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Abstract

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

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

Key-words

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

1. Introduction

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

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

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

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

Moreover, tens of thousands of chemicals chemicals are registered for commercial use in Europe. Monitoring of small sets of micropollutants can overlook highly ecotoxic substances and lead to a bias in the final risk assessment for aquatic organisms. Analytical methods for EPs that are more comprehensive than the routinely used targeted methods are needed (Hug et al., 2014). In this context, high-resolution mass spectrometry is a promising tool that makes it possible to progress from the screening of one hundred to several thousand analytes (Brack et al., 2019). It is increasingly used to carry out the so-called "suspect screening" chemical analysis (Pinasseau et al., 2019; Ccanccapa et al. 2019; Wang et al., 2019). This kind of screening is based on the comparison of molecular characteristics of unknown components detected in the sample with databases of suspect compounds. The correspondence between these characteristics allows the identification of the compounds present. This approach has been developed and applied to identify different families of EPs such as pharmaceuticals, pesticides, surfactants, industrial chemicals (e.g. chemical synthesis intermediates, additives) and their degradation products/metabolites in wastewaters (Deeb et al., 2017; Gros et al., 2017; Hug et al., 2014; Singer et al., 2016; Wang et al., 2019). This strategy was also applied to evaluate the efficiency of WWTP treatments to remove micropollutants, but only based on the comparison of relative peak areas between the WWTP treated and untreated effluents (Deeb et al., 2017; Wang et al., 2019). The qualitative data did not allow the calculation of precise removals, given the differences in matrix complexity of the two types of water. The combination of suspect screening with confirmation and quantification of identified compounds using analytical standards could give a more exhaustive and comprehensive WWTP efficiency assessment, and load 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 °C. Working solutions were prepared in ultra-pure
105 water, stored at 4 °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

characteristics (population connected, incoming annual flow rate, treatments) are provided in Figure 1 and Table 1, respectively. WWTP daily average incoming flow rates vary from 157 to 215092 m3/day, with a fairly good correlation with the incoming pollution load (3025 to 622800 population equivalent (PE)). Only WWTP 3 collects exclusively WW from an industrial area. All the others receive a variable proportion of industrial effluents, between 0.11 and 32.11 %. Regarding WWTP 7, 8, 9 and 10, hospital effluents are also connected to the municipal network. All WWTPs are equipped with three pretreatment systems: a screening, a grit chamber and a grease remover. Three main primary treatments (coagulation-flocculation, sedimentation tank, buffer tank) and three secondary treatments (activated sludges, biofilter, radial flow fluidized filter) are employed, in function of the WWTP. Finally, an advanced tertiary treatment based on a biofiltration (Biostyr®) is implemented in WWTP 10, to reduce suspended solids, carbon and nitrogen pollution.

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

2.3. Conventional physico-chemical analyses

Conventional physico-chemical parameters (such as pH, conductivity, Total Suspended Solids (TSS), Chemical Oxygen Demand (COD)) and anion/cation concentrations (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_2^-, PO_4^{3-}, NO_3^{2-}, SO_4^{2-}) were quantified according to European standards, as described by Perrodin et al. (2016). Anion/cation analyses were performed after filtering the samples through 0.45 µm (dissolved fraction) while the other parameters were measured on the whole effluents.

2.4. Extraction and analysis of EPs

2.4.1. Sample preparation and extraction

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

2.4.2. LC-QToF-MS suspect screening and quantification

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

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

3000 Ultra High Performance Liquid Chromatography (UHPLC) system from Thermo Scientific® (MA, USA) coupled with a Quadrupole Time of Flight mass spectrometer from Bruker Daltonics® Maxis Plus. Analyses were carried out in reverse phase (elution gradient) employing an Acclaim RSLC 120 C18 column (2.2 µm, 100 x 2.1 mm, Thermo Scientific®), protected with a KrudKatcher Ultra In-Line Filter guard column from Phenomenex (Torrence, CA, USA) and heated at 30 °C. The injected volume was 5 µL. Mobile phases consisted of: an aqueous phase (90%/10% ultrapure water/methanol mixture with 5mM ammonium formate and 0.01% formic acid) and an organic phase (methanol with 5mM ammonium formate and 0.01% formic acid). More details on gradient elution and mass spectrometer calibration are available in Pinasseau et al. 2019. For quality assurance and quality control (QA/QC), several laboratory control samples were performed. The accuracy of the mass detector was checked at the start of each cycle and recalibrated if the mass error was more than 0.5 ppm. Every 12 injections, a quality control was injected to verify sensitivity and retention times during data acquisition. A mixture of 10 µl of each extract was prepared and spiked to 500 µg/L with a standard solution of 53 compounds (Table S2). These compounds were also used to adjust retention times in the database. Background signals were identified by analysis of blanks (solvent, procedural, and trip blank). Solvent blanks were also injected to monitor column carryover. All extracts were analyzed in positive electrospray ionization with the following settings: capillary voltage of 3600 V, end plate offset of 500 V, nebulizer pressure of 3 bar (N_2), drying gas of 9 L/min (N_2), and drying temperature of 200 °C. The analysis was performed in broadband Collision Induced Dissociation acquisition mode over the mass range of 80-1000 Da with a scan rate of 2 Hz at 40eV. With this Data Independent Acquisition mode (DIA), all compounds are fragmented in the collision cell, without prior ion selection. Data were acquired with OtofControl 4.1 and Hystar 4.1, Bruker Daltonics® software and processed using TASQ (Target Analysis for Screening and Quantitation) software (version 1.4, Bruker Daltonics®).

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

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

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

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

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

2.4.3 LC-MS/MS confirmation

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

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

2.5. Pollutant mass loads and removal assessment

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

$$DML_x = \frac{Conc_x \times (FR \times 1000)}{10^9} \quad (1)$$

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

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

$$RR = (1 - \frac{DML_{effluent}}{DML_{influent}}) \times 100 \quad (2)$$

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

3. Results and discussion

3.1. Conventional physico-chemical parameters

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

French Order (N°223 09/23/2017) on collective sewerage systems: values respectively below 35 mg/L and 125 mg O_2/L (Pasquini et al., 2014). Moreover, the mean removal rate by WWTPs for these two parameters is respectively 94.47 % and 92.37 %, while this Order imposes an efficiency of up to 90 % and 75 % for the largest WWTPs (BOD5 > 1,2 kg/day). These values show the normal and efficient functioning of the studied WWTPs during the three sampling campaigns. Accordingly, the removal rates for the various detected EPs can be considered as typical and representative of the 10 WWTP's efficiency (Pasquini et al., 2014).

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

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

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

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

3.3. Occurrence and concentrations of emerging pollutants in raw wastewaters

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

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

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

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

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

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

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

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

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

Concerning pesticides, three of them were highly detected into the influents (≥80%): diuron (med. Conc.= 19.46 ng/L), terbutryn (23.99 ng/L) and DEET (295.07 ng/L). These results are in accordance with European scientific literature. For example, Gasperi et al. (2008) detected diuron in all their wastewater samples from Paris, France, with a concentration range of 0.03-0.47 µg/L (present case: < LOQ - 1.43 µg/L). Conversely, they did not detect terbutryn, which is in contradiction with this study, and can be explained by a LOQ higher (60 ng/L) than the current one. Morevover, Köck-Schulmeyer et al. (2013) also observed a diuron median concentration of 42.2 ng/L (detection frequency: 88%) in the influent of Spanish WWTPs. The omnipresence of these two 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 < 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 % < 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 % < 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

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

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

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

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

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

Ecotoxicological hazard related to treated WW pollution can be discussed by comparing the median concentration (See Table S6) of EPs in effluents (Measured Environmental Concentration - MEC) with their environmental threshold values (PNEC - Predicted No Effect Concentration) in order to calculate related median hazard quotients (HQmed = MEC/PNEC) (Gosset et al., 2017). An HQmed value above 1 implies a significant ecotoxicological hazard for aquatic ecosystems. For example, PNEC values determined for atorvastatin (0:.26 ng/L), atenolol (5 ng/L), citalopram (6.35 ng/L), diclofenac (20 ng/L) and telmisartan (26 ng/L) by Orias and Perrodin (2013) and Zhou et al. (2019) led us to calculate HQmed values of 358.42, 73.05, 18.28, 39.27 and 3.42, respectively. These significant and high-hazard values are in accordance with recent studies (e.g. Ramírez-Morales et al., 2020) and illustrate that it would be crucial to assess the final ecotoxicological risk associated with

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

4. Conclusion and perspectives for further study

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

Acknowledgment

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

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

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

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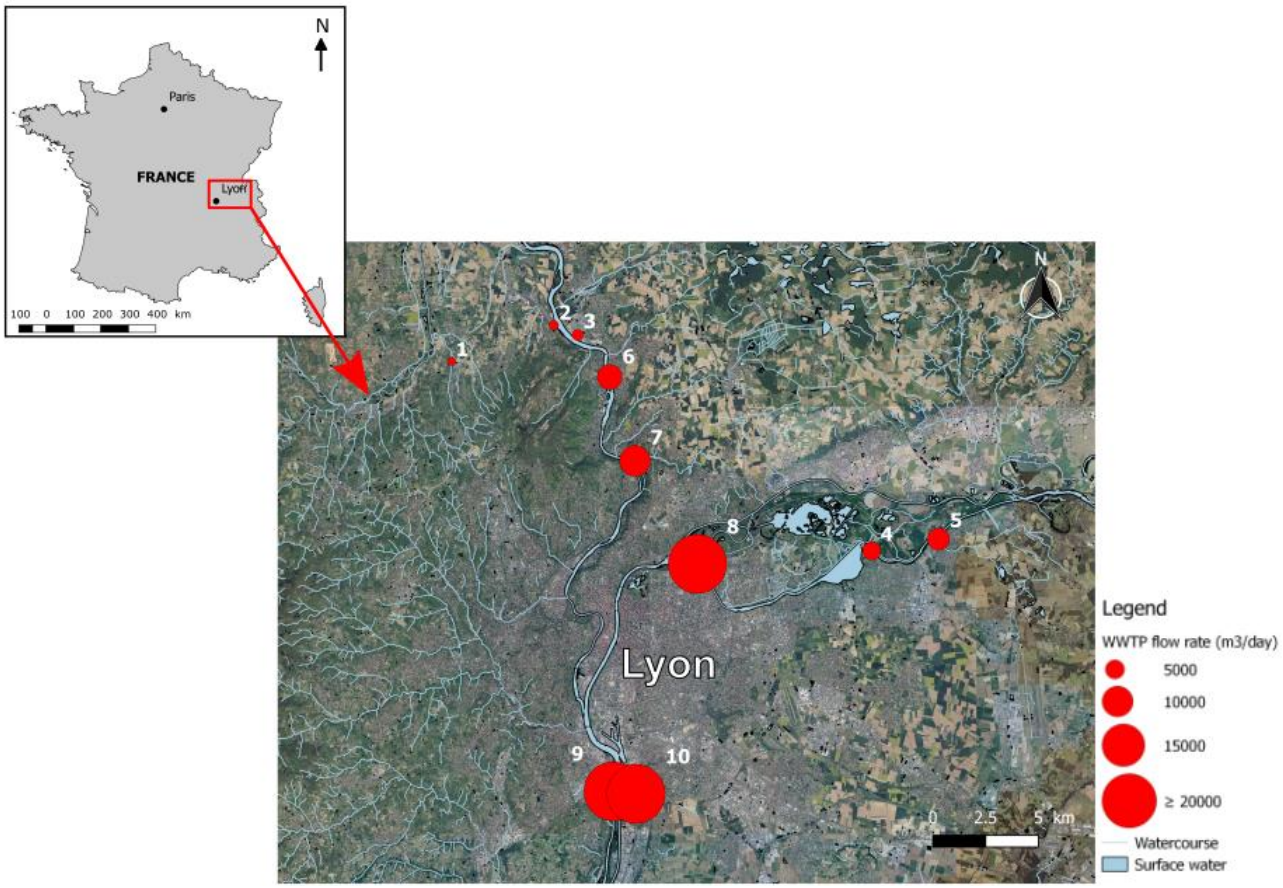

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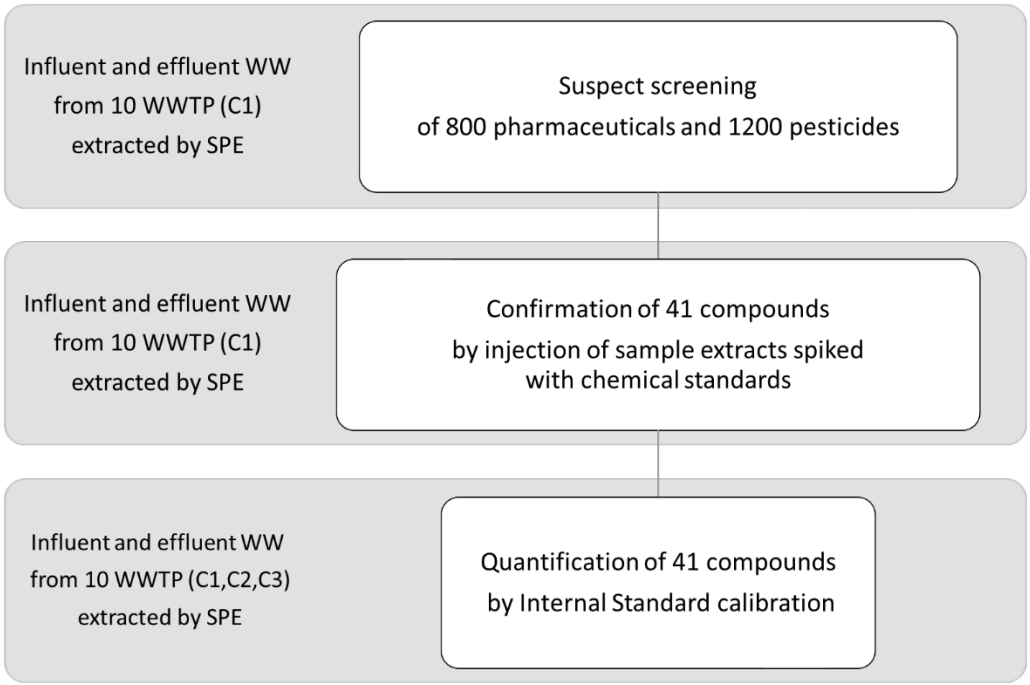

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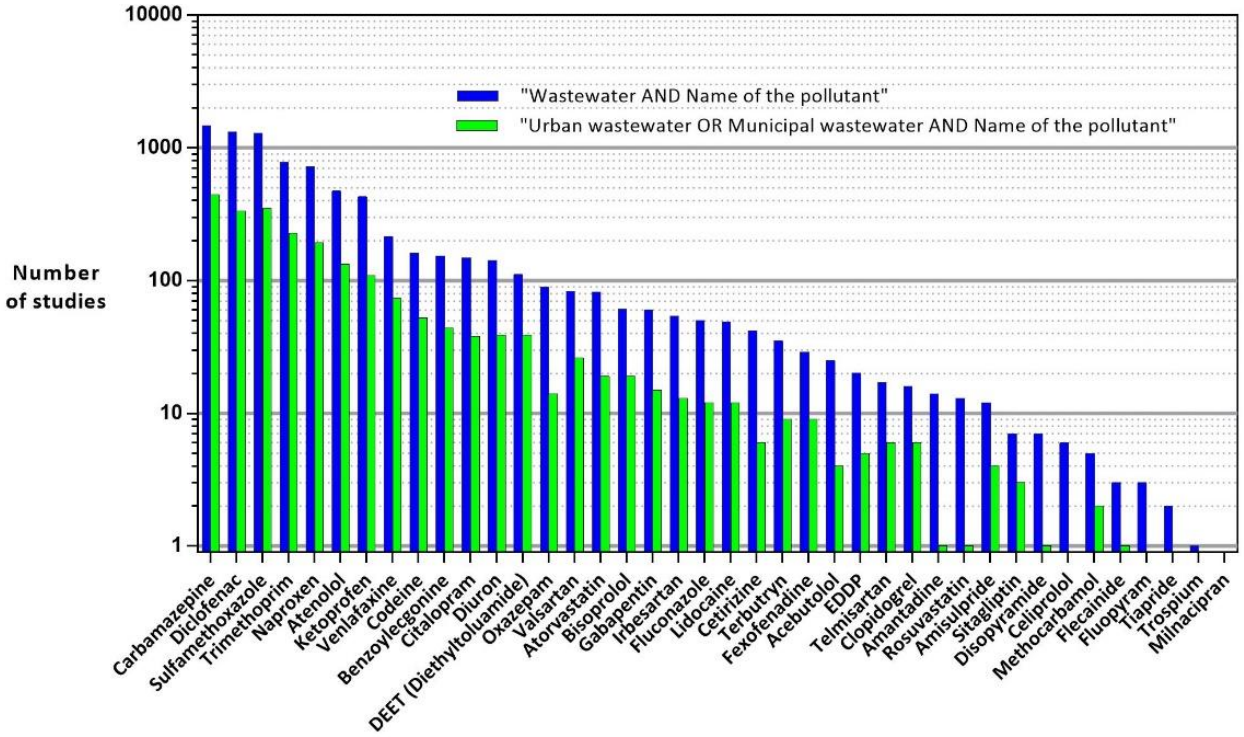

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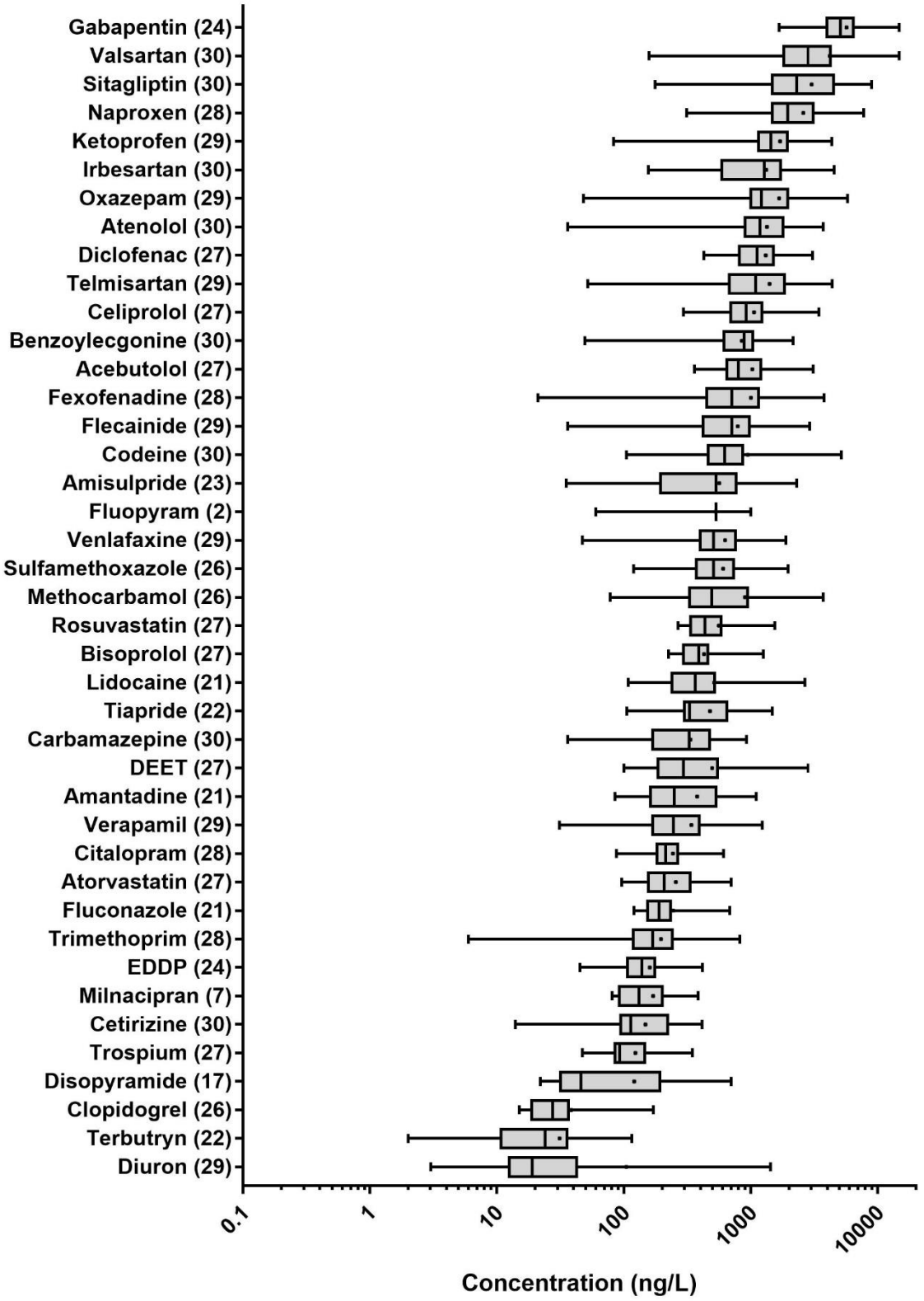

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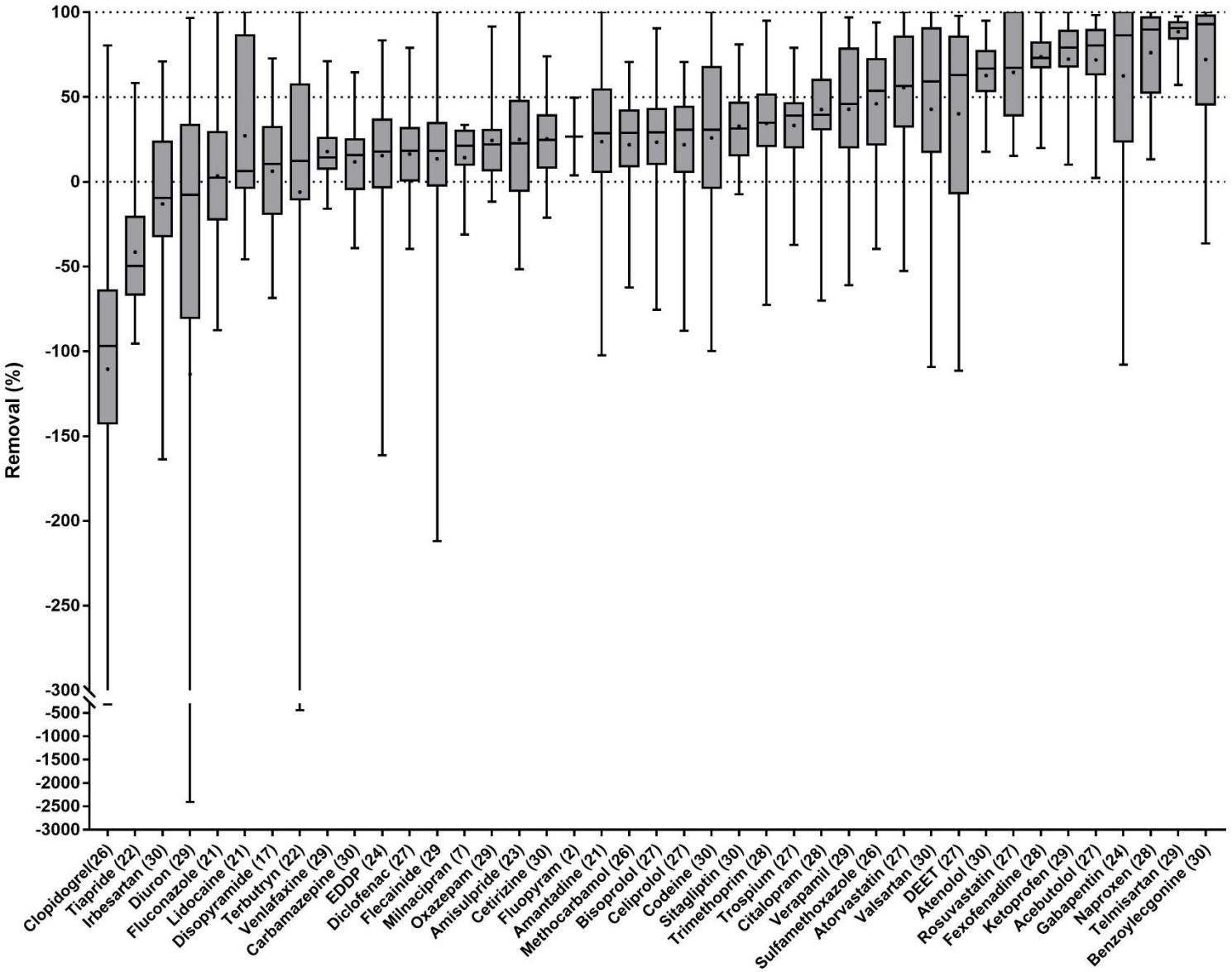

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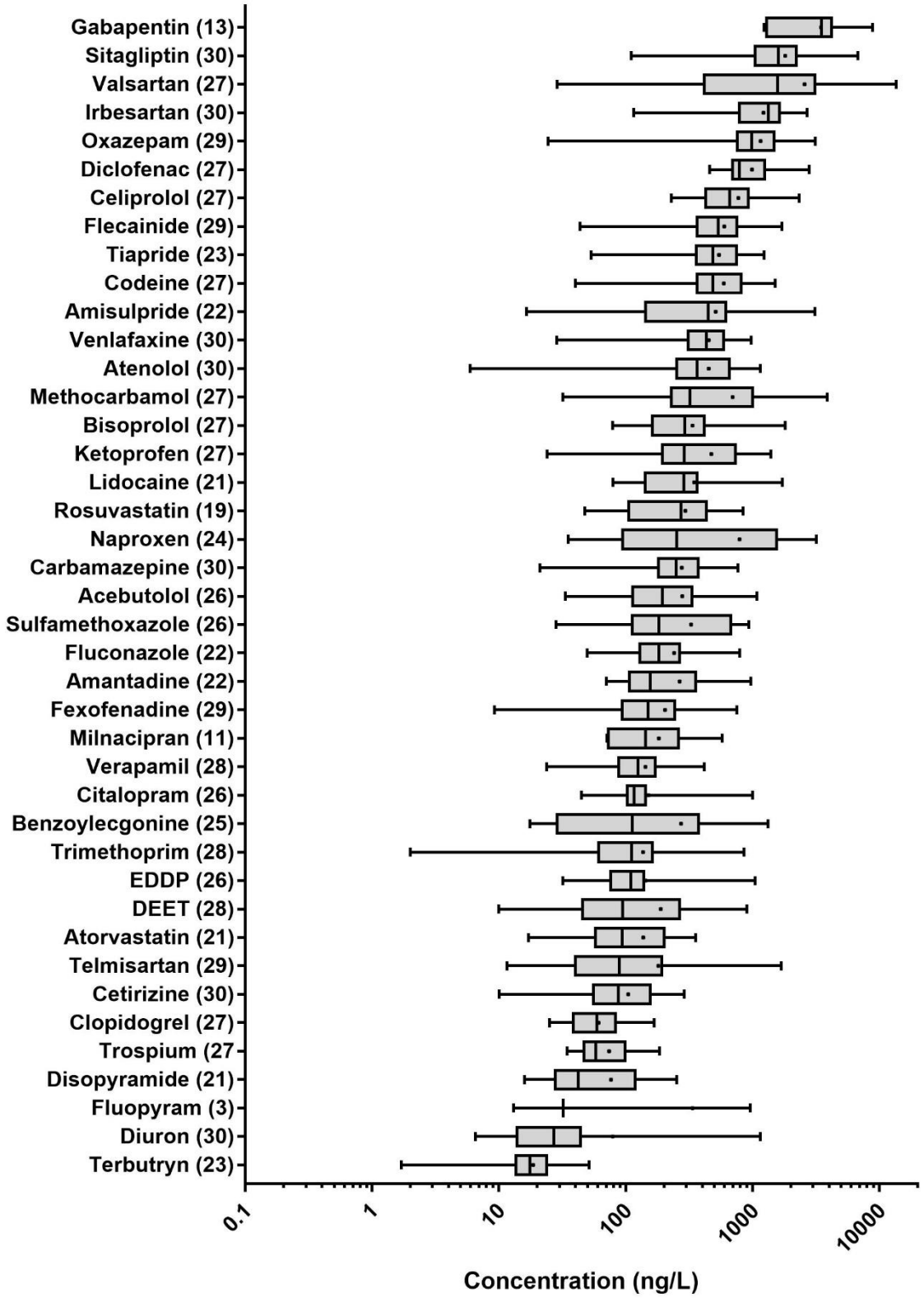

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890 brackets. The dot corresponds to the mean.

891 Table 1: Characteristics of the 10 studied WWTPs

N°	Incoming load in Population Equivalent (PE)	Incoming annual flow rate of the WWTP (m^3/day)	Design capacity (PE)	Design flow rate (m^3/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 intlet	Secondary treatment outlet
3	9150	1300	10000	1300	Screening, grit chamber and grease remover	Buffer tank	Radial flow fluidized filter/ Syncopated aeration	None	WWTP intlet	Secondary treatment outlet
4	25732	4016	33300	8730	Screening, grit chamber and grease remover	Sedimentation tank	Biofilter	None	Pretreatment intlet	Secondary treatment outlet
5	21800	5544	42000	9900	Screening, grit chamber and grease remover	Sedimentation tank	Activated sludges	None	Pretreatment intlet	Secondary treatment outlet
6	16165	6745	34100	18000	Screening, grit chamber and grease remover	Coagulation-flocculation and sedimentation tank	Activated sludges	None	Pretreatment intlet	Secondary treatment outlet
7	44087	8980	30000	9670	Screening, grit chamber and grease remover	Sedimentation tank	Biofilter	None	Pretreatment intlet	Secondary treatment outlet
8	179772	38188	300000	91000	Screening, grit chamber and grease remover	Sedimentation tank	Activated sludges	None	WWTP intlet	Secondary treatment outlet
9	524325	156962	950000	300000	Screening, grit chamber and grease remover	Sedimentation tank	Activated sludges	None	Pretreatment intlet	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 intlet	Tertiary treatment outlet

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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	µS/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_2/L	105.00	616.00	588.37	982.00	30/30	13.00	32.85	41.313	109	30/30
Cl^-	mg/L	57.75	114.45	135.45	361.68	30/30	57.38	121.97	126.82	222.97	30/30
NO_2^-	mg/L	n.d.	0.62	0.75	1.83	6/30	n.d.	1.50	1.61	3.78	9/30
NO_3^-	mg/L	n.d.	6.39	8.64	40.40	25/30	1.87	18.07	32.53	139.38	30/30
PO_4^{3-}	mg/L	n.d.	9.37	9.71	18.62	28/30	n.d.	5.96	6.59	14.90	21/30
SO_4^{2-}	mg/L	39.81	53.99	55.44	110.08	30/30	35.41	51.21	53.53	92.01	30/30
NH_4^+	mg/L	6.68	48.84	47.38	81.66	30/30	0.34	4.67	15.62	63.58	30/30
K^+	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

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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 - Conventionnal 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
Benzoylecgonine	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	Poor removal efficiency (0 % < MRR < 30 %)	29.08	23.34							20.3						36		
Methocarbamol		28.92	21.86	ND														
Amantadine		28.59	23.72									(-109) - 41.7						
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			<0–81.4						<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	<0–62.3			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	Negative removal efficiency (MRR < 0 %)	-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			

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