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Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment

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Abstract

During 8 sampling campaigns carried out over a period of two years, 72 samples, including influent and effluent wastewater, and sludge samples from three conventional wastewater treatment plants (WWTPs), were analyzed to assess the occurrence and fate of 43 pharmaceutical compounds. The selected pharmaceuticals belong to different therapeutic classes (i.e. analgesics and anti-inflammatory drugs, anti-ulcer agent, psychiatric drugs, antiepileptic drug, antibiotics, ß-blockers, diuretics, lipid regulator and cholesterol lowering statin drugs). The obtained results showed the presence of 32 target compounds in wastewater influent and 29 in effluent, in concentrations ranging from low ng/L to a few µg/L (e.g. antiinflamatories). The analysis of sludge samples showed that 21 pharmaceuticals accumulated in sewage sludge from all three WWTPs in concentrations up to 100ng/g. This indicates that even good removal rates obtained in liquid phase (i.e. comparison of influent and effluent wastewater concentrations) do not imply degradation to the same extent. For this reason, the overall removal was estimated as a sum of all the losses of a parent compound produces by different mechanisms of chemical and physical transformation, biodegradation and sorption to solid matter. The target compounds showed very different removal rates and no logical pattern in behavior even if they belong to the same therapeutic groups. What is clear is that the elimination of most of the substances is incomplete and improvements of the wastewater treatment and subsequent treatments of the produced sludge are required to prevent the introduction of these micro-pollutants in the environment.

Keywords: pharmaceuticals, wastewater treatment, wastewater, sludge, removal rate

1. Introduction

Pharmaceuticals are a large and diverse group of compounds designed to prevent, cure and treat disease and improve health. They have long been used in significant quantities throughout the world. Their usage and consumption are increasing consistently due to the discoveries of new illnesses and drugs, the expanding population and the inverting age structure in the general population, as well as due to expiration of patents with resulting availability of less expensive generics (Daughton, 2003). After intake, these highly active compounds undergo metabolic processes in organism. Significant fractions of the parent compound are excreted in unmetabolized form or as an active metabolite to raw sewage and wastewater treatment systems. Sewage treatment plant effluents are discharged to water bodies or reused for irrigation, and biosolids produced are reused in agriculture as soil amendment or disposed to landfill. Thus body metabolization and excretion followed by wastewater treatment is considered to be the primary pathway of pharmaceuticals to the environment.

Continual improvements in analytical equipment and methodologies enable measuring of pharmaceuticals at lower and lower concentration levels in different environmental matrices. Pharmaceuticals and their metabolites in surface waters and aquatic sediment were subject of numerous studies about pharmaceuticals in the environment (Pérez et al., 2007; Bartelt-Hunt et al., 2009; Grujic et al., 2009; Khetan et al., 2007; Miller et al., 2008; Nilsen, 2007; Gros et al., 2006; Hernando et al., 2006; Ellis, 2006; Vazquez-Roig et al., 2010). Several studies investigated occurrence and distribution of pharmaceuticals in soil irrigated with reclaimed water (Gielen et al., 2009; Ternes et al.,

2007; Kinney, 2006)) and soil that received biosolids **(**Carbonell et al., 2009; Sabourin et al., 2009; Lapen et al., 2008; Topp et al., 2008) from urban sewage treatment plants. Results of these studies indicated that wastewater treatment plants are not enough efficient to remove these micropollutants from wastewaters so they find their passage to the environment. Once entered the environment, pharmaceutically active compounds can produce subtle effects on aquatic and terrestrial organisms, especially on the former since they are exposed to long term exposure by wastewater effluents. Several studies investigated and reported on it (Schnell et al., 2009; Pomati et al., 2006; Cleuvers, 2004; Laville et al., 2004; Nentwig et al., 2004).

Therefore, the occurrence of pharmaceutical compounds and the extent to which they can be eliminated during wastewater treatment have become active subject matter of actual research. Conventional systems that use an activated sludge process are still widely employed for wastewater treatment, mostly because they produce an acceptable quality effluent at reasonable operating and maintenance costs. But this type of treatment has limited capability of removing pharmaceuticals from wastewater (Kasprzyk-Hordern et al., 2009; Gros et al., 2010; Wick et al., 2009). Most of the studies on the fate of pharmaceuticals in WWTPs focused only on aqueous phase, and concentrations of the compounds in sludge were rarely determined mainly due to the demanding efforts required in the analysis in this difficult matrix. Out of 117 publications studied by Miege et al (Miège et al., 2009), only 15 reported the concentrations of pharmaceuticals in sludge and 1 in suspended solid, and none of these papers reported removal obtained taking into account both liquid and solid phases of WWTPs. The screening of sewage sludge showed that these micropollutants are very present in this medium (Radjenovic et al., 2009; McClellan et al., 2010; Okuda et al., 2009; Díaz-Cruz et al., 2009; Lillenberg et al., 2009; Nieto et al., 2009).

In this study we aimed to determine the contamination of wastewater and sludge with 43 pharmaceutical compounds in order to obtain more in-depth information on their fate during conventional wastewater treatment. The selected pharmaceuticals belong to different therapeutic groups (i.e. analgesics and anti-inflammatory drugs, anti-ulcer agent, psychiatric drugs, antiepileptic drug, antibiotics, ß-blockers, diuretics, lipid regulator and cholesterol lowering statin drugs, anti-histamines). The samples were provided from three conventional full-scale activated sludge sewage treatment plants with anaerobic digestion of sludge, from the region of Catalonia (Spain). The preparation and analysis of the samples were performed using high performance liquid chromatography coupled to a hybrid triple quadrupole – linear ion trap mass spectrometer (HPLC-QLIT- MS/MS) according to the previously developed multi-residual methodologies for analysis of pharmaceuticals in wastewater and sludge samples (Gros et al., 2009; Jelic et al., 2009).

2. Experimental part

2.1. Chemicals

All the pharmaceutical standards for target compounds were of high purity grade (>90%). Ibuprofen, Naproxen, Ketoprofen, Diclofenac and Gemfibrozil were supplied by Jescuder (Rubí, Spain). Acetaminophen, Indomethacin, Mefenamic acid, Phenazone, Bezifibrate, Mevastatin, Fenofibrate, Pravastatin (as sodium salt), Carbamazepine, Famotidine, Ranitidine (as hydrochloride), Cimetidine (as hydrochloride), Erythromycin (as hydrate), Azithromycin (as dehydrate), Roxitromycin, Clarithromycin, Josamycin, Tylosin A, Sulfamethazine, Trimethoprim, Chloramphenicol, Atenolol, Sotalol, Metoprolol

(as tartrate), Timolol, Pindolol, Nadolol, Salbutamol, Clenbuterol (as hydrochloride), Enalapril (as maleate), Glibenclamide, Furosemide, Hydrochlorothiazide and Metronidazole were purchased from Sigma-Aldrich (Steinheim, Germany). Standard Atorvastatin (as calcium salt) was provided by LGC Promochem (London, UK), while Diazepam, Lorazepam and Butalbital were from Cerilliant (Texas, USA).

The isotopically labelled compounds, used as internal standards, were Sulfamethazine-d_4, Famotidine-$^{13}C_3$, rac-Timolol-d_5 maleate, Clarithromycin-N-methyl-d_3, Atorvastatin-d_5 sodium salt, Fenofibrate-d_6, Metoprolol-d_7, Metronidazole hydroxyl-d_2, Pravastatin-d_3, Ketoprofen-$^{13}CD_3$, Indomethazine-d_4, rac-Naproxen-d_3, Mefenamic acid-d_3, Gemfibrozil-d_6, Bezafibrate-d_4 and Furosemide-d_5 from Toronto Research Chemicals; Diazepam-d_5 and Phenobarbital-d_3 from Cerilliant (Texas, USA); Atenolol-d_7, Carbamazepine-d_{10}, Ibuprofen-d_3, Clotrimazole-d_5, Enalapril–d_5, Hydrochlorothiazide-d_2, Glyburide-d_3, Albuterol-d_3, Cimetidine-d_3, Antipyrine-d_3, Acetaminophen-d_4, Diclofenac-d_4, Clofibric-d_4 acid, Hydrochlorothiazide-3,3-d_2 from CDN Isotopes (Quebec, Canada); Sotalol hydrochloride d_6 from Dr. Ehrenstorfer (Augsburg, Germany) and Erythromycin-$^{13}C,d_3$ (N-Methyl-$^{13}C,d_3$) from Isotec (Ohio, USA).

The solvents, HPLC grade methanol, acetonitrile, water (Lichrosolv) and formic acid 98% were provided by Merck (Darmstadt, Germany). Nitrogen used for drying from Air Liquide (Spain) was of 99.995 % purity.

The cartridges used for solid phase extraction were Oasis® HLB (200mg, 6mL) from Waters Corporation (Milford, MA, USA). The syringe filters of 0.45µm pore size were purchased from Pall Corp (USA).

The individual standard solutions as well as isotopically labelled internal standard solutions were prepared on a weight basis in methanol. Furosemide and Butalbital were

obtained as solutions in acetonitrile, while Lorazepam and Diazepam were dissolved in methanol, at a concentration of 1 mg/mL. The solutions were stored at -20ºC. Fresh stock solutions of antibiotics were prepared monthly due to their limited stability while stock solutions for the rest of substances was renewed every three months. A mixture of all pharmaceuticals was prepared by appropriate dilution of individual stock solutions in methanol-water (25:75, v/v) and it was renewed before each analytical run. A separate mixture of isotopically labelled internal standards, used for internal standard quantification, was prepared in methanol and further diluted in methanol-water (25:75, v/v) mixture.

2.2. Sample collection

Samples (i.e. influent and effluent wastewater, and sewage sludge) were obtained from three full-scale wastewater treatment plants (WWTPs) in the region of Catalonia (Spain). All the samples were collected in eight sampling campaigns between July 2007 and March 2009, in campaign intervals of 2 to 3 months. Composite influent and effluent waste water samples (24h) were collected in 1L amber glass bottles and kept on 4ºC until extraction (within 48 hours). Prior to extraction, the water was vacuum filtered through 1µm glass fiber filters, followed by 0.45µm nylon membrane filters (Teknokroma, Barcelona, Spain). Sludge samples were freeze-dried (LioAlfa 6, Telstar) at -50 ºC and under 0.044 bar vacuum and stored at -20 ºC until the analysis.

Composite wastewater samples were collected at the entrance of the treatment plant i.e. influent wastewater and at the exit of the plants i.e. effluent wastewater (after the secondary treatment at WWTP2 and WWTP3, and after the tertiary one in WWTP1). The

analyzed samples of sludge were collected at the final phase of the process, i.e. treated sewage sludge.

In Table 1 are summarized some characteristics of the three investigated wastewater treatment plants. WWTP1 and WWTP2 treat predominantly municipal waste water, while the WWTP3 influent has an important industrial contribution. The WWTP1 is designed for 210000 equivalent inhabitants (eq.inh.) and to treat up to 47500 m^3/day of wastewater. It is situated in the tourist coastal area where the amount and the quality of water entering the plant are significantly affected by the seasonal population growth. The wastewater flow in WWTP1 changes from 15000, during the winter months, to 32000 m^3/day during the summer months. The WWTP 2 can treat up to 35000m^3/day of wastewater serving a population equivalent of around 170000. It usually works with 80% of designed treatment capacity, with fairly constant flow rate of water of approx.25000 m^3/day (in 2009). The WWTP3 treated an average of 25000m^3/day in 2008, which is about 80% of the total treatment capacity of the plant. The wastewater treatments in all the plants include primary and secondary treatment, and in the case of WWTP1 an additional tertiary treatment. The plants employ biological activated sludge process for wastewater treatment. Sludge generated during primary and secondary treatment is thickened and blended and fed to anaerobic digester system in WWTP2 and WWTP3 and, in the case of the WWTP1, centrifuged and sent to composting.

2.3. Sample preparation

Procedures for preparation of water and sludge samples for instrumental analysis were described in detail previously (Gros et al., 2009; Jelic et al., 2009).

In brief, in the filtered-aliquots of wastewater (100ml for influent and 200ml for effluent) Na_2EDTA was added to a concentration of 0.1vol%. Then the target compounds were separated by solid phase extraction (Oasis HLB cartridges, 6 cc, 200 mg; Waters Corp., Milford, MA) using a Baker vacuum system (J.T. Baker, Deventer, The Netherlands), and concentrated via elution with pure methanol. The 8ml eluents were evaporated under a stream of nitrogen and reconstituted in a methanol-water mixture (25:75). Prior to instrumental analysis, these samples were fortified by a mixture of internal standard to a final concentration of 20ng/ml.

Sludge samples were extracted using an accelerated solvent extraction (ASE) (Dionex ASE 200, Dionex; Sunnyvale, CA). The extractions were carried out using a methanol-water mixture (1:2) as extraction solvent, at 1500 psi and 100 ºC in 3 static cycles, each lasting 5 minutes. Finally, the cell was flushed with 100% cell volume of fresh solvent. Concentrated extracts were dissolved in water in order to reduce the content of methanol (< 5 vol%) and processed further as water samples. Instrumental analysis of all samples was done by HPLC-QLIT-MS/MS.

2.4. Instrumental analysis

The analytical method used in this study was already developed by M. Gros et al (Gros et al., 2009). Samples were analysed using high performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS). LC analysis was performed using Symbiosis™ Pico (SP104.002, Spark, Holland), equipped with an autosampler and connected in series with a 4000 QTRAP Hybrid Triple Quadrupole - Linear Ion Trap mass spectrometer equipped with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster

City, CA, USA). Chromatographic separation was achieved with a Purospher Star RP-18 endcapped column (125mm x 2.0 mm, particle size 5µm) preceded by a C_{18} guard column (4 x 4, 5µm), both supplied by Merck (Darmstadt, Germany).

The mobile phases for the analysis in negative ionization (NI) mode were a mixture of acetonitrile-methanol (1:1, v/v) (i.e. eluent A), and HPLC grade water (i.e. eluent B). The analysis in positive ionization (PI) mode was performed using acetonitrile as eluent A and HPLC grade water with 0.1% formic acid as eluent B. The target compounds were scanned in MRM, monitoring two transitions between the precursor ion and the most abundant fragment ions for each compound. Further information on the methodology and its performances can be found elsewhere (Gros et al., 2009; Jelic et al., 2009).

2.5. Removal rate calculation

In this study we employed a mass balance approach in order to asses quantitatively the removal of the selected pharmaceuticals during wastewater treatment. Even when dealing with such a complex system, we can assume that the WWTP behaves as a black-box with only one entrance (i.e. influent water) and two outlets (i.e. effluent water and treated sludge) and operates at steady state over the studied period of two years. Then, from the measured concentrations and the operation parameters (i.e. flow rates of influent, $\dot{V}_{influent}$, and effluent, $\dot{V}_{effluent}$, and sludge production, $\dot{P}_{sludge}$) could be written as follows:

$$\dot{R}_{Overall} = \dot{m}_{in} - \dot{m}_{out} \quad (1)$$

$$\dot{m}_{in} = \dot{m}_{influent} \quad (2)$$

$$\dot{m}_{out} = \dot{m}_{effluent} + \dot{m}_{sludge} \quad (3)$$

$$\dot{V}_{influent} = \dot{V}_{effluent} = \dot{V}_l \quad (4)$$

$$\dot{R}_{Overall} = c_{influent} \times \dot{V}_l - \left(c_{effluent} \times \dot{V}_l + c_{sludge} \times \dot{P}_{sludge}\right) \quad (5)$$

where $\dot{m}_{in}$, $\dot{m}_{out}$, $\dot{m}_{influent}$, $\dot{m}_{effluent}$ and $\dot{m}_{sludge}$ are the mass flow rate (in g/day) of inlet, outlet, influent liquid, effluent liquid and sludge, respectively. $\dot{R}_{Overall}$ (g/day) is the mass load lost per unit of time due to the sum of all processes that can occur during wastewater treatment. Mass flow rates of pharmaceutical compounds in influent and effluent streams were calculated by multiplying the measured concentrations in a given stream by the appropriate flow rate of that stream. Thus, the concentration of each pharmaceutical in the daily influent and effluent samples ($c_{influent}$ or $c_{effluent}$, [g/m^3]) was multiplied by the flow rate for that day (i.e. $\dot{V}_l$, [m^3/day]) to give the mass of the pharmaceutical entering or leaving the plant that day (g/day) (i.e. daily mass load). Similarly, the concentration of pharmaceuticals in the treated sludge (c_{sludge}, [ng/g d.w.]) was multiplied by the production rate of sludge (tons/day) to determine the mass of pharmaceuticals removed with the sludge (g/day). From these data, both removal from liquid-phase, $R_{Liquid\ phase}$(%), and overall removal (i.e. mass loss), $R_{Overall}$(%), of the target compounds were calculated according to the equations 3 and 4, respectively, and the results are presented in Figure 2:

$$R_{Liquid\ phase}(\%) = 100 \times \frac{c_{influent} \times \dot{V}_l - c_{effluent} \times \dot{V}_l}{c_{influent} \times \dot{V}_l} \quad (6)$$

$$R_{Overall}(\%) = 100 \times \frac{R_{Overall}}{c_{influent} \times \dot{V}_l} \quad (7)$$

3. Results and discussion

3.1. Occurrence of pharmaceuticals in wastewater and sludge

In Table 2 are shown the frequencies of detection and the measured concentrations of the pharmaceutical compounds detected in wastewater and sludge from the studied WWTPs. Out of 43 analyzed pharmaceutical compounds, 32 were detected in influent, 29 in effluent and 21 in sludge samples. The analysis of samples from different campaigns of a given plant showed variation in concentration levels, which is due to changes of the composition of influent waters in different seasons, weather conditions and operational conditions of the plant. For easier interpretation of the results, the concentrations of each pharmaceutical are given as the median and maximum values of concentrations measured for eight sampling campaigns. The concentrations lower than the method detection limits are marked with n.d. (i.e. not detected).

According to the daily loads and population served by each plant, the amount of pharmaceuticals disposed in these plants is estimated to be 5.6, 2.0 and 0.4 g/day/1000 equivalent inhabitants for WWTP1, WWTP2 and WWTP3, respectively (Figure 1). The highest levels at the influent of all three WWTPs were observed for non-steroidal anti-inflammatory drugs (NSAIDs) that were expected due to their high consumption. In addition, topical application of the NSAIDs results in greater discharge of these compounds in unmodified forms. This result is in fairly good agreement with previously reported studies (Gracia-Lor et al., 2010, Miège et al., 2009). At the influent of the plants, this group accounts for ca. 65% of all the therapeutic groups analyzed, as can be seen in the Figure 1.

Naproxen, ketoprofen and diclofenac were detected in all the samples in concentration ranges 4.2-7.2μg/L, 1.1-2.3μg/L and 0.4-1.5μg/L, respectively. Ibuprofen and acetaminophen were not included in the discussion because they yielded to high concentrations which can be due to the strong matrix effect and/or to interactions that may produce false identification and thus incorrect concentration values. Lower but still significant levels of diuretics (~9%), lipid regulators (~9%) and beta-blockers (~6%) were detected entering these WWTPs. Furosemide, bezafibrate, atenolol and anticonvulsant carbamazepine were quantified in all the influent samples from the three WWTPs in concentrations ranging from 0.4 to 1.4μg/L.

The amount found in effluent or sludge depended on the removal efficiency of plant and/or the properties of the compounds. As the influent concentrations give us information about the consumption of the pharmaceuticals, the effluent and the sludge concentrations are important from the environmental point of view, since the pharmaceuticals find their way to the environment through discharges of treated waters to rivers, or disposal of sludge to agricultural and forest land. In the effluent waters, the analgesics and antiinflamatories were present in the highest percentage, i.e. 39%, followed by the lipid regulators (~37%) and psychiatric drugs (~18%) (Figure1). The highest concentrations in the effluents of all the WWTPs were found for naproxen, diclofenac and carbamazepine, and they ranged from 0.4 to 1μg/L depending on the compound and the removal efficiency of the plant. In the treated effluent of WWTP2, ketoprofen, bezafibrate, atenolol and furosemide were detected in much higher concentrations (0.7, 0.4, 0.4 and 0.9μg/L, respectively) than in the other two plants. Analysis of sludge samples showed the presence of 21 out of 43 analyzed pharmaceuticals, where diuretics accounted for ca. 19%, antibiotics ca. 18% and lipid regulators ca. 16% of all the pharmaceuticals analyzed. Hydrochlorthiazide, furosemide,

atorvastatine, clarithormycin, carbamazepine and diclofenac were ubiquitous in samples from all three WWTPs, in concentrations from 30 to 60ng/g. On the other hand, β-blockers, β-antagonists and histamine H_2-antagonists were found in very low concentrations in sludge. The total loads of analyzed pharmaceuticals that leave the plants unmodified (including sludge and effluent water) were calculated to equal 1.1, 0.9 and 0.1 g/day/1000 equivalent inhabitants for WWTP1, WWTP2 and WWTP3, respectively, of which only 3-9% (depending on the plant) was retained by sludge (Figure 1). The amount of pharmaceutical compounds detected in this study exiting the plants is not of great concern if we compare it with the results from some other studies done in this field (Zorita et al., 2009; Castiglioni et al., 2005).

3.2. Overall removal of pharmaceuticals during wastewater treatment

The daily mass loads of target compounds in wastewater influent and effluent, and in sludge, in g/day, were calculated as explained previously, and these values were used for the estimation and the comparison of the liquid phase and the overall removal rates (Figure 2). Considering the fact that pharmaceuticals are grouped by the therapeutical applications for which they are used and not on the basis of their physico-chemical similarity, their removal during treatment is expected to be diverse. Here the term removal refers to the conversion of a pharmaceutical to a compound different than the analyzed one (i.e. the parent compound). Thus, the overall removal refers to all the losses of a parent compound produced by different mechanisms of chemical and physical transformation, biodegradation and sorption to solid matter.

High liquid-phase removal rates for some compounds (i.e. lipid regulator fenofibrate and hystamin H2 receptor antagonist famotidine) would suggest very good removal of these compounds during the wastewater treatments. But, as shown in the Figure 2, only a certain percent of the total mass input is really lost during the treatments (overall removal). The rest was accumulated in sludge or discharged with the effluent. Sorption of fenofibrate, atorvastatine, diazepam and clarithromycin contributed to the elimination from the liquid phase with more than 20% related to the amount of these compounds at the influent. This finding clearly indicates the importance of the analysis of sludge when studying wastewater treatment performances. Since many of the analyzed compounds were found in the sludge samples, the overall removal rate was the parameter used to compare the removal performances of the studied treatment plants.

In general, the removal rates varied strongly without evident correlation to the compound structure, as can be seen in the Figure 2. The antihypertensive enalapril and NSAIDs ketoprofen and naproxen were removed in all the three cases with very good removal efficiency (>80%) and they did not accumulate in sludge. Similar removal of these compounds from liquid phase, under conventional treatment conditions, was observed in various studies on this topic (Zorita et al., 2009; Gros et al., 2010; Sim et al., 2010; Lishman et al., 2006). But then, the most analyzed anticonvulsant carbamazepine showed very low removal (<25%) regardless of the treatment applied. The results concerning its persistence and ubiquitous occurrence match with those from previous studies (Joss et al., 2005; Radjenovic et al., 2007; Pérez et al., 2007). No significant overall removal during the studied treatments (<30%) was observed for antibiotics trimethoprim and metronidazole, NSAIDs mefenamic acid, hystamin H2 receptor antagonist famotidine and benzodiazepine lorazepam. A benzodiazepine diazepam and antimicrobial chloramphenicol were detected

352 in concentrations close to their corresponding LOQs thus no reliable conclusion could be
353 made on their behaviour.

354 Cholesterol lowering statin drugs pravastatin and mevastatin, antibiotics
355 sulfamethazine and metronidazole, β-blockers metaprolol and timolol, β-antagonist
356 salbutamol were not acumulated in sludge and they showed a variety of removal rates
357 between 30 and 80%. Inconsistent overall removal was also observed for NSAIDs
358 diclofenac and indometacin, hystamin H_2 receptor antagonists cimetidine and ranitidine,
359 and diuretic furosemide. It seems that for the mentioned compounds, removal was mainly
360 influenced by operational conditions and treatment technology used. Comparing to the
361 other two plants, WWTP1 offers much better removal for the majority of the analyzed
362 compounds (Figure 2). This activated sludge plant featured by a tertiary treatment in
363 WWTP1 improves the removal of diclofenac to 60%, while in the other two plants removal
364 is much lower (<24%). Low removals of diclofenac were already reported in some
365 publications on this topic (Quintana et al., 2005; Kimura et al., 2007; Cirja et al., 2008)
366 imputed its persistence to the presence of chlorine group in the molecule. Some studies on
367 removal during wastewater treatment showed no influence of solid retention time on the
368 removal of diclofenac (Clara et al., 2005; Kreuzinger et al., 2004; Lishman et al., 2006).
369 Furosemide, pravastatin, and ranitidine that were eliminated with removal ca. 80% and
370 60% in WWTP1 and WWTP3, respectively, marked very low (ca.30%) removal rates in
371 WWTP2. Better performances of WWTP1 and WWTP3 may be due to longer both
372 hydraulic and solid retention times which are proved to influence the elimination of most of
373 the pharmaceuticals during sewage treatment (Clara et al., 2005; Göbel et al., 2007; Reif et
374 al., 2008; Suárez S et al., 2005). If a compound spends more time in reactors then bacteria

growth is promoted so biological transformation may occur to a greater extent (Reif et al., 2008).

The negative values of removal rates (omitted in the Figure 2) refer to an increase in the concentration of an analyzed parent compound during treatment. This phenomenon of "negative removal" for some compounds was already reported in the literature (Gros et al., 2009; Wick et al., 2009; Joss et al., 2005). Hydrochlorothiazide was not detected in influent neither effluent water samples, but it was detected in sludge. This was not at all expected according to its low logP and the fact that >95% of the dose of this pharmaceutical is excreted unchanged (EMEA/CHMP/471165/2009). Lipid-regulating agent gemfibrozil was detected in higher concentration in the effluent than in the influent water samples. Similar was observed for macrolide clarythromycin, anti-diabetic glibenclamide, lipid regulators fenofibrate and atrorvastatin, as well as for carbamazepine in one of the plants, which yielded higher concentration levels at the exit of a plant (i.e. including effluent and sludge) than at its entrance. This could be explained by the formation of unmeasured products of human metabolism and/or transformation products (e.g. glucuronide conjugate, methylates, glycinates etc.) that passing through the plant convert back to the parent compounds. This can be considered as a reasonable assumption since the metabolites and some derivates of the mentioned compounds are well-known (e.g. hydroxy and epoxy-derivatives of carbamazepine; 4-trans-hydroxy and 3-cis-hydroxy derivatives of glibenclamide; ortho- and parahydroxylated derivatives of atorvastatine; gemfibrozil acyl glucuronide etc.) (Miao et al., 2005; Shipkova et al., 2005; Aviram et al., 1998). Gobel et al. (Göbel et al., 2007) proposed gradual release of the macrolides (e.g. clarithormycin) from feces particles during biological treatment as an explanation for the possible negative removal rates for these antibiotics. During complex metabolic processes in human body and bio-chemical in

399 wastewater treatment, various scenarios of transformation from parent compound to
400 metabolite and derivatives and vice versa can occur. These metabolites can be just as active
401 as their parent compounds (http://www.rxlist.com). Therefore, the occurrence of
402 metabolites and transformation products and pathways should be included in the future
403 studies in order to obtain accurate information on removal of pharmaceuticals during
404 treatment and to determine treatment plant capabilities.
405
406 **4. Conclusion**
407
408 This work presents the results obtained in a two year study on the occurrence and
409 fate of the selected pharmaceuticals during conventional wastewater treatment. Out of 43
410 analyzed pharmaceuticals, 32 compounds were detected in wastewaters in concentrations
411 ranging from low ng/L to a few μg/L (e.g. antiinflamatories). The variety of the compounds
412 detected in effluent wastewaters indicates that WWTP outlets are important contributors of
413 pharmaceuticals in the aquatic environment. This study showed that 21 of 43 analyzed
414 compounds accumulated in sludge in concentrations up to 100ng/g. Thus the disposal of
415 sludge to the agricultural land can be another mechanism for the reintroduction of these
416 micro-contaminants into the environment through the WWTPs. For this reason the results
417 of this and similar studies can be considered as very useful for rough estimations of the
418 magnitude of the pharmaceuticals releases from WWTPs into the environment. A simple
419 mass balance involving data for influent and effluent wastewater and sludge, for the
420 pharmaceuticals in the three monitored WWTPs, was employed for calculation of the
421 removal rates. Varying removals and no evident pattern in behavior were observed even for
422 the compounds belonging to the same therapeutic group. Significantly low and even

423 negative removals were observed for some compounds (carbamazepine,
424 hydrochlorothiazide etc.) which can be result of the formation of unmeasured
425 transformation products that passing through the plant convert back to the parent
426 compounds. There are many factors that should be considered while studying wastewater
427 treatment performances. The screening of the metabolites and some other transformation
428 products (such as conjugates: glucuronides, methylates etc.) should be naturally included in
429 the study of wastewater treatment processes. What is clear from the results is that, even
430 though the WWTPs meet the regulatory requirements for wastewater treatment (Directive
431 91/271/EEC), they are only moderately effective in removing pharmaceutical compounds.
432 This is an issue of great importance especially when attempting to reuse wastewater and
433 dispose sludge to agricultural areas and landfills.
434

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446

447 **References**

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449 Aviram, M., Rosenblat, M., Bisgaier, C. L. and Newton, R. S. 1998. Atorvastatin and
450 gemfibrozil metabolites, but not the parent drugs, are potent antioxidants against
451 lipoprotein oxidation. *Atherosclerosis*, 138 (2) 271-280.
452 Bartelt-Hunt, S. L., Snow, D. D., Damon, T., Shockley, J. and Hoagland, K. 2009. The
453 occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and
454 surface waters in Nebraska. *Environmental Pollution*, 157 (3) 786-791.
455 Carbonell, G., Pro, J., Gómez, N., Babín, M. M., Fernández, C., Alonso, E. and Tarazona,
456 J. V. 2009. Sewage sludge applied to agricultural soil: Ecotoxicological effects on
457 representative soil organisms. *Ecotoxicology and Environmental Safety*, 72 (4)
458 1309-1319.
459 Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D. and Zuccato, E. 2005.
460 Removal of Pharmaceuticals in Sewage Treatment Plants in Italy. *Environmental*
461 *Science & Technology*, 40 (1) 357-363.
462 Cirja, M., Ivashechkin, P., Schäffer, A. and Corvini, P. 2008. Factors affecting the removal
463 of organic micropollutants from wastewater in conventional treatment plants (CTP)
464 and membrane bioreactors (MBR) *Reviews in Environmental Science and*
465 *Biotechnology*, 7 (1) 61-78.
466 Clara, M., Kreuzinger, N., Strenn, B., Gans, O. and Kroiss, H. 2005. The solids retention
467 time--a suitable design parameter to evaluate the capacity of wastewater treatment
468 plants to remove micropollutants. *Water Research*, 39 (1) 97-106.

469 Cleuvers, M. 2004. Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen,
470 naproxen, and acetylsalicylic acid. *Ecotoxicology and Environmental Safety*, 59 (3)
471 309-315.
472 Daughton, C. G. 2003. Cradle-to-cradle stewardship of drugs for minimizing their
473 environmental disposition while promoting human health. I. Rational for and
474 avenues toward a green pharmacy. *Environ Health Perspect*, 111 (5) 757–774.
475 Díaz-Cruz, M. S., García-Galán, M. J., Guerra, P., Jelic, A., Postigo, C., Eljarrat, E., Farré,
476 M., López de Alda, M. J., Petrovic, M., Barceló, D., Petrovic, M. and Barceló, D.
477 2009. Analysis of selected emerging contaminants in sewage sludge. *TrAC Trends*
478 *in Analytical Chemistry*, 28 (11) 1263-1275.
479 Ellis, J. B. 2006. Pharmaceutical and personal care products (PPCPs) in urban receiving
480 waters. *Environmental Pollution*, 144 (1) 184-189.
481 European Medicines Agency - Evaluation of Medicines for Human Use,
482 Doc.Ref: EMEA/CHMP/471165/2009 (www.ema.europa.eu/humandocs)
483 Gielen, G. J. H. P., Heuvel, M. R. v. d., Clinton, P. W. and Greenfield, L. G. 2009. Factors
484 impacting on pharmaceutical leaching following sewage application to land.
485 *Chemosphere*, 74 (4) 537-542.
486 Göbel, A., McArdell, C. S., Joss, A., Siegrist, H. and Giger, W. 2007. Fate of sulfonamides,
487 macrolides, and trimethoprim in different wastewater treatment technologies.
488 *Science of The Total Environment*, 372 (2-3) 361-371.
489 Gracia-Lor, E., Sancho, J. V. and Hernández, F. 2010. Simultaneous determination of
490 acidic, neutral and basic pharmaceuticals in urban wastewater by ultra high-pressure
491 liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*,
492 1217 (5) 622-632.

493 Gros, M., Petrovic, M. and Barceló, D. 2006. Development of a multi-residue analytical
494 methodology based on liquid chromatography-tandem mass spectrometry (LC-
495 MS/MS) for screening and trace level determination of pharmaceuticals in surface
496 and wastewaters. *Talanta*, 70 (4) 678-690.
497 Gros, M., Petrovic, M., Ginebreda, A. and Barceló, D. 2010. Removal of pharmaceuticals
498 during wastewater treatment and environmental risk assessment using hazard
499 indexes. *Environment International*, 36 (1) 15-26.
500 Gros, M., Petrovic, M., and Barceló, D. 2009. Tracing Pharmaceutical Residues of
501 Different Therapeutic Classes in Environmental Waters by Using Liquid
502 Chromatography/Quadrupole-Linear Ion Trap Mass Spectrometry and Automated
503 Library Searching. *Analytical Chemistry*, 81 (3) 898-912.
504 Grujic, S., Vasiljevic, T. and Lausevic, M. 2009. Determination of multiple pharmaceutical
505 classes in surface and ground waters by liquid chromatography-ion trap-tandem
506 mass spectrometry. *Journal of Chromatography A*, 1216 (25) 4989-5000.
507 Hernando, M. D., Mezcua, M., Fernández-Alba, A. R. and Barceló, D. 2006.
508 Environmental risk assessment of pharmaceutical residues in wastewater effluents,
509 surface waters and sediments. *Talanta*, 69 (2) 334-342.
510 http://www.rxlist.com
511 Jelic, A., Petrovic, M. and Barceló, D. 2009. Multi-residue method for trace level
512 determination of pharmaceuticals in solid samples using pressurized liquid
513 extraction followed by liquid chromatography/quadrupole-linear ion trap mass
514 spectrometry. *Talanta*, 80 (1) 363-371.

515 Joss, A., Keller, E., Alder, A. C., Göbel, A., McArdell, C. S., Ternes, T. and Siegrist, H.
516 2005. Removal of pharmaceuticals and fragrances in biological wastewater
517 treatment. *Water Research*, 39 (14) 3139-3152.
518 Kasprzyk-Hordern, B., Dinsdale, R. M. and Guwy, A. J. 2009. The removal of
519 pharmaceuticals, personal care products, endocrine disruptors and illicit drugs
520 during wastewater treatment and its impact on the quality of receiving waters.
521 *Water Research*, 43 (2) 363-380.
522 Khetan, S. K. and Collins, T. J. 2007. Human Pharmaceuticals in the Aquatic Environment:
523 A Challenge to Green Chemistry. *Chemical Reviews*, 107 (6) 2319-2364.
524 Kimura, K., Hara, H. and Watanabe, Y. 2007. Elimination of Selected Acidic
525 Pharmaceuticals from Municipal Wastewater by an Activated Sludge System and
526 Membrane Bioreactors. *Environmental Science & Technology*, 41 (10) 3708-3714.
527 Kinney, C. A., Furlong, E.T., Werner, S.L., and Cahill, J.D. 2006. Presence and distribution
528 of wastewater-derived pharmaceuticals in soil irrigated with reclaimed water.
529 *Environmental Toxicology and Chemistry*, 25 (2) 317-326.
530 Kreuzinger, N., Clara, M., Strenn, B. and Kroiss, H. 2004. Relevance of the sludge
531 retention time (SRT) as design criteria for wastewater treatment plants for the
532 removal of endocrine disruptors and pharmaceuticals from wastewater. *Water*
533 *Science and Technology*, 50 (5) 149-156.
534 Lapen, D. R., Topp, E., Metcalfe, C. D., Li, H., Edwards, M., Gottschall, N., Bolton, P.,
535 Curnoe, W., Payne, M. and Beck, A. 2008. Pharmaceutical and personal care
536 products in tile drainage following land application of municipal biosolids. *Science*
537 *of The Total Environment*, 399 (1-3) 50-65.

538 Laville, N., Aït-Aïssa, S., Gomez, E., Casellas, C. and Porcher, J. M. 2004. Effects of
539 human pharmaceuticals on cytotoxicity, EROD activity and ROS production in fish
540 hepatocytes. *Toxicology*, 196 (1-2) 41-55.
541 Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Sepp, K., Lõhmus, R. and
542 Nei, L. 2009. Simultaneous determination of fluoroquinolones, sulfonamides and
543 tetracyclines in sewage sludge by pressurized liquid extraction and liquid
544 chromatography electrospray ionization-mass spectrometry. *Journal of*
545 *Chromatography A*, 1216 (32) 5949-5954.
546 Lishman, L., Smyth, S. A., Sarafin, K., Kleywegt, S., Toito, J., Peart, T., Lee, B., Servos,
547 M., Beland, M. and Seto, P. 2006. Occurrence and reductions of pharmaceuticals
548 and personal care products and estrogens by municipal wastewater treatment plants
549 in Ontario, Canada. *Science of The Total Environment*, 367 (2-3) 544-558.
550 McClellan, K. and Halden, R. U. 2010. Pharmaceuticals and personal care products in
551 archived U.S. biosolids from the 2001 EPA national sewage sludge survey. *Water*
552 *Research*, 44 (2) 658-668
553 Miao, X.S., Yang, J.J. & Metcalfe, C. D. 2005. Carbamazepine and Its Metabolites in
554 Wastewater and in Biosolids in a Municipal Wastewater Treatment Plant.
555 *Environmental Science & Technology*, 39 (19) 7469-7475.
556 Miège, C., Choubert, J. M., Ribeiro, L., Eusèbe, M. and Coquery, M. 2009. Fate of
557 pharmaceuticals and personal care products in wastewater treatment plants -
558 Conception of a database and first results. Environmental Pollution, 157 (5) 1721-
559 1726
560 Miller, T. R., Heidler, J., Chillrud, S. N., DeLaquil, A., Ritchie, J. C., Mihalic, J. N., Bopp,
561 R. & Halden, R. U. 2008. Fate of Triclosan and Evidence for Reductive

562 Dechlorination of Triclocarban in Estuarine Sediments. *Environmental Science &*
563 *Technology*, 42 (12) 4570-4576.
564 Nentwig, G., Oetken, M. and Oehlmann, J. 2004. Effects of pharmaceuticals on aquatic
565 invertebrates—the example of carbamazepine and clofibric acid. *In: Kümmerer K*
566 *(ed.): Pharmaceuticals in the Environment. Sources, Fate, Effects and Risks. (2ed)*
567 195–207
568 Nieto, A., Borrull, F., Marcé, R. M. and Pocurull, E. 2009. Determination of personal care
569 products in sewage sludge by pressurized liquid extraction and ultra high
570 performance liquid chromatography-tandem mass spectrometry, *Journal of*
571 *Chromatography A*, 1216 (30) 5619-5625.
572 Nilsen, E. B., Rosenbauer, R.R., Furlong, E.T., Burkhardt, M.R., Werner, S.L., Greaser, L.,
573 and Noriega, M. 2007. Pharmaceuticals, personal care products and anthropogenic
574 waste indicators detected in streambed sediments of the lower Columbia River and
575 selected tributaries, *National Ground Water Association, Paper 4483*, p. 15.
576 Okuda, T., Yamashita, N., Tanaka, H., Matsukawa, H. and Tanabe, K. 2009. Development
577 of extraction method of pharmaceuticals and their occurrences found in Japanese
578 wastewater treatment plants. *Environment International*, 35 (5) 815-820.
579 Pérez, S. and Barceló, D. 2007. Application of advanced MS techniques to analysis and
580 identification of human and microbial metabolites of pharmaceuticals in the aquatic
581 environment. *TrAC Trends in Analytical Chemistry*, 26 (6) 494-514.
582 Pomati, F., Castiglioni, S., Zuccato, E., Fanelli, R., Vigetti, D., Rossetti, C. and Calamari,
583 D. 2006. Effects of a Complex Mixture of Therapeutic Drugs at Environmental
584 Levels on Human Embryonic Cells. *Environmental Science & Technology*, 40 (7)
585 2442-2447.

586 Quintana, J. B., Weiss, S. and Reemtsma, T. 2005. Pathways and metabolites of microbial
587 degradation of selected acidic pharmaceutical and their occurrence in municipal
588 wastewater treated by a membrane bioreactor. *Water Research*, 39 (12) 2654-2664.
589 Radjenovic, J., Petrovic, M. and Barceló, D. 2007. Analysis of pharmaceuticals in
590 wastewater and removal using a membrane bioreactor. *Analytical and Bioanalytical*
591 *Chemistry*, 387, 1365-1377.
592 Radjenovic, J., Petrovic, M. and Barceló, D. 2009. Fate and distribution of pharmaceuticals
593 in wastewater and sewage sludge of the conventional activated sludge (CAS) and
594 advanced membrane bioreactor (MBR) treatment. *Water Research*, 43 (3) 831-841.
595 Reif, R., Suárez, S., Omil, F. and Lema, J. M. 2008. Fate of pharmaceuticals and cosmetic
596 ingredients during the operation of a MBR treating sewage. *Desalination*, 221 (1-3)
597 511-517.
598 Sabourin, L., Beck, A., Duenk, P. W., Kleywegt, S., Lapen, D. R., Li, H., Metcalfe, C. D.,
599 Payne, M. and Topp, E. 2009. Runoff of pharmaceuticals and personal care
600 products following application of dewatered municipal biosolids to an agricultural
601 field. *Science of The Total Environment*, 407 (16) 4596-4604.
602 Schnell, S., Bols, N. C., Barata, C. and Porte, C. 2009. Single and combined toxicity of
603 pharmaceuticals and personal care products (PPCPs) on the rainbow trout liver cell
604 line RTL-W1. *Aquatic Toxicology*, 93 (4) 244-252.
605 Shipkova, M. and Wieland, E. 2005. Glucuronidation in therapeutic drug monitoring.
606 *Clinica Chimica Acta*, 358 (1) 2-23.
607 Sim, W. J., Lee, J. W. and Oh, J. E. 2010. Occurrence and fate of pharmaceuticals in
608 wastewater treatment plants and rivers in Korea. *Environmental Pollution*, 158 (5)
609 1938-1947.

610 Suárez S, Ramil M, Omil F. and Lema J.M., 2005. Removal of pharmaceutically active
611 compounds in nitrifying-denitrifying plants. *Water Science and Technology*, 52 (8)
612 9-14.
613 Ternes, T. A., Bonerz, M., Herrmann, N., Teiser, B. and Andersen, H. R. 2007. Irrigation of
614 treated wastewater in Braunschweig, Germany: An option to remove
615 pharmaceuticals and musk fragrances. *Chemosphere*, 66 (5) 894-904.
616 Topp, E., Monteiro, S. C., Beck, A., Coelho, B. B., Boxall, A. B. A., Duenk, P. W.,
617 Kleywegt, S., Lapen, D. R., Payne, M., Sabourin, L., Li, H. and Metcalfe, C. D.
618 2008. Runoff of pharmaceuticals and personal care products following application
619 of biosolids to an agricultural field. *Science of The Total Environment*, 396 (1) 52-
620 59.
621 Vazquez-Roig, P., Segarra, R., Blasco, C., Andreu, V. and Picó, Y. 2010. Determination of
622 pharmaceuticals in soils and sediments by pressurized liquid extraction and liquid
623 chromatography tandem mass spectrometry. *Journal of Chromatography A*, 1217
624 (16) 2471-83
625 Wick, A., Fink, G., Joss, A., Siegrist, H. and Ternes, T. A. 2009. Fate of beta blockers and
626 psycho-active drugs in conventional wastewater treatment. *Water Research*, 43 (4)
627 1060-1074.
628 Zorita, S., Mårtensson, L. and Mathiasson, L. 2009. Occurrence and removal of
629 pharmaceuticals in a municipal sewage treatment system in the south of Sweden.
630 *Science of The Total Environment*, 407 (8) 2760-2770.
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Table 1. Characteristics of the studied wastewater treatment plants (WWTPs)

	Type of treatment	Designed Treatment Capacity (m^3/day)	Average Flow Treated in 2008 (m^3/day)	Population Equivalent	Sludge Treatment	Disposal of Sludge	Sludge produced in 2008 (t/year)
WWTP 1	Biological + Tertiary	47500	25000	74000	Composting	Disposal to soil; Agricultural usage	9000 (1800 d.m.)
WWTP 2	Biological	35000	26000	170000	Anaerobic digestion	Disposal to soil; Inceneration	8500 (2000 d.m.)
WWTP 3	Biological with Phosphorus and Nitrogen Removal	25000	21000	400000	Anaerobic digestion + Drying	Controlled disposal to landfill	11400 (2900 d.m.)

*d.m. dry matter (dry weight)

Table 1. Characteristics of the studied wastewater treatment plants (WWTPs)

A) Treatment characteristics

	Type of treatment	HRT (h)	SRT (days)	Designed Treatment Capacity (m^3/day)	Average Flow (m^3/day)	Population Equivavalent	Sludge tretment	Disposal of sludge	Sludge production (t/year)	Dry matter (t/year)	Organic matter (%)
WWTP1	Biological + Tertiary	26-40	10	47500	25000	74000	Composting	Disposal to soil; Agricultural usage	9000	1800	75
WWTP2	Biological	20	6	35000	26000	170000	Anaerobic digestion	Disposal to soil; Inceneration	8500	2000	65
WWTP3	Biological with P and N removal	40	16	25000	21000	400000	Anaerobic digestion + Drying	Controlled disposal to landfill	11400	2900	53

B) Wastewater and sludge characteristics

	Wastewater													**Sludge**					
	$SS_{Influent}$ (mg/L)	$SS_{Effluent}$ (mg/L)	$BOD5_{In}$ (mg/L)	$BOD5_{Out}$ (mg/L)	COD_{In} (mg/L)	COD_{Out} (mg/L)	Nt_{In} (mg/L)	Nt_{Out} (mg/L)	Pt_{In} (mg/L)	Pt_{Out} (mg/L)	$T_{effluent}$ °C	$pH_{Influent}$	$pH_{effluent}$	Nt_{Sludge} (%)	$N_{amoniuim}$ (%)	$N_{organic}$ (%)	P (P2O5) (%)	K (K2O) (%)	pH_{Sludge}
WWTP1	191	43	175	19	393	82	39	25	6	4	n.d.	7.7	7.3	5.4	1.2	4.2	3	0.3	6.5
WWTP2	330	20	390	14	700	85	70	42	8	3	20 ± 6	n.d.	7.2	5.9	2.9	2.9	5	0.3	6.4
WWTP3	750	10	1130	6	1800	36	126	11	16	1	22 ± 5	7.5	7.2	5.1	1.1	4.1	3.6	0.2	7.6

SPM - Suspended particulate matter
BOD5 - Biochemical Oxygen Demand
COD - Chemical Oxygen Demand

Nt - Total Nitrogen
Pt - Total Phosphorus

Table 2. Frequency of detection (%) and median and maximum (max) concentrations of pharmaceuticals detected in wastewater influent (WWI), effluent (WWE) and sewage sludge from the studied wastewater treatment plants (WWTP1, WWTP2 and WWTP3) during 8 sampling campaigns

Compounds	Frequency of detection, %			WWTP 1						WWTP2						WWTP3					
	WWI	WWE	Sludge	$c_{Influent}$, ng/L		$c_{Effluent}$, ng/L		c_{Sludge}, ng/g		$c_{Influent}$, ng/L		$c_{Effluent}$, ng/L		c_{Sludge}, ng/g		$c_{Influent}$, ng/L		$c_{Effluent}$, ng/L		c_{Sludge}, ng/g	
				Median	Max	Median	Max	Median	Max	Median	Max	Median	Max	Median	Max	Median	Max	Median	Max	Median	Max
Ketoprofen	100	54	0	2244	3301	160	340	n.d.	n.d.	2270	6007	690	948	n.d.	n.d.	1100	1720	70	80	n.d.	n.d.
Naproxen	100	88	0	7129	8862	455	1446	n.d.	n.d.	4802	10150	1126	2624	n.d.	n.d.	4161	5545	105	307	n.d.	n.d.
Diclofenac	92	100	100	1532	1709	456	743	61	97	1090	1674	785	1100	23	36	385	1250	336	900	40	75
Indomethacine	65	58	0	166	997	74	495	n.d.	n.d.	175	873	128	622	n.d.	n.d.	74	187	43	440	n.d.	n.d.
Mefenamic acid	54	77	83	130	212	58	113	19	81	68	132	20	30	15	21	50	50	10	15	29	56
Bezafibrate	100	100	100	436	1969	80	240	17	23	503	1346	441	500	18	27	204	583	48	100	3.1	7.4
Fenofibrate	42	0	79	27	30	n.d.	n.d.	6.7	65	5.2	25	n.d.	n.d.	22	45	9.1	9.1	n.d.	n.d.	16	21
Gemfibrozil	38	58	50	107	135	378	525	2.6	3.8	42	145	139	1772	4.2	6.6	283	1040	31	45	2.3	3.2
Atorvastatin	100	77	96	65	159	15	64	20	70	79	180	53	395	34	56	22	110	5.4	5.4	33	46
Pravastatin	73	65	0	179	558	22	69	n.d.	n.d.	123	710	68	619	n.d.	n.d.	49	50	19	92	n.d.	n.d.
Mevastatin	12	8	0	1353	1353	476	476	n.d.	n.d.	208	208	287	287	n.d.	n.d.	135	135	n.d.	n.d.	n.d.	n.d.
Diazepam	54	54	88	7.4	8.8	3.5	5.0	6.5	9.3	3.7	7.1	5.1	7.4	6.1	11	3.2	3.2	5.2	5.7	4.5	7.0
Lorazepam	81	85	79	64	70	50	75	11	15	93	126	64	208	10	19	49	160	31	116	8	11
Carbamazepine	100	100	100	782	949	539	705	29	68	664	814	509	665	23	35	327	561	367	518	31	46
Clarithromycin	73	85	83	86	113	33	55	55	91	57	501	50	184	40	75	44	333	30	116	44	54
Cimetidine	100	69	88	210	534	10	10	7	12	140	207	80	132	6.9	12	41	185	14	47	5.6	8.7
Ranitidine	92	88	92	113	456	9	63	8	19	221	840	179	401	4.6	6	127	323	34	76	4.6	20
Famotidine	19	8	96	42	45	n.d.	n.d.	13	15	19	21	16	18	6.6	14	14	14	n.d.	n.d.	7.5	27
Sulfamethazine	58	65	33	6.1	6.1	3.2	3.2	n.d.	n.d.	10	24	1.7	8.1	n.d.	n.d.	20	586	12	195	12	23
Trimethoprim	100	96	88	155	237	42	147	30	36	176	645	101	255	12	80	56	95	29	86	12	20
Metronidazole	62	62	0	47	392	30	187	n.d.	n.d.	104	285	118	295	n.d.	n.d.	15	19	13	13	n.d.	n.d.
Chloramphenicol	12	46	0	23	23	17	21	n.d.	n.d.	4.0	4.0	4.0	14	n.d.	n.d.	n.d.	n.d.	2.3	2.3	n.d.	n.d.
Atenolol	100	100	88	490	1451	129	574	9	17	1195	1436	383	1160	7.8	28	382	1310	63	139	4.3	8.1
Sotalol	65	58	54	88	859	49	147	12	20	72	157	63	92	9.4	23	86	193	42	65	9.3	16
Metoprolol	35	62	0	131	196	82	141	n.d.	n.d.	15	17	10	35	n.d.	n.d.	n.d.	n.d.	3.6	6.9	n.d.	n.d.
Timolol	42	65	0	10	10	4.6	6.7	n.d.	n.d.	11	12	7	10	n.d.	n.d.	7.4	7.4	4.2	4.8	n.d.	n.d.
Nadolol	100	69	54	20	48	5.4	7.6	2.0	2.3	22	82	13	78	3.2	6.3	7.8	17	n.d.	n.d.	6.4	7.0
Salbutamol	69	58	0	44	67	5.8	24	n.d.	n.d.	45	48	19	23	n.d.	n.d.	12	15	6.7	10	n.d.	n.d.
Enalapril	96	46	0	165	567	1.2	10	n.d.	n.d.	344	1309	6.5	1041	n.d.	n.d.	214	773	8.5	8.5	n.d.	n.d.
Glibenclamide	85	65	92	32	100	18	38	20	88	60	200	58	162	19	32	10	103	11	100	34	49
Furosemide	100	96	83	1371	1530	142	491	36	75	1374	2437	865	1371	24	52	865	925	212	443	43	54
Hydrochlorthiazide	0	0	100	n.d.	n.d.	n.d.	n.d.	37	53	n.d.	n.d	n.d.	n.d.	39	65	n.d.	n.d.	n.d.	n.d.	32	42

Table 2. Frequency of detection (%) and limits of quantification (LOQ) of pharmaceuticals detected in wastewater influent (WWI), effluent (WWE) and sewage sludge from the studied WWTPs during 8 sampling campaigns

Compounds	Frequency of detection, %			LOQ (ng/L)		LOQ (ng/g)
	WWI	WWE	Sludge	WWI	WWE	Sludge
Ketoprofen (KTP)	100	54	0	13	7.0	1.3
Naproxen (NPR)	100	88	0	21	3.0	0.9
Diclofenac (DCL)	92	100	100	4.0	4.0	2.0
Indomethacine (INM)	65	58	0	3.0	2.0	1.0
Mefenamic acid (MFA)	54	77	83	16	5.0	0.4
Bezafibrate (BZF)	100	100	100	4.0	0.4	0.4
Fenofibrate (FNB)	42	0	79	0.5	0.5	2.5
Gemfibrozil (GMB)	38	58	50	3.0	1.0	1.7
Atorvastatin (ATR)	100	77	96	4.0	2.0	2.5
Pravastatin (PRV)	73	65	0	25	9.0	2.4
Mevastatin (MVS)	12	8	0	2.0	2.0	4.5
Diazepam (DZP)	54	54	88	3.0	1.2	4.1
Lorazepam (LRZ)	81	85	79	7.0	4.0	5.1
Carbamazepine (CBZ)	100	100	100	2.0	2.0	0.2
Clarithromycin (CLR)	73	85	83	5.0	4.0	7.1
Cimetidine (CMTD)	100	69	88	0.6	0.4	0.2
Ranitidine (RNTD)	92	88	92	3.0	2.0	0.3
Famotidine (FMTD)	19	8	96	1.0	0.7	0.1
Sulfamethazine (SLFM)	58	65	33	2.0	1.0	0.8
Trimethoprim (TRM)	100	96	88	1.0	0.4	0.6
Metronidazole (MTR)	62	62	0	6.0	0.7	5.6
Chloramphenicol (CHLR)	12	46	0	2.0	0.6	0.2
Atenolol (ATN)	100	100	88	9.0	9.0	0.7
Sotalol (STL)	65	58	54	5.0	2.0	0.4
Metoprolol (MTP)	35	62	0	2.0	2.0	1.2
Timolol (TML)	42	65	0	0.3	0.3	0.9
Nadolol (NDL)	100	69	54	0.8	0.2	0.3
Salbutamol (SLB)	69	58	0	0.3	0.2	0.3
Enalapril (ENL)	96	46	0	5.0	0.7	0.4
Glibenclamide (GLB)	85	65	92	5.0	4.0	3.5
Furosemide (FRS)	100	96	83	4.0	2.0	1.0
Hydrochlorthiazide (HCRT)	0	0	100	13	6.0	0.5

Figure 1. Daily mass loads of different therapeutic groups at the influent and effluent, and in the sludge from the studied WWTPs

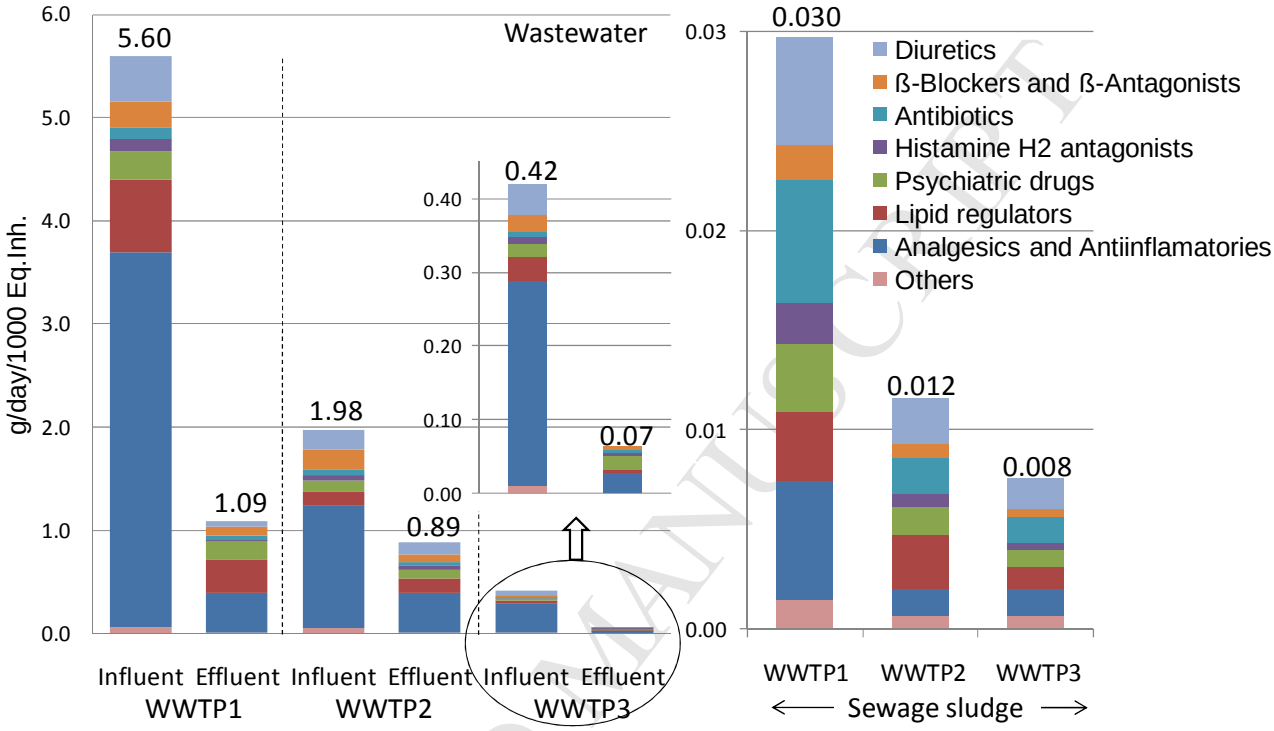

Figure 1. Schematic diagram of the studied wastewater treatment plants.

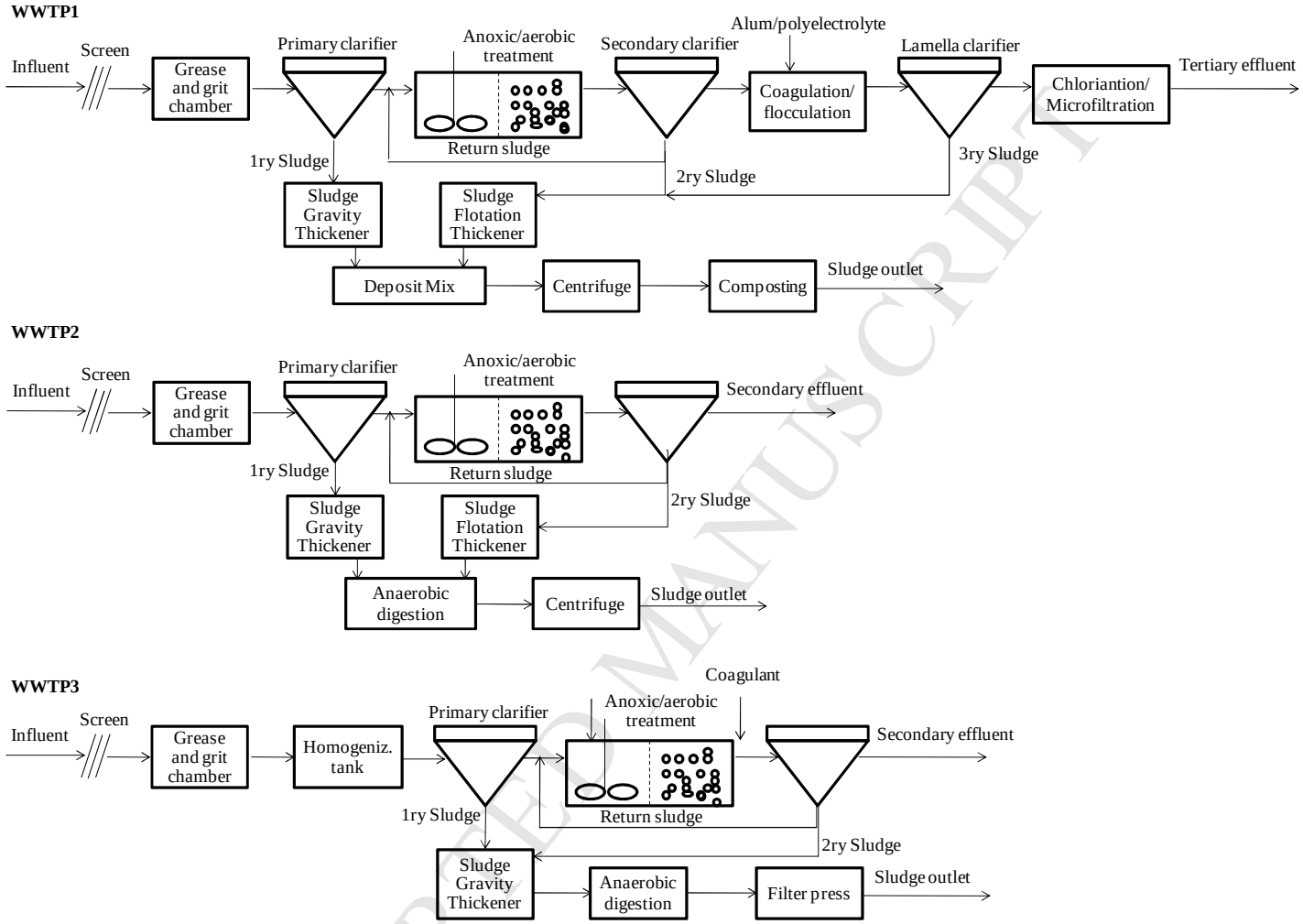

Figure 2. Normalized mass loads of the selected pharmaceuticals entering the studied WWTPs

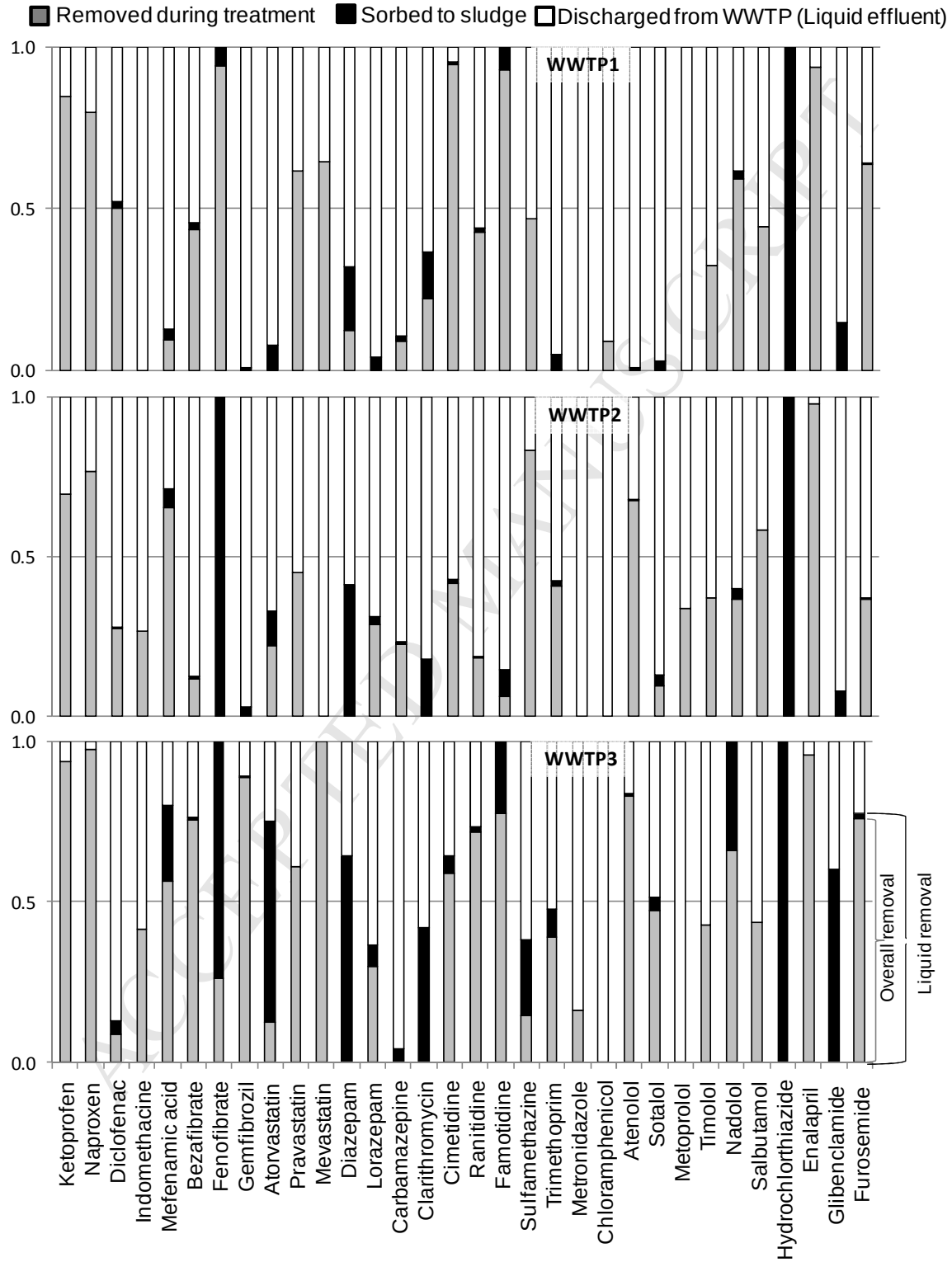

Figure 2. Box plots of concentration ranges (Min (-), P 0.25, Median, P 0.75 and Max (▲) of the pharmaceuticals detected in wastewater influent (IN), effluent (OUT) and sewage sludge from the studied wastewater treatment plants (WWTP1, WWTP2 and WWTP3) during 8 sampling campaigns (compound abbreviations are indicated in Table 2)

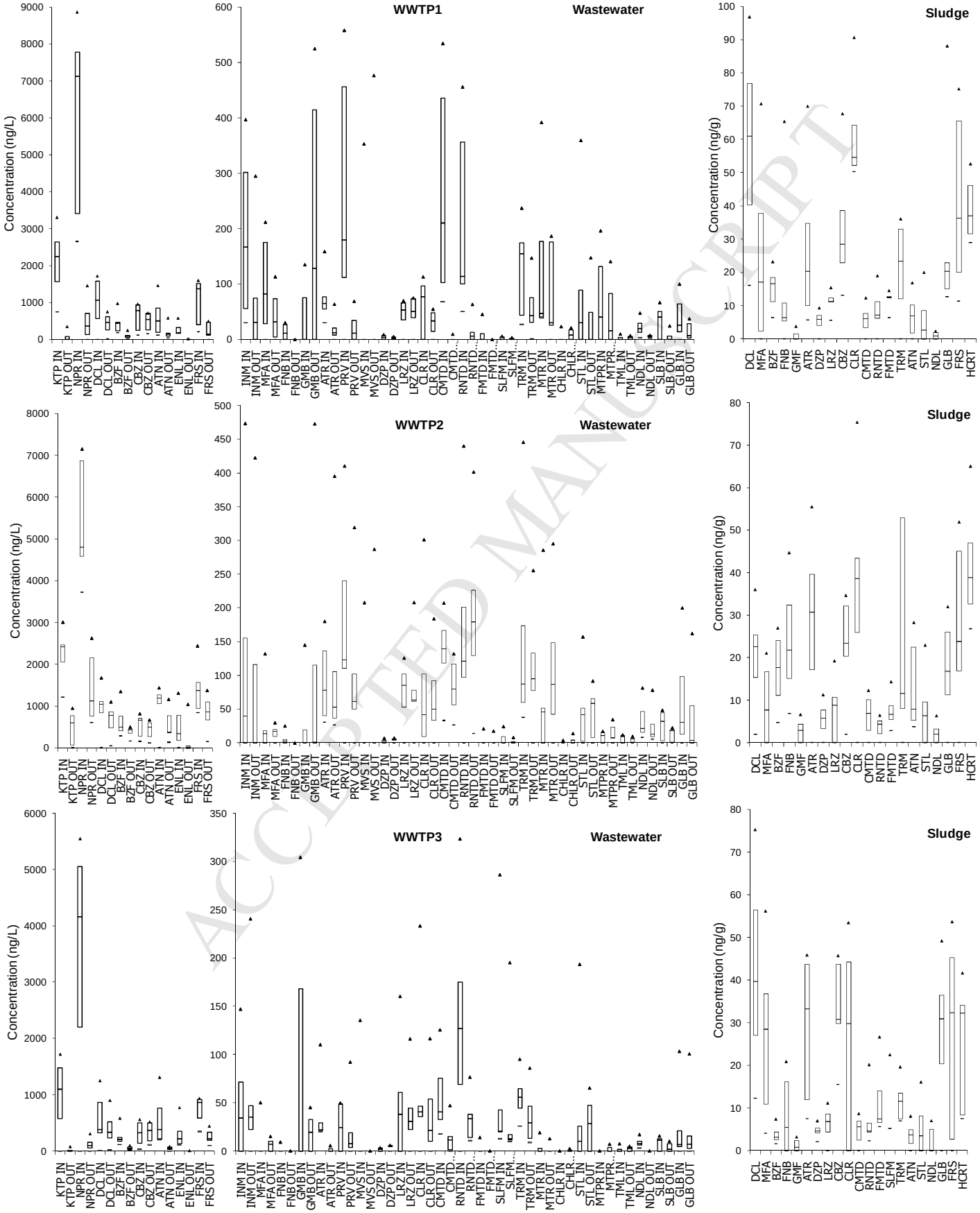

Figure 3. Daily mass loads (g/day per 1000 eq.inh.) of different therapeutic groups at the influent and effluent, and in the sludge from the studied WWTPs

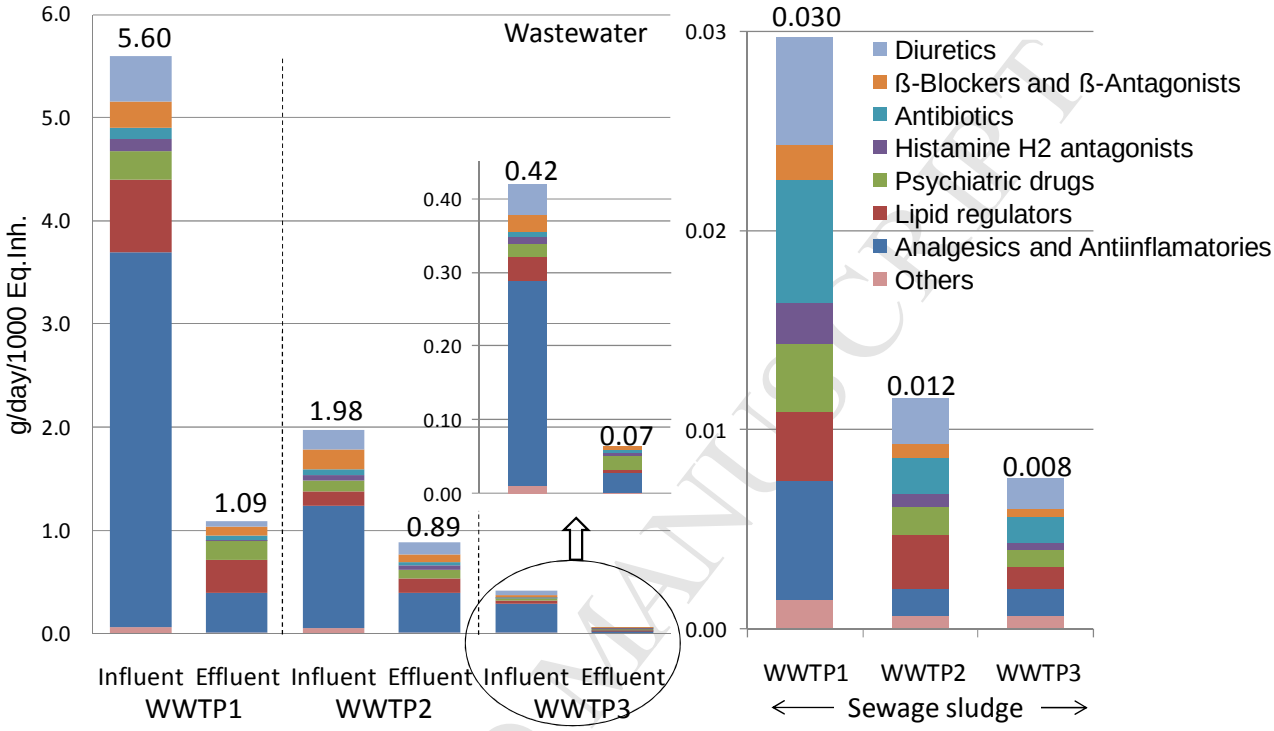

Figure 4. Normalized mass loads of the selected pharmaceuticals entering the studied WWTPs (i.e. fraction discharged with effluent, sorbed to sludge, and removed during treatment (overall removal rate))