environ. Sci. Process. Impact* 19(9), 1185-1196.

642 Fulazzaky, M. A., Abdullah, N. H., Yusoff, A. R. M., Paul, E., 2015. Conditioning the alternating  
643 aerobic–anoxic process to enhance the removal of inorganic nitrogen pollution from a municipal  
644 wastewater in France. *J. Clean. Prod.* 100, 195-201.

645 Gasperi, J., Garnaud, S., Rocher, V., Moilleron, R., 2008. Priority pollutants in wastewater and  
646 combined sewer overflow. *Sci. Total Environ.* 407(1), 263-272.

647 Gasperi, J., Zgheib, S., Cladière, M., Rocher, V., Moilleron, R., Chebbo, G., 2012. Priority pollutants  
648 in urban stormwater: Part 2–Case of combined sewers. *Water Res.* 46(20), 6693-6703.

649 Gavrilescu, M., Demnerová, K., Aamand, J., Agathos, S., Fava, F., 2015. Emerging pollutants in the  
650 environment: present and future challenges in biomonitoring, ecological risks and bioremediation.  
651 *New Biotechnol.* 32(1), 147-156.

652 Göbel, A., McArdell, C. S., Joss, A., Siegrist, H., Giger, W., 2007. Fate of sulfonamides, macrolides,  
653 and trimethoprim in different wastewater treatment technologies. *Sci. Total Environ.* 372(2-3), 361-  
654 371.

655 Golovko, O., Kumar, V., Fedorova, G., Randak, T., Grabic, R., 2014. Seasonal changes in antibiotics,  
656 antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant.  
657 *Chemosphere*, 111, 418-426.

658 Gosset, A., Durrieu, C., Orias, F., Bayard, R., Perrodin, Y., 2017. Identification and assessment of  
659 ecotoxicological hazards attributable to pollutants in urban wet weather discharges. *Environ. Sci.*  
660 *Process. Impact* 19(9), 1150-1168.

661 Gosset, A., Polomé, P., Perrodin, Y., 2020. Ecotoxicological risk assessment of micropollutants from  
662 treated urban wastewater effluents for watercourses at a territorial scale: Application and comparison  
663 of two approaches. *Int. J. Hyg. Environ. Health* 224, 113437.

664 Gros, M., Blum, K. M., Jernstedt, H., Renman, G., Rodríguez-Mozaz, S., Haglund, P., Andersson,  
665 P.L., Wiberg, K., Ahrens, L., 2017. Screening and prioritization of micropollutants in wastewaters  
666 from on-site sewage treatment facilities. *J. Hazard. Mater.* 328, 37-45.

667 Gros, M., Petrović, M., Ginebreda, A., Barceló, D., 2010. Removal of pharmaceuticals during  
668 wastewater treatment and environmental risk assessment using hazard indexes. *Environ. Int.* 36(1),  
669 15-26.

670 Guillosoy, R., Le Roux, J., Mailler, R., Vulliet, E., Morlay, C., Nauleau, F., Gasperi, J., Rocher, V.,  
671 2019. Organic micropollutants in a large wastewater treatment plant: What are the benefits of an  
672 advanced treatment by activated carbon adsorption in comparison to conventional treatment?.  
673 *Chemosphere* 218, 1050-1060.

674 Gurke, R., Rößler, M., Marx, C., Diamond, S., Schubert, S., Oertel, R., Fauler, J., 2015. Occurrence  
675 and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater  
676 of a sewage treatment plant. *Sci. Total Environ.* 532, 762-770.

677 Hug, C., Ulrich, N., Schulze, T., Brack, W., Krauss, M., 2014. Identification of novel micropollutants  
678 in wastewater by a combination of suspect and nontarget screening. *Environ. Pollut.* 184, 25-32.

679 INERIS,2020. Portail Substances Chimiques (French national database)  
680 <https://substances.ineris.fr/fr/> (Last consultation : 11-03-2020)

681 Iranzo, M., Gamón, M., Boluda, R., Mormeneo, S., 2018. Analysis of pharmaceutical biodegradation  
682 of WWTP sludge using composting and identification of certain microorganisms involved in the  
683 process. *Sci. Total Environ.* 640, 840-848.

684 Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R., Ventura, F., Petrovic, M., Barcelo, D.,  
685 2011. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during  
686 wastewater treatment. *Water Res.* 45(3), 1165-1176.

687 Kimura, K., Hara, H., Watanabe, Y. (2005). Removal of pharmaceutical compounds by submerged  
688 membrane bioreactors (MBRs). *Desalination*, 178(1-3), 135-140.

689 Köck-Schulmeyer, M., Villagrasa, M., de Alda, M. L., Céspedes-Sánchez, R., Ventura, F., Barceló,  
690 D., 2013. Occurrence and behavior of pesticides in wastewater treatment plants and their  
691 environmental impact. *Sci. Total Environ.* 458, 466-476.

692 Krauss, M., Hug, C., Bloch, R., Schulze, T., Brack, W., 2019. Prioritising site-specific  
693 micropollutants in surface water from LC-HRMS non-target screening data using a rarity score. *Env.*  
694 *Sci. Eur.* 31(1), 45.

695 Kümmerer, K., Dionysiou, D.D., Olsson, O., Fatta-Kassinos, D., 2019. Reducing aquatic  
696 micropollutants - Increasing the focus on input prevention and integrated emission management. *Sci.*  
697 *Total Environ.* 652, 836-850

698 Laquaz, M., Dagot, C., Wiest, L., Bazin, C., Gaschet, M., Perrodin, Y. 2020. Ecotoxicity and  
699 antibiotic resistance of wastewater during transport in an urban sewage network. *Environ. Sci. Pollut.*  
700 *Res.*

701 López-Pacheco, I., Silva-Núñez, A., Salinas-Salazar, C., Arévalo-Gallegos, A., Lizarazo-Holguin,  
702 L. A., Barceló, D., Iqbal, H. M., Parra-Saldívar, R., 2019. Anthropogenic contaminants of high  
703 concern: Existence in water resources and their adverse effects. *Sci. Total Environ.* 690, 1068-1088.

704 Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S., Wang, X. C., 2014. A  
705 review on the occurrence of micropollutants in the aquatic environment and their fate and removal  
706 during wastewater treatment. *Sci. Total Environ.* 473, 619-641.

707 Martín, J., Camacho-Muñoz, D., Santos, J. L., Aparicio, I., Alonso, E., 2012. Occurrence of  
708 pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: removal and  
709 ecotoxicological impact of wastewater discharges and sludge disposal. *J. Hazard. Mater.* 239, 40-47.

710 Merel S., Snyder S. A., 2016. Critical assessment of the ubiquitous occurrence and fate of the insect  
711 repellent N,N-diethyl-m-toluamide in water. *Environ. Int.* 96, 98-117.

712 Miège, C., Favier, M., Brosse, C., Canler, J. P., Coquery, M., 2006. Occurrence of betablockers in  
713 effluents of wastewater treatment plants from the Lyon area (France) and risk assessment for the  
714 downstream rivers. *Talanta*, 70(4), 739-744.

715 Mohan, S., Balakrishnan, P., 2019. Triclosan in treated wastewater from a city wastewater treatment  
716 plant and its environmental risk assessment. *Water Air Soil Pollut.* 230(3), 69.

717 Nannou, C., Ofrydopoulou, A., Evgenidou, E., Heath, D., Heath, E., Lambropoulou, D., 2020.  
718 Antiviral drugs in aquatic environment and wastewater treatment plants: A review on occurrence,  
719 fate, removal and ecotoxicity. *Sci. Total Environ.* 699, 134322.

720 Nefau, T., Karolak, S., Castillo, L., Boireau, V., Levi, Y., 2013. Presence of illicit drugs and  
721 metabolites in influents and effluents of 25 sewage water treatment plants and map of drug  
722 consumption in France. *Sci. Total Environ.* 461, 712-722.

723 Orias, F., Perrodin, Y., 2013. Characterisation of the ecotoxicity of hospital effluents: a review. *Sci.*  
724 *Total Environ.* 454, 250-276.

725 Östman, M., Björleinius, B., Fick, J., Tysklind, M., 2019. Effect of full-scale ozonation and pilot-  
726 scale granular activated carbon on the removal of biocides, antimycotics and antibiotics in a sewage  
727 treatment plant. *Sci. Total Environ.* 649, 1117-1123.

728 Palli, L., Spina, F., Varese, G. C., Vincenzi, M., Aragno, M., Arcangeli, G., Mucci, N., Santianni,  
729 D., Caffaz, S., Gori, R., 2019. Occurrence of selected pharmaceuticals in wastewater treatment plants  
730 of Tuscany: An effect-based approach to evaluate the potential environmental impact. *Int. J. Hyg.*  
731 *Environ. Health* 222(4), 717-725.

732 Pasquini, L., Munoz, J. F., Pons, M. N., Yvon, J., Dauchy, X., France, X., Le, N.D., France-Lanord,  
733 C., Görner, T., 2014. Occurrence of eight household micropollutants in urban wastewater and their  
734 fate in a wastewater treatment plant. Statistical evaluation. *Sci. Total Environ.* 481, 459-468.

735 Perrodin, Y., Bazin, C., Orias, F., Wigh, A., Bastide, T., Berlioz-Barbier, A., Vulliet, E., Wiest, L.,  
736 2016. A posteriori assessment of ecotoxicological risks linked to building a hospital. *Chemosphere*  
737 144, 440-445.

738 Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2015. A review on emerging contaminants in  
739 wastewaters and the environment: current knowledge, understudied areas and recommendations for  
740 future monitoring. *Water Res.* 72, 3-27.

741 Pinasseau, L., Wiest, L., Fildier, A., Volatier, L., Fones, G. R., Mills, G. A., Mermillod-Blondin, F.,  
742 Vulliet, E., 2019. Use of passive sampling and high resolution mass spectrometry using a suspect  
743 screening approach to characterise emerging pollutants in contaminated groundwater and runoff. *Sci.*  
744 *Total Environ.* 672, 253-263.

745 Ramírez-Morales, D., Masís-Mora, M., Montiel-Mora, J. R., Cambronero-Heinrichs, J. C., Briceño-  
746 Guevara, S., Rojas-Sánchez, C. E., Rodríguez-Rodríguez, C. E., 2020. Occurrence of  
747 pharmaceuticals, hazard assessment and ecotoxicological evaluation of wastewater treatment plants  
748 in Costa Rica. *Sci. Total Environ.* 746, 141200.

749 Repice, C., Dal Grande, M., Maggi, R., Pedrazzani, R., 2013. Licit and illicit drugs in a wastewater  
750 treatment plant in Verona, Italy. *Sci. Total Environ.* 463, 27-34.

751 Rogers, H. R., 1996. Sources, behaviour and fate of organic contaminants during sewage treatment  
752 and in sewage sludges. *Sci. Total Environ.* 185(1-3), 3-26.

753 Santos, L. H., Gros, M., Rodriguez-Mozaz, S., Delerue-Matos, C., Pena, A., Barceló, D.,  
754 Montenegro, M. C. B., 2013. Contribution of hospital effluents to the load of pharmaceuticals in  
755 urban wastewaters: identification of ecologically relevant pharmaceuticals. *Sci. Total Environ.* 461,  
756 302-316.

757 Saussereau, E., Lacroix, C., Guerbet, M., Cellier, D., Spiroux, J., Goullé, J. P., 2013. Determination  
758 of levels of current drugs in hospital and urban wastewater. *Bull. Environ. Contam. Toxicol.* 91(2),  
759 171-176.

760 Schymanski, E.L., Jeon, J., Gulde R, Fenner, K., Ruff, M., Singer, H.P., Hollender, J. (2014).  
761 Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence.  
762 *Environ. Sci. Technol.* 48, 2097-2098

763 Singer, H. P., Wössner, A. E., Mc Ardell, C. S., Fenner, K., 2016. Rapid screening for exposure to  
764 “non-target” pharmaceuticals from wastewater effluents by combining HRMS-based suspect  
765 screening and exposure modeling. *Environ. Sci. Technol.* 50(13), 6698-6707.

766 Sjerps, R. M., Vughs, D., van Leerdam, J. A., ter Laak, T. L., van Wezel, A. P., 2016. Data-driven  
767 prioritization of chemicals for various water types using suspect screening LC-HRMS. *Water Res.*  
768 93, 254-264.

769 Tlili, A., Hollender, J., Kienle, C., Behra, R., 2017. Micropollutant-induced tolerance of in situ  
770 periphyton: establishing causality in wastewater-impacted streams. *Water Res.* 111, 185-194

771 Tran, N. H., Reinhard, M., Gin, K. Y. H., 2018. Occurrence and fate of emerging contaminants in  
772 municipal wastewater treatment plants from different geographical regions—a review. *Water Res.* 133,  
773 182-207.

774 Tsui, M. M., Leung, H. W., Lam, P. K., Murphy, M. B., 2014. Seasonal occurrence, removal  
775 efficiencies and preliminary risk assessment of multiple classes of organic UV filters in wastewater  
776 treatment plants. *Water Res.* 53, 58-67.

777 Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban  
778 wastewater: removal, mass load and environmental risk after a secondary treatment—a review. *Sci.*  
779 *Total Environ.* 429, 123-155.

780 Vidal, 2020. Le site de référence des professionnels de santé/The reference site for healthcare  
781 professionals. (Last consultation: 11-03-2020). <https://www.vidal.fr/>

782 Vieno, N., Sillanpää, M., 2014. Fate of diclofenac in municipal wastewater treatment plant—a  
783 review. *Environ. Int.* 69, 28-39.

784 Vulliet, E., Cren-Olivé, C., 2011. Screening of pharmaceuticals and hormones at the regional scale,  
785 in surface and groundwaters intended to human consumption. *Environ. Pollut.* 159(10), 2929-2934.

786 Wang, Y., Gao, W., Wang, Y., Jiang, G., 2019. Suspect screening analysis of the occurrence and  
787 removal of micropollutants by GC-QTOF MS during wastewater treatment processes. *J. Hazard.*  
788 *Mater.* 376, 153-159.

789 Wiest, L., Chonova, T., Bergé, A., Baudot, R., Bessueille-Barbier, F., Ayouni-Derouiche, L., Vulliet,  
790 E., 2018. Two-year survey of specific hospital wastewater treatment and its impact on  
791 pharmaceutical discharges. *Environ. Sci. Pollut. Res.* 25(10), 9207-9218.

792 Wigh, A., Devaux, A., Brosselin, V., Gonzalez-Ospina, A., Domenjoud, B., Aït-Aïssa, S., Creusot,  
793 N., Gosset, A., Bazin, C., Bony, S., 2016. Proposal to optimize ecotoxicological evaluation of  
794 wastewater treated by conventional biological and ozonation processes. *Environ. Sci. Pollut. Res.*  
795 23(4), 3008-3017.

796 Yadav, M. K., Short, M. D., Gerber, C., van den Akker, B., Aryal, R., Saint, C. P., 2019. Occurrence,  
797 removal and environmental risk of markers of five drugs of abuse in urban wastewater systems in  
798 South Australia. *Environ. Sci. Pollut. Res.* 26(33), 33816-33826.

799 Yan, Q., Gao, X., Chen, Y. P., Peng, X. Y., Zhang, Y. X., Gan, X. M., Zi, C.-F., Guo, J. S., 2014.  
800 Occurrence, fate and ecotoxicological assessment of pharmaceutically active compounds in  
801 wastewater and sludge from wastewater treatment plants in Chongqing, the Three Gorges Reservoir  
802 Area. *Sci. Total Environ.* 470, 618-630.

803 Zhou, S., Di Paolo, C., Wu, X., Shao, Y., Seiler, T. B., Hollert, H. (2019). Optimization of screening-  
804 level risk assessment and priority selection of emerging pollutants–The case of pharmaceuticals in  
805 European surface waters. *Environ. Int.* 128, 1-10.

806 Zorita, S., Mårtensson, L., Mathiasson, L., 2009. Occurrence and removal of pharmaceuticals in a  
807 municipal sewage treatment system in the south of Sweden. *Sci. Total Environ.* 407(8), 2760-2770.

808

809

810

811

812

813

814

815

816

817 List of the figures

818 Figure 1: Location of the 10 studied WWTPs on the Lyon (France) urbanized area.

819 Figure 2: Schematic of performed LC-QToF-MS analyses

820 Figure 3: Number of scientific studies devoted to each detected compound and wastewaters or  
821 urban/municipal wastewaters (Web of Knowledge search, last consultation: 04-01-2020).

822 Figure 4: Box plot (min., quartiles, median, max.) of the concentrations of each identified EP in  
823 influent for all WWTPs and sampling campaigns (n=30). Only concentrations above the limit of  
824 quantification (LOQ) are considered. The specific number of values for each EP is specified in the  
825 brackets. The dot corresponds to the mean.

826 Figure 5: Box plot (min., quartiles, median, max.) of the overall removal rates (%) of each EP  
827 identified in this study for all WWTPs and sampling campaigns (n=30). The specific number of  
828 calculated values for each EP is specified in the brackets. The dot corresponds to the mean.

829 Figure 6: Box plot (min., quartiles, median, max.) of the concentrations of each identified EP in  
830 effluent for all WWTPs and sampling campaigns (n=30). Only concentrations above the limit of  
831 quantification (LOQ) are considered. The specific number of values for each EP is specified in the  
832 brackets. The dot corresponds to the mean

833

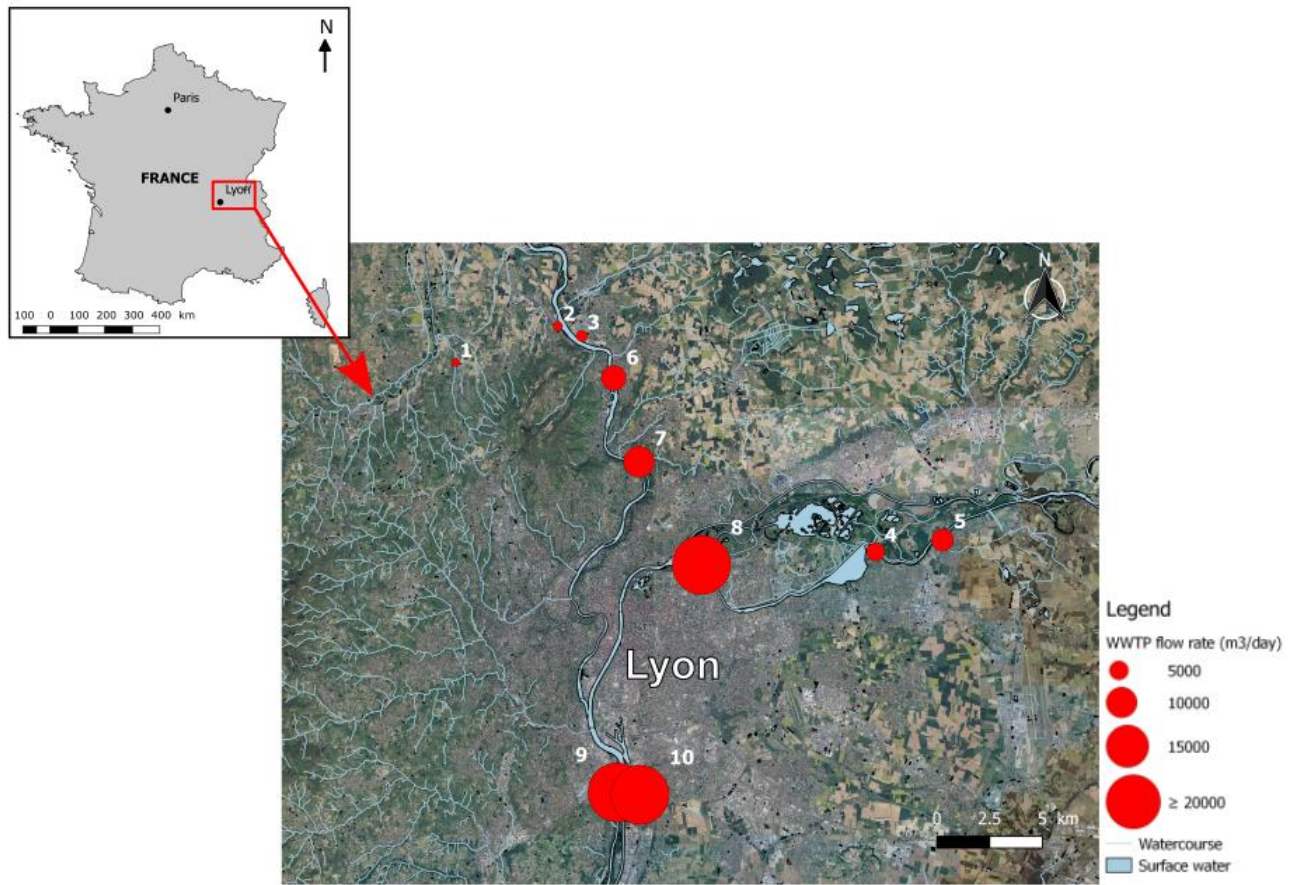
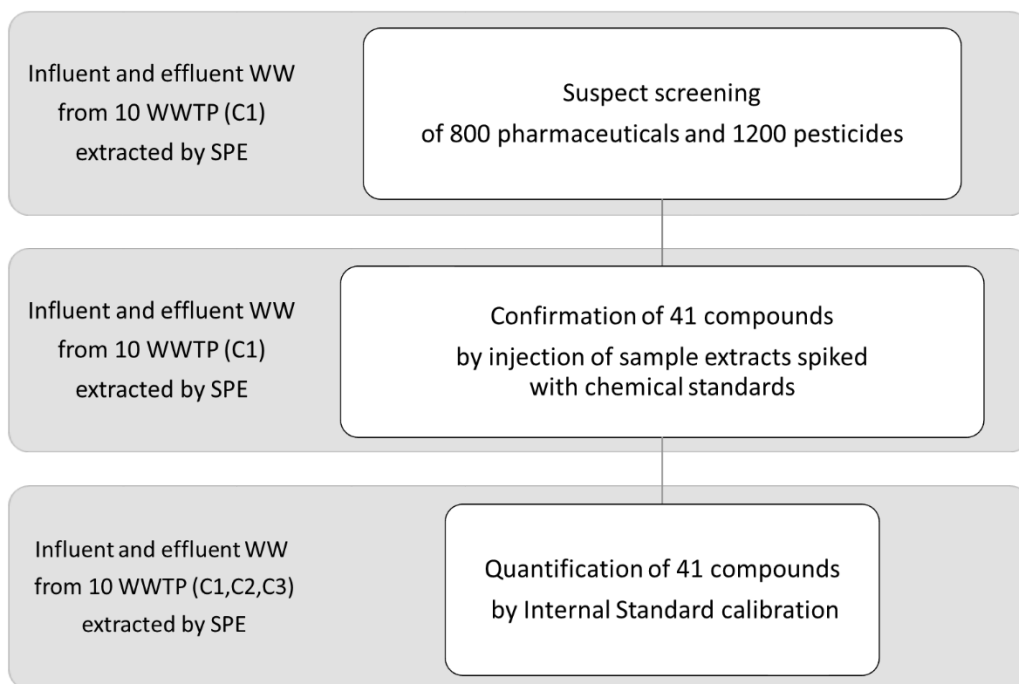


Figure 1: Location of the 10 studied WWTPs on the Lyon (France) urbanized area.





843

844 Figure 2: Schematic of performed LC-QToF-MS analyses.

845

846

847

848

849

850

851

852

853

854

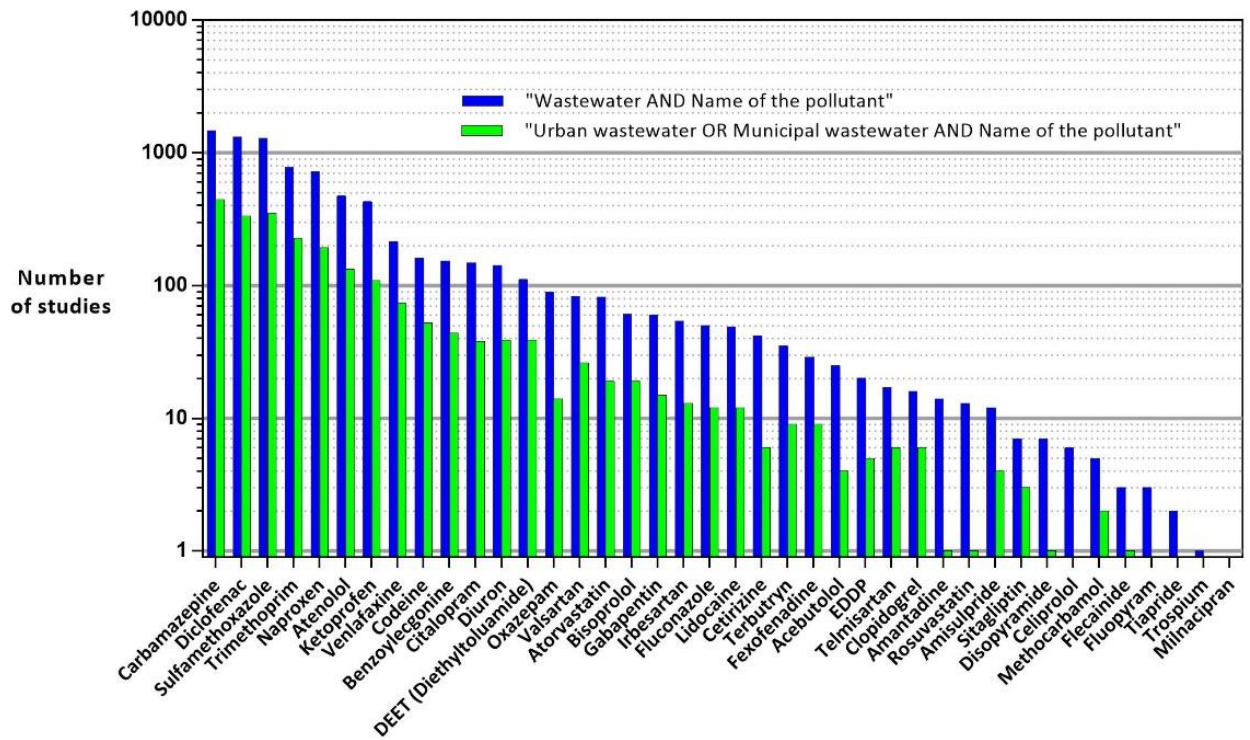
855

856

857

858

859



860 Figure 3: Number of scientific studies devoted to each detected compound and wastewaters or  
 861 urban/municipal wastewaters (Web of Knowledge search, last consultation: 04-01-2020).

862

863

864

865

866

867

868

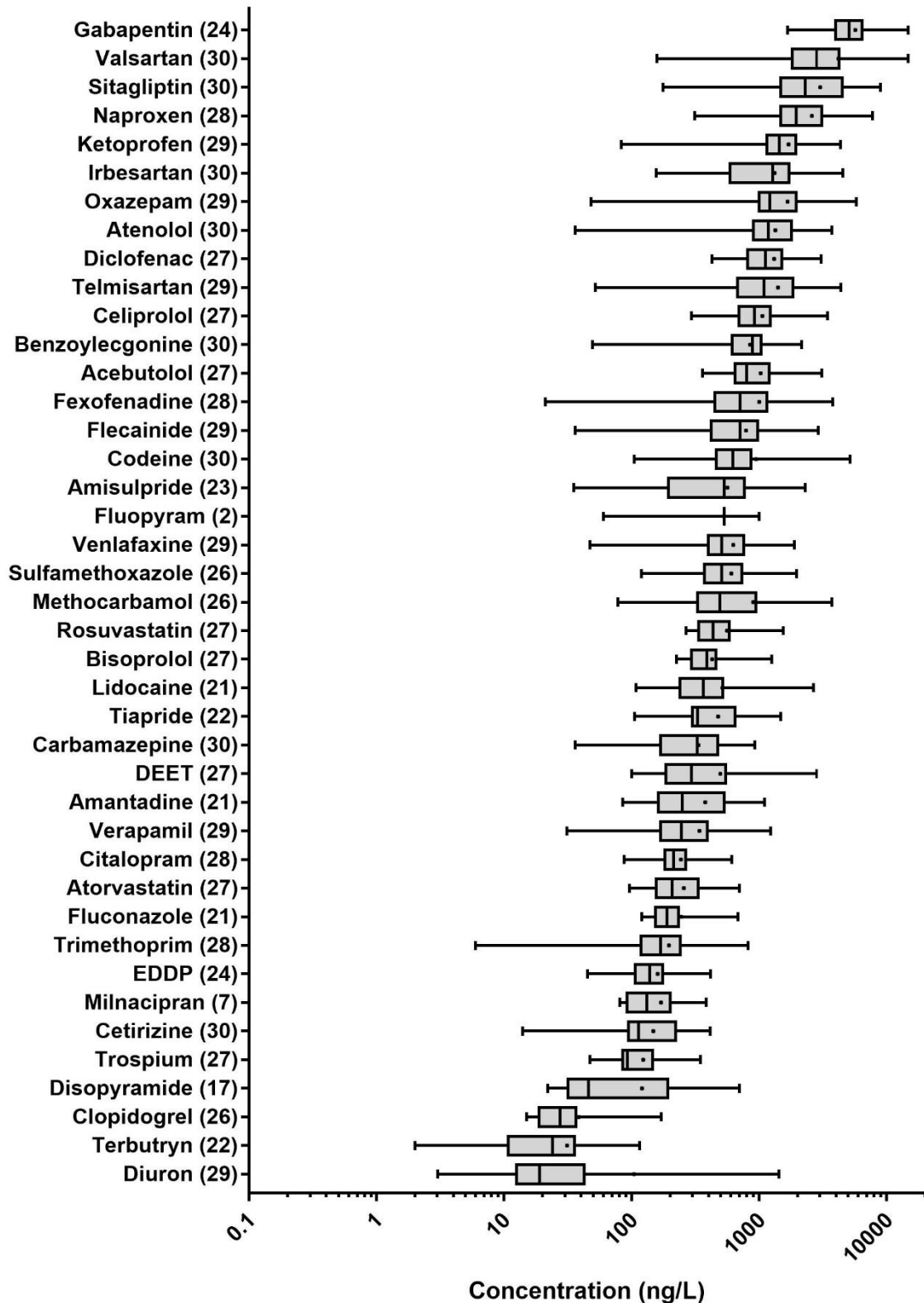
869

870

871

872

873



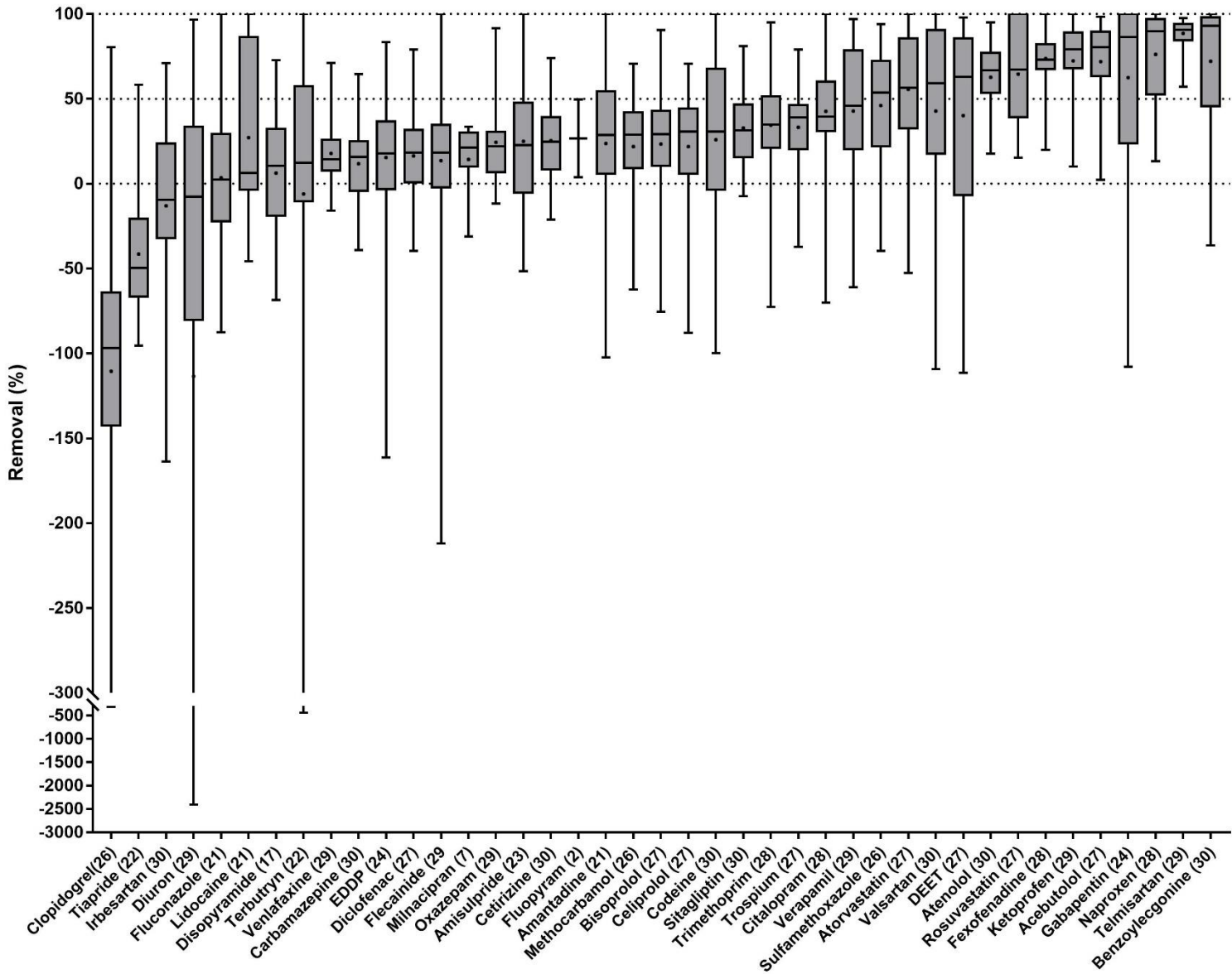
874

875 Figure 4: Box plot (min., quartiles, median, max.) of the concentrations of each identified EP in  
 876 influent for all WWTPs and sampling campaigns (n=30). Only concentrations above the limit of  
 877 quantification (LOQ) are considered. The specific number of values for each EP is specified in the  
 878 brackets. The dot corresponds to the mean.

879

880

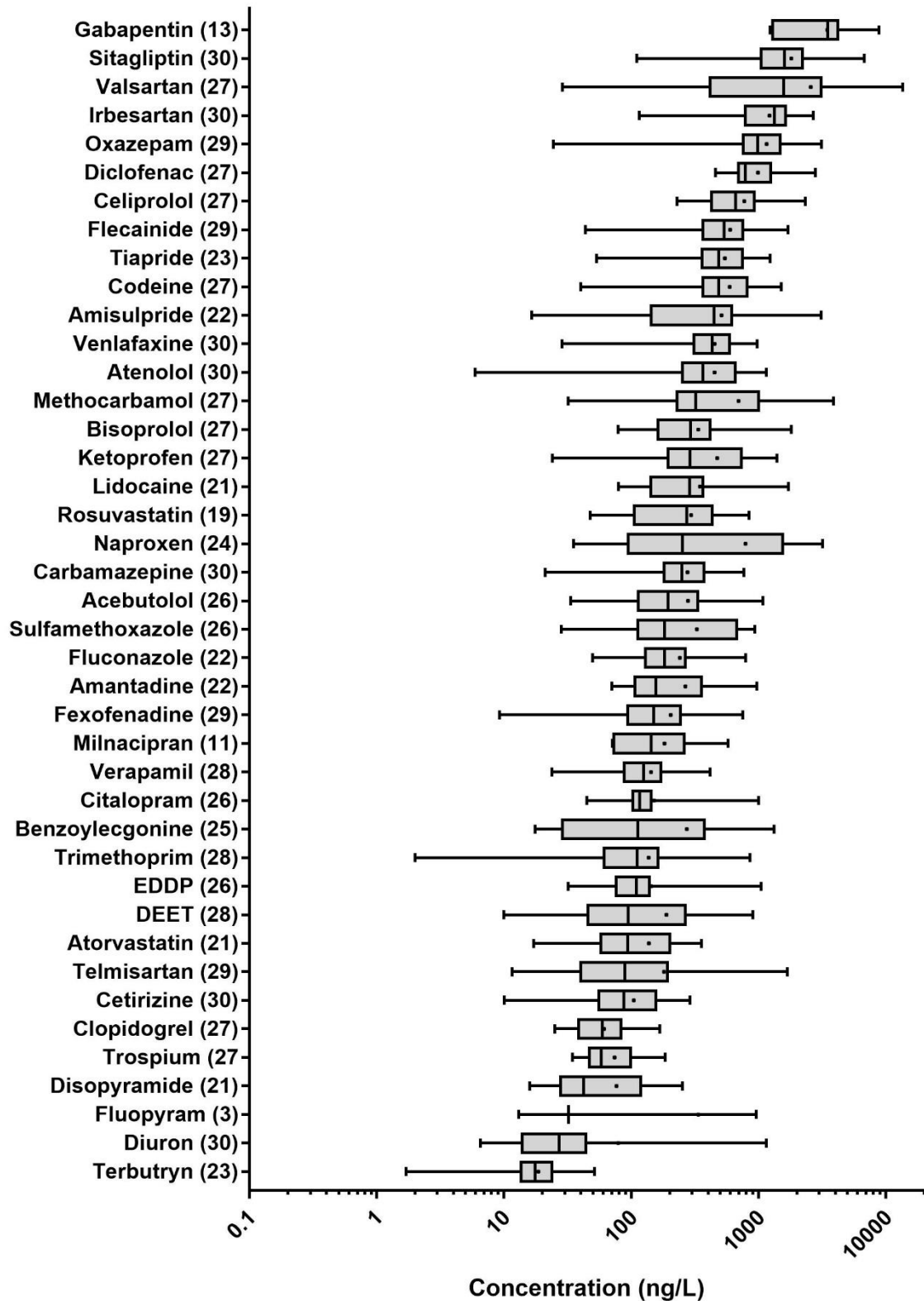
881



882

883 Figure 5: Box plot (min., quartiles, median, max.) of the overall removal rates (%) of each EP  
884 identified in this study for all WWTPs and sampling campaigns (n=30). The specific number of  
885 calculated values for each EP is specified in the brackets. The dot corresponds to the mean.

886



887 Figure 6: Box plot (min., quartiles, median, max.) of the concentrations of each identified EP in  
 888 effluent for all WWTPs and sampling campaigns (n=30). Only concentrations above the limit of  
 889 quantification (LOQ) are considered. The specific number of values for each EP is specified in the  
 890 brackets. The dot corresponds to the mean.

Table 1: Characteristics of the 10 studied WWTPs

N°	Incoming load in Population Equivalent (PE)	Incoming annual flow rate of the WWTP (m <sup>3</sup> /day)	Design capacity (PE)	Design flow rate (m <sup>3</sup> /day)	Pretreatment	Primary treatment	Secondary treatment	Tertiary treatment	Influent - Sampling location	Effluent - Sampling location
1	772	235	1433	320	Screening, grit chamber and grease remover	None	Activated sludges	None	Pretreatment outlet	Secondary treatment outlet
2	2843	679	3830	900	Screening, grit chamber and grease remover	Sedimentation tank	Activated sludges	None	Pretreatment inlet	Secondary treatment outlet
3	9150	1300	10000	1300	Screening, grit chamber and grease remover	Buffer tank	Radial flow fluidized filter/ Syncopated aeration	None	WWTP inlet	Secondary treatment outlet
4	25732	4016	33300	8730	Screening, grit chamber and grease remover	Sedimentation tank	Biofilter	None	Pretreatment inlet	Secondary treatment outlet
5	21800	5544	42000	9900	Screening, grit chamber and grease remover	Sedimentation tank	Activated sludges	None	Pretreatment inlet	Secondary treatment outlet
6	16165	6745	34100	18000	Screening, grit chamber and grease remover	Coagulation-flocculation and sedimentation tank	Activated sludges	None	Pretreatment inlet	Secondary treatment outlet
7	44087	8980	30000	9670	Screening, grit chamber and grease remover	Sedimentation tank	Biofilter	None	Pretreatment inlet	Secondary treatment outlet
8	179772	38188	300000	91000	Screening, grit chamber and grease remover	Sedimentation tank	Activated sludges	None	WWTP inlet	Secondary treatment outlet
9	524325	156962	950000	300000	Screening, grit chamber and grease remover	Sedimentation tank	Activated sludges	None	Pretreatment inlet	Secondary treatment outlet
10	622800	215092	983000	554000	Screening, grit chamber and grease remover	Sedimentation tank	Activated sludges	Biofiltration (Suspended solids, carbon and nitrogen pollution removal)	WWTP inlet	Tertiary treatment outlet

892

893 Table 2: Classical physico-chemical parameters measured on influent and effluent samples.

Parameter	Unit	Influents					Effluents				
		Min	Median	Mean	Max	Freq.	Min	Median	Mean	Max	Freq.
pH	pH unit	7.40	7.80	7.81	8.00	X	7.50	7.90	7.88	8.10	X
Conductivity	$\mu\text{S/cm}$ at 25°C	989.00	1160.00	1243.40	1793.00	X	617.00	953.5	994.9	1258.00	X
TSS	mg/L	81.00	279.00	270.80	450.00	30/30	3.00	8	12.9	36	30/30
COD	mg O <sub>2</sub> /L	105.00	616.00	588.37	982.00	30/30	13.00	32.85	41.313	109	30/30
Cl <sup>-</sup>	mg/L	57.75	114.45	135.45	361.68	30/30	57.38	121.97	126.82	222.97	30/30
NO <sub>2</sub> <sup>-</sup>	mg/L	n.d.	0.62	0.75	1.83	6/30	n.d.	1.50	1.61	3.78	9/30
NO <sub>3</sub> <sup>-</sup>	mg/L	n.d.	6.39	8.64	40.40	25/30	1.87	18.07	32.53	139.38	30/30
PO <sub>4</sub> <sup>3-</sup>	mg/L	n.d.	9.37	9.71	18.62	28/30	n.d.	5.96	6.59	14.90	21/30
SO <sub>4</sub> <sup>2-</sup>	mg/L	39.81	53.99	55.44	110.08	30/30	35.41	51.21	53.53	92.01	30/30
NH <sub>4</sub> <sup>+</sup>	mg/L	6.68	48.84	47.38	81.66	30/30	0.34	4.67	15.62	63.58	30/30
K <sup>+</sup>	mg/L	5.85	17.57	18.02	27.34	30/30	4.83	15.68	16.31	32.73	30/30

894 n.d.: Not detected.; Freq.: Number of values different from "n.d."; COD: Chemical Oxygen Demand; TSS; Total Suspended Solids

895

896

897

898

899 Table 3: Overall removal (%) of identified emerging pollutants in this study compared to urban and conventional WWTPs worldwide. ND: No data in literature.

900 MRR: Median removal rate.

	This study			Archer et al., 2017	Burns et al., 2018	Campo et al., 2013	Couto et al., 2019	Deblonde et al., 2011	Golovko et al., 2014	Gurke et al., 2015	Luo et al., 2014	Nannou et al., 2020	Repice et al., 2013	Santos et al., 2013	Saussereau et al., 2013	Tran et al., 2018	Yadav et al., 2019		
	Urban WWTPs			Urban WWTP - South Africa	Urban WWTPs - United Kingdom	Urban WWTPs - Spain	Review - Municipal WWTPs worldwide	Review - All types of WWTPs worldwide	Urban WWTP - Czech Republic	Urban WWTP - Germany	Review - Conventional WWTPs worldwide	Review - All types of WWTPs worldwide	Urban WWTP - Italie	Urban WWTP - Portugal	Urban WWTP - France	Review - Full-scale WWTPs worldwide	Urban WWTPs - Australia		
Removal (%)	Removal category	Median removal	Mean removal	Mean	Range	Mean	Range	Mean	Mean	Mean	Range	Range	Mean	Range	Mean	Annual removal	Range	Mean	
Benzoyllecgonine	High removal efficiency (MRR >70 %)	92.94	72.18	98									90					75	
Telmisartan		90.74	88.53						45.5										
Naproxen		89.71	76.21	47				0-90	81.6			43.3-98.6		<0-90	53			<0-99.3	
Gabapentin		86.33	62.50			87.4-97.9				6.4								<0-95.6	
Acebutolol		80.35	71.91						58.2							52			
Ketoprofen		79.17	72.40	77				98.7	31.1			10.8-100			35-68	53		51.5-91.9	
Fexofenadine		73.07	73.80	49		0.47-22.9				11									
Rosuvastatin	Moderate removal efficiency (30 % < MRR < 70 %)	67.31	64.53						68										
Atenolol		66.80	62.75	75		90.7-94.8		48-100	56.7		22.6	<0-85.1		<0-21	<0		<0-96		
DEET		62.82	40.17									65.6-79.5						27-100	
Valsartan		59.21	42.82	90							24.4			<0-100	52				
Atorvastatin		56.48	55.64	67				66.7		93									
Sulfamethoxazole		53.76	46.15	18		37.2-92.8		36-68	17.5	58	42.4	4-88.9		<0-41	12			<0-99	
Verapamil		45.89	42.74			20-20								18-75	45	57			
Citalopram		39.57	42.64			(-7.2)-30.3				18	6.3			<0-28	<0				
Trospium		39.06	33.21	ND															
Trimethoprim		34.86	34.49	80		56.7-74.7		1-99	1.4		-10.6	<0-81.6			<0-20	<0			
Sitagliptin		31.42	32.76			24.4-44.1													
Codeine		30.76	25.93	74		93.5-95.5									1-93	38	4.8	<0-98	75
Celiprolol	30.69	21.90						36.4		-1.1						7.8			



Bisoprolol		29.08	23.34						20.3						36			
Methocarbamol		28.92	21.86	ND														
Amantadine		28.59	23.72															
Fluopyram		26.74	26.74	ND														
Cetirizine		24.71	25.34												8.1			
Amisulpride		22.76	24.93	ND														
Oxazepam		21.97	24.41		(-26)- 38.7		39.8		-17						7.5			
Milnacipran		21.38	14.34	ND														
Flecainide		18.47	13.65												2.8			
Diclofenac		18.36	16.39	47			46.8-94	34.6									<0-98	
EDDP		17.86	15.50	5														
Carbamazepine		15.75	11.80	13	0.36- 25.1		0-94.9	-5.7	-12	-6.6					6-31	19	<0	<0-83
Venlafaxine		14.55	17.80	60	16.8- 66.3				1	7.7					<0-11	<0		
Terbutryn		12.34	-6.07			24,19												
Disopyramide		10.59	6.25													0		
Lidocaine		6.49	27.10		11.7- 27.4												<0	
Fluconazole		2.49	3.50							15.4								
Diuron		-7.55	-113.45												26.7-71.9			
Irbesartan		-9.49	-12.99	28						8.1					<0-88	<0		
Tiapride		-49.80	-41.54	ND														
Clopidogrel		-96.73	-110.46												<0-69	21		

901

902

903