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Abstract

The occurrence of 15 pharmaceutically active compounds (PACs) in an urban wastewater treatment plant (WWTP) was assessed in both influent and effluent samples. PACs were quantified by gas chromatography coupled to mass spectrometry. The sampling campaign was carried out during summer (n = 13) to assess the variation in both the influent concentrations and the removal efficiencies in similar climatic conditions. Among the selected PACs, all were quantified in influent samples but two of them were not systematically detected mainly due to the high quantification limit. PACs were detected at $\mu g.L^{-1}$ levels with a maximum concentration of 96.7 $\mu g.L^{-1}$ for Acetaminophen. The mean mass balance of the whole PAC pool during tracking was 448.5 and 26.3 $g.day^{-1}$ in influents and effluents respectively. However, the removal efficiency varied depending on the sample (e.g. between -20 and 50 % for Diclofenac). The fate of PACs during water treatment therefore depends on the removal quality in general, highlighted by the removal of nitrogen or BOD_5. As a result, effluent concentrations were variable, unlike influent concentrations which were used to correctly assess the consumption behavior of the

population around the sampled WWTP. Although the daily mass loads were comparable with those found in other studies in Europe for the same type of WWTP, the estimated consumption sometimes exhibited significant differences with the theoretical one. These differences depend on the mode of consumption, i.e. whether the therapeutic class treats chronic or episodic diseases, and on the scale gap between estimated and theoretical concentrations.

1. Introduction

In recent decades Pharmaceutically Active Compounds (PACs) have been extensively investigated as they represent a common and persistent form of pollution in numerous water compartments, from wastewaters to drinking water (Mompelat et al., 2009; Ternes, 1998). Due to their high frequency of detection (Loos et al., 2010; Lopez et al., 2015; Vulliet and Cren-Olivé, 2011) and their significant concentrations in natural waters (i.e. from $ng.L^{-1}$ to $\mu g.L^{-1}$), they are now considered as a potential hazard for numerous living beings, including humans (de Jongh et al., 2012; Grabicova et al., 2014). A study has recently demonstrated at field-relevant concentration (i.e. tested at $1.8\mu g.L^{-1}$, for a field concentration of 0.58 $\mu g.L^{-1}$) the impact of a benzodiazepine on the behaviour of the European Perch (Brodin et al., 2013).

The main origin of these contaminants is human and cattle therapies which lead to variable rates of excretion via urine and faeces, depending on the PACs characteristics (Lienert et al., 2007). Due to the continuous increase in drug consumption during the XX^{th} century, the levels of contamination raise serious questions about the amounts of PACs prescribed (Daughton, 2014).

Activated sludge treatment plants are the most common and therefore the most widely studied type of Wastewater Treatment Plant (WWTP) around the world (Collado et al., 2014; Lindberg et al., 2014; Yan et al., 2014). This type of plant allows a significant removal of classical chemical parameters such as BOD_5 or TP (Jones et al., 2007). However, the removal of PACs remains insufficient (Petrie et al., 2013; Verlicchi et al., 2012). The discrepancy between this insufficient removal and the increase in drug consumption means that contamination by PACs will remain a problem for the foreseeable future.

Several studies propose innovative tertiary treatments in order to improve the removal of PACs (Altmann et al., 2015; Gerrity et al., 2016; Lee et al., 2016; Thiebault et al., 2016a). Their use is nevertheless expensive and it seems important to better understand the origin of this lack of efficiency in activated sludge treatment.

Estimating the link between the consumption of a drug and the contamination level in effluent is important for field managers in order to assess the level of pollution in real time. Tracking the concentration of PACs in influents can also provide information on the consumption behaviour of the population concerned in order to determine some site-specific features (Baz-Lomba et al., 2016). To address this issue, influents and effluents were sampled within the same season. Several studies have demonstrated that the removal efficiency is not constant throughout the year, due to a seasonal effect (Papageorgiou et al., 2016; Sui et al., 2011). It also appeared important to assess the variability in removal efficiency within a single season, in our case summer, in order to determine whether removal remains constant during the same climatic event and what the origin of these variations is, such as for example variation in the efficiency of treatment step.

The analytical method selected in the present work was GC-MS. Previous studies have demonstrated that, although not widely used, this technique is suitable for the analysis of pharmaceutical residues at field-relevant concentrations (Jones et al., 2007; Togola and Budzinski, 2008). While GC-MS presents some disadvantages compared to HPLC-MS^2 (analytical duration and sample preparation), the equipment is widely available and the method has certain advantages (weak matrix effect, low analytical cost) that offset its drawbacks (Hao et al., 2007).

The aim of this study was therefore to optimize this technique for the analysis of PACs generated by human consumption in both influents and effluents in order to assess the fate

of PACs during activated sludge treatment within the same season. The results obtained should suggest new tools for a better prediction of removal assessment and consequently of environmental contamination.

2. Materials and Methods

2.1. Site settings

The WWTP investigated is one of the three main plants that serve the town of Orléans. The purification capacity of the installation is 93,933 population equivalent (PE). Influents arrive at the WWTP by two routes: the first collects the industrial effluents of a paper mill and the second collects domestic waste. Since 1989, the effluents have been discharged into the Loire river.

The treatment chain of domestic effluents consists in a conventional activated sludge treatment (Figure S1) with a hydraulic retention time of 2 days and a solid retention time between 10 and 20 days.

2.2 Sample Collection

Influents and effluents were collected by an automatic sampler indexed to the flow between April and August 2015 (n = 13). Each sample was a 24h-composite and was collected in 5-liter glass jars. After collection, samples were filtered with glass fiber filters (GF/A and GF/F, Whatman) and 0.45µm filters (Millipore) within 2 hours. The filters were previously heated at 105°C during 24 hours to eliminate any residual water. Residues were stored in the fridge before solid-phase extraction, carried out within the following 2 days.

The physico-chemical parameters (BOD_5, COD, etc.) of the collected samples were also analyzed and are summarized in Table S1.

2.3. Chemical Reagents

The 15 PAC standards (purity grade > 98 %; see Table 1 for details) were obtained from Sigma-Aldrich for Acetaminophen (ACM), Atenolol (ATE), Carbamazepine (CBZ), Codeine (COD), Diazepam (DIA), Doxepin (DOX), Gemfibrozil (GEM), Ketoprofen (KET), Metoprolol (MET), Naproxen (NAP), Oxazepam (OXA), Salicylic acid (SCA), Tramadol (TRA), and from Acros Organics for Diclofenac (DIC) and Ibuprofen (IBU). The PAC standards were selected from various therapeutic classes: analgesics (ACM and SCA), anti-inflammatory drugs (DIC, IBU, KET and NAP), psychotropic drugs (CBZ, DIA, DOX and OXA), β-blockers (ATE and MET) and lipid regulators (GEM). The internal standards Tramadol-d6 and 5α-cholestane were purchased from Sigma-Aldrich.

Chemical reagents of analytical grade, methanol (MeOH) and pyridine were purchased from Fisher Scientific. N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA, 95 %) was supplied by Sigma-Aldrich.

2.4. Residues concentration and analysis

Leachate solutions were concentrated by Solid-Phase Extraction (SPE) and analyzed by Gas Chromatography coupled to Mass Spectrometry (GC-MS). This methodology was already used in previous studies (Thiebault et al., 2016a, 2016b) but was optimized here for low concentrations.

PAC concentration was carried out on a 6 mL glass cartridge filled with HR-X phase (Macherey-Nagel). Cartridges were conditioned with 5 mL of MeOH followed by 5 mL of ultra-pure water. Columns were filled with 100 mL of sample, previously spiked with the

appropriate amount of the first internal standard (i.e. Tramadol-d6), and then rinsed with 5 mL of ultra-pure water before drying for 30 minutes under vacuum. Finally, elution was performed with 3 x 5 mL of MeOH. Thereafter, the second internal standard (i.e. 5α-cholestane) was added to organic layers in order to control the conservation and the injection of the samples. Then, organic layers were evaporated under reduced pressure. Residues were finally derivatized in a pyridine-MTBSTFA mix (60:40) at 60°C during 60 minutes.

Analyses were performed on a Trace GC Ultra gas chromatograph (GC) coupled to a TSQ Quantum XLS mass spectrometer equipped with an AS 3000 autosampler (both from Thermo Scientific). The GC was fitted with a Thermo Trace Gold TG-5 MS capillary column (60 m, 0.25 mm i.d., 0.25 µm film thickness).

The temperature of the column was held at 50°C for 3 min, increased from 50 to 120°C at 30°C min^{-1}, and from 120 to 310°C at 3°C min^{-1} with a final isothermal hold at 310°C for 21 min. 2 µL of sample was injected in splitless mode at 280°C. Helium was the carrier gas (1 mL min^{-1}). The mass spectrometer was operated in EI mode at 70 eV, from m/z 50 to 500.

Calibration curves were realized following the same preparation procedure as for the samples. The Method Quantification Limit (MQL) was estimated by using a signal to noise ratio of up to 10 (Jelic et al., 2011).

2.5. Processing of Results

The use of raw concentrations is not a consistent way to assess the removal efficiency of WWTPs and the daily variation in the amount of PACs. The irregularity of the flow of both influents and effluents impacts the assessment of the removal efficiency based on concentrations. It is therefore necessary to calculate both influent and effluent load. In the

present study, they were two reasons for this irregularity: (i) the flow generated by the industrial installation is not regular over the week since the factory closes during the weekend; (ii) influents are contaminated by rainwater despite the splitter network. By taking into account the flow, it is possible to calculate the load of PACs that passed through the WWTP for each sampling campaign.

$$\text{load} = C \; x \; F$$

with load, the mass load of PACs in $mg.day^{-1}$, *C*, the concentration in $\mu g.L^{-1}$, and *F*, the flow in $m^3.day^{-1}$

The removal efficiencies were hereafter calculated based on loads.

$$Removal = 100 - \frac{(\text{load}_{eff} \; x \; 100)}{\text{load}_{inf}}$$

with *Removal* the removal efficiency in %

Another mandatory back-calculation is to normalize the load by the number of PE.

$$\text{DML} = \frac{\text{load}}{n_{PE}}$$

with DML, the daily mass load in $mg.day^{-1}.PE^{-1}$ and n_{PE}, the population-equivalent number around the WWTP.

Lastly, to calculate the consumption of each PAC, a correction factor must be applied by taking into account the sorption on suspended particles and the molar ratio between the parent and the targeted residue (Baker et al., 2014; Gracia-Lor et al., 2016). In the present study, two PACs are concerned by this molar ratio variation, SCA and OXA, in order to calculate the consumption of acetylsalicylic acid and DIA respectively. The use of by-product derived from the original PAC is justified when the former exhibits a better

stability than the latter (Baker et al., 2014). The consumption was back-calculated on influent DML only.

$$Consumption = \frac{\mathrm{DML}_{inf}}{ExR} \text{ x } \frac{Mwp}{Mwr} \text{x } (\frac{100}{100 - SSP})$$

with *Consumption*, the estimated consumption in mg.day^{-1}.PE^{-1}, *ExR*, the excretion rate in %, *Mwp*, the molecular weight of the parent compound in g.mol^{-1}, *Mwr*, the molecular weight of the targeted residue in g.mol^{-1} and *SSP* the sorption onto suspended particles in %.

Correlation matrix and pairwise *p*-values were performed by using the package FactoMineR of the R software (Lê et al., 2008). Statistical analyses were performed on removal values calculated based on loads. The Pearson correlation was used to assess both the correlation matrix and *p*-values.

Table 1: Studied compounds with various parameters (CAS-Number, Log Kow the octanol/water partition, pKa and MW the molecular weight in g.mol^{-1}), and method validation data: m/z ratio (quantification and confirmation), RR, the recovery ratio obtained by SPE ± the relative standard deviation of triplicates in %, r² the linearity of calibration curves, MQL the method quantification limit for influent and effluent in µg.L^{-1}, and RSD the standard deviation of analytical triplicates in %

Class	PAC	CAS-Number	pKa	Log Kow	TR	MW	m/z ratio	RR	r²	MQL_{inf}	MQL_{eff}	RSD
Analgesics												
	ACM	103-90-2	9.4	0.46	41.71	151.2	322 248	85.0 ± 0.49	0.993	0.067	0.008	6
	SCA	69-72-7	3.5	1.19	37.36	138.1	309 195	82.5 ± 0.96	0.996	0.045	0.006	5
β-blockers												
	ATE	29122-68-7	9.6	0.16	61.99	266.3	437 72	68 ± 2.07	0.992	0.243	0.078	8
	MET	56392-17-7	9.6	1.79	47.86	267.4	223 324	70.8 ± 1.98	0.994	0.155	0.040	11
Psychotropic drugs												
	CBZ	298-46-4	13.9	2.25	53.56	236.3	193 237	73.3 ± 0.80	0.997	0.032	0.003	10
	DIA	439-14-5	3.4	2.82	53.34	284.7	256 221	62.2 ± 1.96	0.992	0.282	0.025	19
	DOX	1229-29-4	8.9	3.86	46.98	279.4	58 313	116 ± 2.51	0.995	0.058	0.018	8
	OXA	604-75-1	1.7- 11.6	2.31	60.16	287.0	457 147	73.2 ± 0.86	0.999	0.036	0.002	5
Anti-inflammatory drugs												

	DIC	15307-79-6	4.2	4.06	54.61	296.2	352 214	85.0 ± 0.82	0.999	0.022	0.006	7
	IBU	15687-27-1	4.9	3.72	33.78	206.3	263 303	78.7 ± 1.09	0.990	0.025	0.005	6
	KET	22071-15-4	4.5	2.81	51.71	254.3	311 295	65.4 ± 1.02	0.999	0.042	0.005	10
	NAP	22204-53-1	4.2	3.00	48.18	230.3	287 185	85.1 ± 2.09	0.998	0.153	0.032	8
Lipid regulator												
	GEM	25812-30-0	4.8	3.40	44.41	250.3	243 185	70.8 ± 1.96	0.999	0.368	0.080	17
Opioids												
	COD	76-57-3	8.21	1.20	59.33	299.4	313 235	71.5 ± 5.2	0.998	0.030	0.008	6
	TRA	27203-92-5	9.4	2.51	38.06	263.4	58 263	98.7 ± 2.82	0.994	0.064	0.016	8

3. Results

3.1. Occurrence of PACs

Table 2: Minimum, median and maximum concentrations (in $\mu g.L^{-1}$) and number of detections (n = 13 = 100 %) of each selected PAC in influent and effluent samples; n.d corresponds to non-detected concentration

Class	**PAC**	**Influent**				**Effluent**			
		min	med	max	*n*	min	med	max	*n*
Analgesics									
	ACM	22.6	55.8	96.7	13	n.d	0.013	0.172	11
	SCA	2.36	10.6	25.5	13	0.007	0.096	0.423	13
β-blockers									
	ATE	3.56	16.4	26.5	13	n.d	0.893	9.32	12
	MET	0.277	1.26	2.76	13	n.d	0.121	1.75	8
Psychotropic drugs									
	CBZ	0.051	0.215	0.937	13	0.005	0.163	0.357	13
	DIA	n.d	n.d	0.420	1	n.d	n.d	0.030	1
	DOX	0.092	0.279	1.02	13	n.d	0.028	0.299	11
	OXA	0.154	1.20	2.02	13	0.005	0.499	1.13	13
Anti-inflammatory drugs									
	DIC	0.063	0.245	1.19	13	0.043	0.079	1.38	13
	IBU	1.56	2.27	7.28	13	0.006	0.038	0.284	13
	KET	0.149	1.70	6.56	13	0.015	0.047	0.176	13
	NAP	0.457	1.33	4.74	13	n.d	0.058	0.238	12

Lipid regulator									
	GEM	n.d	n.d	0.648	4	n.d	n.d	0.105	2
Opioids									
	COD	0.279	0.933	2.11	13	n.d	0.137	0.518	12
	TRA	1.35	1.63	9.86	13	0.072	0.273	1.19	13

The occurrence of the selected PACs in influent and effluent samples is presented in Table 2.

In influent samples, three PACs presented a median concentration up to 10 µg.L^{-1}, namely ACM, SCA and ATE. They were also among the most abundant compounds with a frequency of detection of 100 %. Only two PACs, DIA and GEM, exhibited a frequency of detection less than 100 % during the tracking in influents. The MQL of these two compounds is significantly high (Table 1), which could explain this weak frequency of detection.

In effluent samples, the most abundant PACs were OXA, ATE and TRA (Table 2), with median concentrations up to 0.2 µg.L^{-1}. Other compounds, such as MET and DIC, presented occurrences up to µg.L^{-1} but the median concentrations remained below 0.2 µg.L^{-1}. Unsurprisingly, the frequency of detection in effluent samples was lower than in influents, as illustrated for example by ACM (n = 11), the most abundant compound in influents. However, the frequency of detection for most compounds, such as CBZ and DIC, was 100% in both influent and effluent (Table 2).

3.2. Load assessment

Apart from some extreme values (e.g. DOX) or significant variations in concentration for DIC and MET (i.e. above one order of magnitude), the influent mass balances varied only slightly during this seasonal monitoring, confirming several studies (Birošová et al., 2014; Sui et al., 2011; Thiebault et al., 2017). However, the variability rarely exceeds one order of magnitude (Figure 1).

Conversely, significant variations were observed in effluent concentrations and removal efficiency, two interdependent values, with effluent concentrations extending over two or three orders of magnitude. For example the effluent load of ATE varied from <MQL to 59 g.day^{-1} (Figure 1).

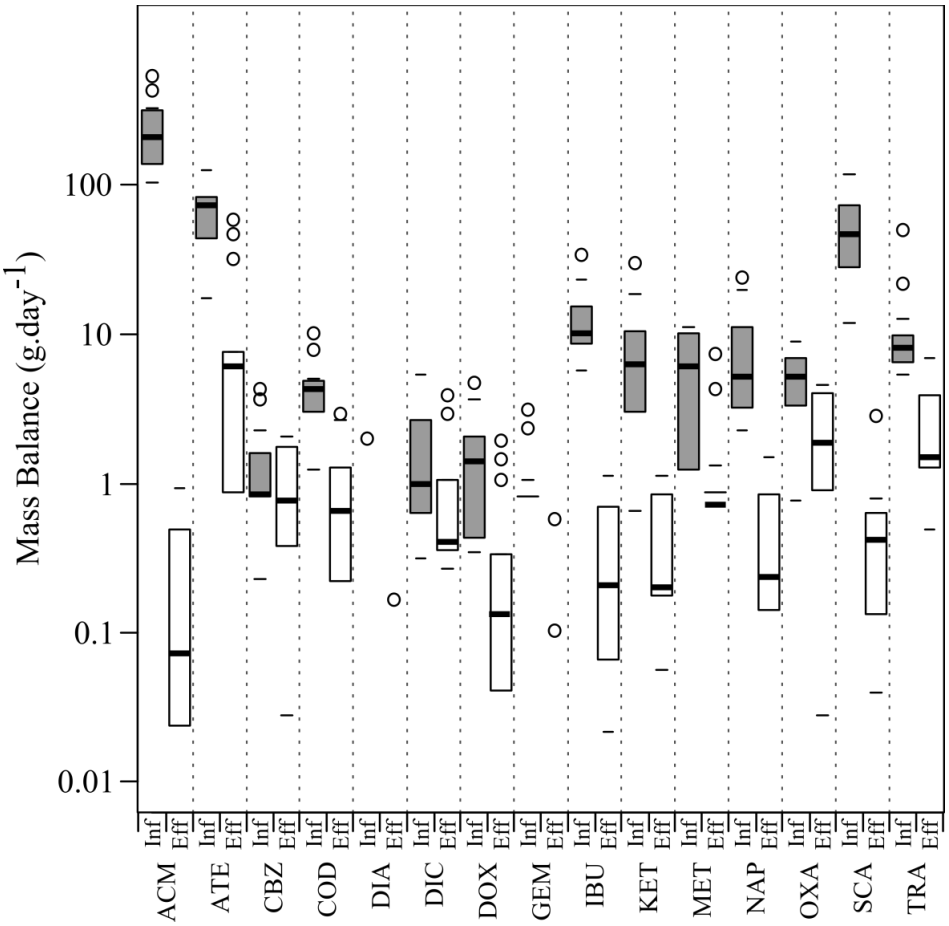

Figure 1: Boxplots of loads for each PAC in influent and effluent samples. The line within the box marks the median, boundaries indicate the 25th and 75th percentiles**,** error bars indicate the maximum and the minimum load ± 1.5 standard deviation and white squares indicate values outside this range

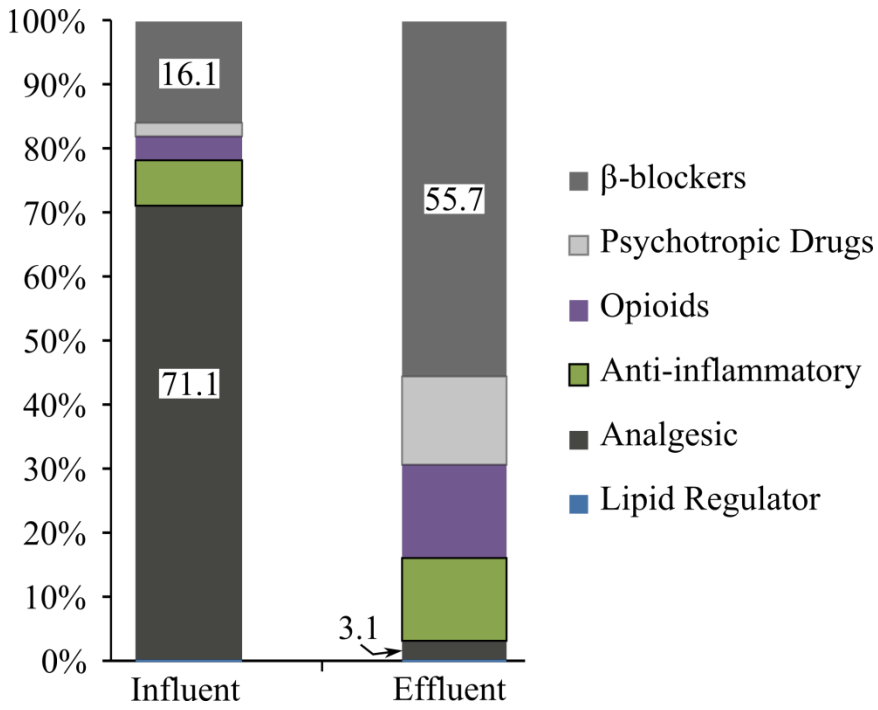

Figure 2: Relative percentage of each therapeutic class in both influent and effluent based on the mean load value

Raw data give mean loads of 448.5 and 26.3 $g.day^{-1}$ in influent and effluent respectively, corresponding to a mean removal of 94% when adding the load of each selected PAC. By separating the total load values into therapeutic classes, it is possible to assess the relative contribution of each class to the influent and effluent composition (Figure 2).

It can be seen that whereas analgesics predominate in influents with a relative percentage of 71%, their contribution drops to 3.1% of the total load of PACs in effluents. The opposite trend is observed for other classes whose contribution significantly increases between influent and effluent. β-blockers are especially concerned, with a relative percentage of 16.1% in influent which increases to 55.7% in effluent, indicating that this class represents the predominant load in effluents of this WWTP, a case already mentioned in other plants (Behera et al., 2011).

It should be mentioned that some therapeutic classes, such as antibiotics, are not present in this study due to the methodology employed. These classes may potentially make a significant contribution to the total load.

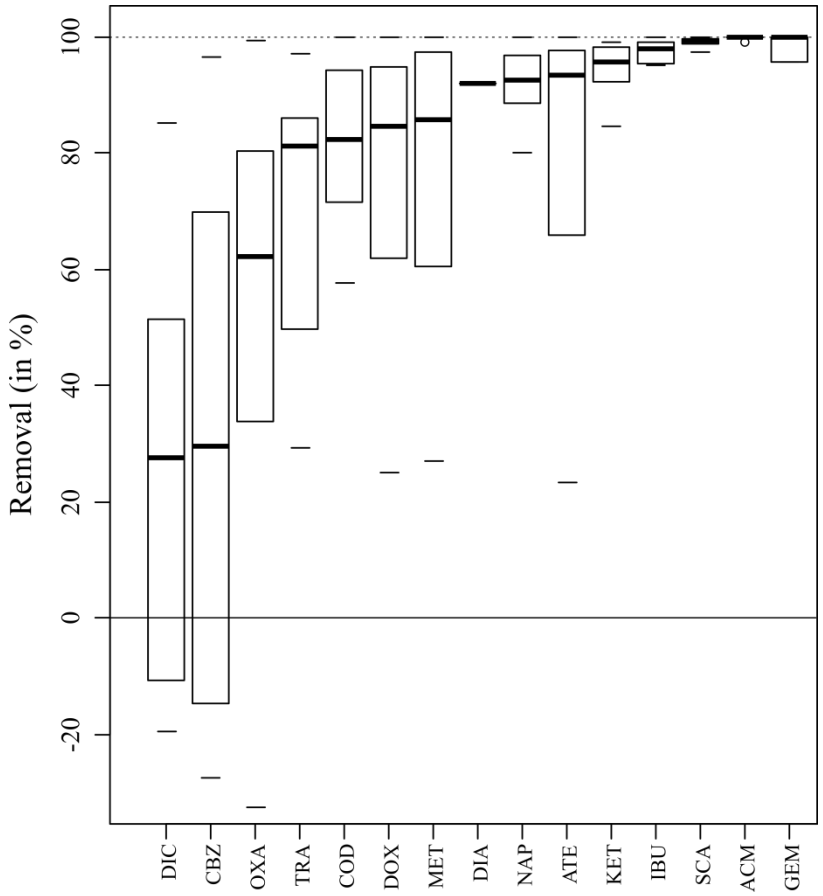

Figure 3: Removal efficiencies of each PAC for all samples calculated on load values. The line within the box marks the median, boundaries indicate the 25th and 75th percentiles and error bars indicate the maximum and the minimum removal values

3.3. Removal of PACs

As noted in previous studies, the removal efficiency was variable in time, especially for low concentrations of PACs for which the analytical error is proportionally higher. For the lower removal efficiencies such as DIC, the values varied between -20 % and 50 % (Figure 3). This is consistent with literature data, even in the same WWTP (Verlicchi et al., 2012). For the most concentrated compounds, the removal varied from 95 to 100 % (e.g. ACM and SCA).

These results revealed that beyond a seasonal effect regularly pointed out in the literature as impacting the removal efficiency (Sui et al., 2011; Zhang et al., 2015), there is also a significant removal variability within the same season. Thus, it seems risky to estimate the removal efficiency in a particular season based on the analysis of only one or two samples.

This high variability could be caused by changes in the chemical parameters of the influent water that could potentially affect the water-treatment efficiency, since while the influent concentrations of PACs remained fairly stable, the chemical parameters of the raw waters changed (Table S1).

The removal of classical wastewater parameters was also calculated to assess the stability of the removal quality during the tracking (Table S2). Their removal is stable, especially concerning the carbonaceous organic matter, whereas the removal of nitrogen is far more variable (i.e. between 30 and 99%; Table S2).

4. Discussion

4.1. Fate of PACs during treatment

The considerable variation in the removal efficiency of PACs in the selected WWTP raises questions about the regularity in time of the removal mechanisms (Figure 3). In activated sludge treatment, two main mechanisms remove PACs. Firstly, biodegradation generated by several types of bacteria (i.e. for the removal of carbonaceous organic matter or nitrogen) and then, flocculation/precipitation that is mostly responsible of the removal of phosphorus (Joss et al., 2005).

The statistical correlation between the removal efficiency of PACs and the removal efficiency of classical wastewater quality parameters (Table S2) could therefore point out the mechanism which is responsible for the degradation of each PAC (Table 3).

Table 3: Correlation coefficients describe the relationship between removal efficiencies of each detected PAC and removal efficiencies of classical parameters of water treatment, DIA and GEM are not presented due to their low frequency of detection [1]

	ACM	ATE	CBZ	COD	DIC	DOX	IBU	KET	MET	NAP	OXA	SCA	TRA
BOD_5	-0.02	-0.04	0.60**	-0.13	-0.22	-0.49**	-0.17	0.09	0.57**	-0.24	-0.11	0.03	-0.35
COD	-0.09	0.00	0.21	-0.27	-0.21	-0.43*	-0.47*	-0.22	0.68***	-0.50**	-0.30	-0.35	-0.50***
SS	-0.23	0.29	0.06	0.24	0.35	0.15	-0.39*	0.08	0.09	0.05	0.00	-0.06	0.01
GLN	-0.10	0.75***	-0.13	0.61**	0.39*	0.64***	0.07	-0.05	-0.35	0.49*	0.77***	0.10	0.59*
NH_4^+	0.14	0.76***	-0.14	0.09	-0.12	0.10	-0.22	-0.09	-0.01	0.12	0.51**	-0.21	0.18
NO_2^-	0.20	0.55**	0.16	0.01	-0.05	-0.12	-0.32	0.00	0.39*	-0.01	0.25	-0.16	-0.06
NO_3^-	-0.09	0.33	0.08	0.20	0.53**	0.26	-0.19	-0.01	0.27	0.48*	0.45*	0.05	0.45*
TKN	-0.15	0.69***	-0.19	0.35	0.17	0.33	-0.25	-0.02	-0.21	0.24	0.48*	-0.10	0.23
TP	-0.26	0.00	-0.38	0.22	0.02	0.46*	0.20	-0.07	-0.53**	-0.04	-0.14	-0.03	0.02

Highly removed PACs such as ACM and SCA (i.e. > 99.9%, Figure 1) do not exhibit any statistically significant correlation with other parameters. Yet, these two PACs are known be highly biodegradable and photodegradable micropollutants and variations in the WWTP operating conditions should not affect their removal (Andreozzi et al., 2003; Petrie et al., 2015).

Moderately removed PACs such as ATE, MET and TRA (i.e. median removal between 80 and 99%) exhibit several statistically significant correlations that could indicate the process which is responsible for their removal (Miège et al., 2009). MET is known to be sensitive to the biodegradation induced by various type of bacteria (Velázquez and Nacheva, 2017), and the correlation with both the removal of BOD_5 and NO_2^- indicates that the quality of oxic processes (i.e. oxic degradation of carbonaceous organic matter, and oxidation of nitrites into nitrates) impacts the removal of MET. Global nitrogen removal is also presented as a key factor concerning the removal efficiency of several PACs such as ATE (Miège et al., 2009; Vieno et al., 2007). This assumption is verified by the statistically significant correlation between the removal of ATE and the removal of global nitrogen (i.e. GLN) in our results (Table 3).

*, **, *** correspond to *p*-values < 0.05, 0.01 and 0.001 respectively

Tracking the removal of nitrogen in a plant could therefore be a solution in order to estimate the removal of several PACs such as ATE but also COD, DOX, NAP, OXA, DIC and TRA, respectively (Table 3).

CBZ is considered as a persistent compound in conventional wastewater treatment (Bahlmann et al., 2014; Clara et al., 2005). The results presented in this study are in accordance with this, as one of the lowest removal values was found for CBZ (Figure 3). However, the limited removal of CBZ would be caused by aerobic biodegradation (Kosjek et al., 2009; Petrie et al., 2013). This assumption is confirmed by the statistically significant correlation between the removal of CBZ and the removal of BOD_5 (Table 4). Although low, the removal of CBZ remains significant and is probably enhanced by the selected season (i.e. end of spring and summer) which maximized biological activity (Collado et al., 2014).

Lastly, the flocculation/precipitation could impact the removal of PACs. Even if the sorption of PACs is recognized as minor in the treatment process (Jones et al., 2006; Luo et al., 2014; Radjenović et al., 2009), some hydrophobic PACs could be impacted by this mechanism, mainly responsible for the removal of phosphorus. Only the removal of DOX demonstrates a statistically significant correlation with the removal of phosphorus. DOX is one of the most hydrophobic contaminants among the selected PACs with DIC and IBU (Table 1). But unlike DIC and IBU (i.e. anionic forms), DOX is in cationic form due to the slightly alkaline pH (Table S1). Yet, the sorption of cationic pollutants onto negatively charged surfaces of microorganisms and sludge is proposed as an efficient removal factor especially for hydrophobic compounds (Giebułtowicz and Nałęcz-Jawecki, 2014; Ternes et al., 2004). This difference of charge state between DOX, DIC and IBU therefore explains the difference in the statistical correlation between the removal of these PACs and the removal of phosphorus.

Beyond the link between the removal efficiencies of PACs and chemical parameters, negative removal values also raise questions. The PACs concerned are DIC, CBZ and OXA. Negative removal values for these PACs are frequently observed but can be attributed to various reasons. For both DIC and CBZ, the most common reason given is that they are partially excreted in conjugated form. Yet, they can be transformed back to parent compound during treatment explaining their negative removal values (Bahlmann et al., 2014; Ternes, 1998). Concerning the negative removal values of OXA, the main reason put forward for its persistence is its position at the end of the degradation pattern of benzodiazepines such as DIA and temazepam (Hummel et al., 2006), which may artificially increase the concentration of this product. Although we analysed the occurrence of DIA in this study, only one occurrence was found, mainly due to the high MQL (Table 1). However, concentrations of OXA were greater than those of DIA (Table 2).

4.2. Daily mass loads of PACs

The mean DML obtained in this study can be compared with those obtained in previous ones to assess the average impact per inhabitant in Orléans with respect to other places in the world. Another possibility is to assess the type of influents by comparing their PAC loads. For example, the study by Collado et al. (2014) was carried out in a WWTP drained mainly by industrial influents. The account of PE was therefore difficult and probably overestimated. This is the probable reason why DML influents in this study are systematically the lowest among the selected literature (Figure 4).

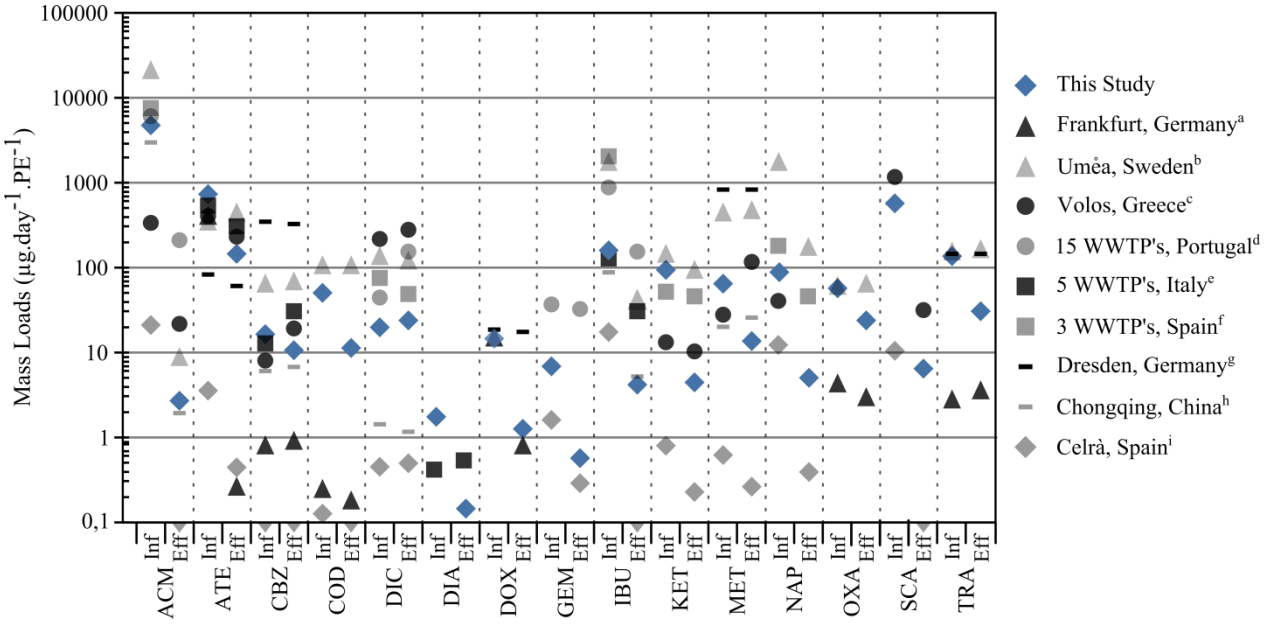

Figure 4: Mean DML in influent (Inf) and effluent (Eff) samples from the selected WWTP compared with other studies; triangles indicate values below 0.1 µg.day^{-1}.PE^{-1}

Data from:
Gurke et al. (2015)[a]
Lindberg et al. (2014)[b]
Papageorgiou et al. (2016)[c]
Pereira et al. (2016)[d]
Castiglioni et al. (2006)[e]
Gracia-Lor et al. (2012)[f]
Wick et al. (2009)[g]
Yan et al. (2014)[h]
Collado et al. (2014)[i]

In the present study, the industrial impacts on influents were withdrawn for the calculation of the DML. The results are consistent with the literature published on the subject. By comparison with other predominantly domestic WWTPs, the DML are often in the same order of magnitude (Figure 4). However, some extreme values were observed, depending on the location of the WWTP, indicating variations at different scales.

Except data from Collado et al. (2014), the WWTPs studied in the selected literature served medium-sized towns to large cities (i.e. from 10^5 to 10^6 inhabitants) characterized by a prevalence of domestic waste. No systematic rule can be drawn for the comparison of DML

(Figure 4). SCA and ACM are the most loaded PACs in influent whatever the studied WWTP (between 320 to 21,456 µg.day^{-1}.PE^{-1} in Greece and Sweden, respectively for ACM) and are also highly removed in all cases (i.e. two orders of magnitude, Figure 4). For these two PACs, the results of this study are in the same order of magnitude for both influent and effluent load. The influent loads of DIC and CBZ are not particularly high, but their effluent load remains close to their influent load (i.e. in the same order of magnitude), whatever the studied plant. This indicates that the difficulty in removing these specific PACs is not limited to the WWTP investigated in this study. By contrast, MET and TRA are moderately removed in the present work whereas they are poorly removed in other studies such as in Germany or Sweden indicating that the removal of some PACs can vary strongly depending on the WWTP investigated.

However, the influent DML of several PACs varies between and within each country. For example, while France is one of the largest consumers of PACs in weight, there are some differences in the consumption behavior of particular classes of medication. The influent loads should therefore be valuable in order to assess the consumption of PACs.

4.3. Consumption assessment

The back-calculation of the mean consumption in the targeted site is given in Table 4. These values were compared with published data on the amount of sold PACs in France in 2014. There is a lack of consumption data on a national scale for three contaminants, GEM, COD and DOX.

The comparison between experimental and theoretical values for other PACs shows two trends. For widely consumed PACs, the estimated experimental and theoretical consumption gives values in the same order of magnitude, with for example an estimated consumption of 117 and 0.887 mg.day^{-1}.PE^{-1} and a theoretical consumption of 137 and 0.761 mg.day^{-1}.PE^{-1} for ACM and ATE respectively (Table 4). While there is a good

correlation between experimental and theoretical consumption for several PACs, such as β-blockers or analgesics, considerable variations can be observed for other PACs such as CBZ, DIC and IBU. For these three compounds, the estimated consumption values are significantly lower than theoretical ones (i.e. up to one order of magnitude). These variations could come from two factors.

Firstly, the comparison is between national scale and local scale data for theoretical and experimental values respectively. This change in the scale could explain the significant variation in consumption, especially for CBZ. As CBZ is often used to treat chronic diseases, the seasonal impact on consumption can be considered negligible.

Then, the consumption of anti-inflammatory drugs (e.g. IBU and DIC) differs greatly between cold and hot seasons (Sui et al., 2011; Vieno et al., 2005). As the sampling campaign was carried out only in the hot season (end of spring and summer), the low consumption of anti-inflammatory drugs when compared with yearly consumption may result from the significant seasonal differences in their consumption. However, the consumption of IBU has already been noticed as significantly lower than theoretical one in the same region (Thiebault et al., 2017).

Table 4: Excretion Rate of targeted pharmaceuticals, calculation of the mean amount consumed and comparison with the theoretical estimation with ExR the excretion rate, SSP, the sorption on suspended solids, the estimated consumption, based on the effluent mean DML value, the theoretical consumption data in France in 2014 according to the literature, and n.d corresponding to a lack of data

PAC	ExR (%)	SSP (%)	Estimated Consumption $mg.day^{-1}.PE^{-1}$	Theoretical Consumption in France [h] $mg.day^{-1}.PE^{-1}$
Analgesics				
ACM	4[a]	0.0	117	137
SCA	8[a]	-	7.22	16.4

β-blockers				
ATE	83[a]	9.0[f]	0.887	0.761
MET	11[a]	0.0[g]	0.599	0.364
Psychotropic drugs				
CBZ	16[b]	0.0[f]	0.099	1.39
DIA	8[a]	42.0[g]	0.037	0.022
DOX	25	-	0.072	n.d
OXA	75[d]	5.4[c]	0.105	0.257
Anti-inflammatory drugs				
DIC	16[a]	0.0[f]	0.125	1.62
IBU	30[a]	5.0[f]	0.492	9.97
KET	10[e]	0.0[g]	0.950	0.900
NAP	10[e]	0.0[g]	0.895	1.55
Lipid regulator				
GEM	76[e]	0.0[g]	0.009	n.d
Opioids				
COD	64[c]	1.0[c]	0.079	n.d
TRA	32[g]	1.0[g]	0.420	1.07

Data from:

Lienert et al. (2007)[a]

Bahlmann et al. (2014)[b]

Baker et al. (2014)[c]

Carballa et al. (2008)[d]

Khan and Ongerth (2004)[e]

Hörsing et al. (2011)[f]

Jelic et al. (2011)[g]

Chiffre et al. (2016)[h]

5. Conclusion

This work has demonstrated that the quantification of PACs by GC-MS is feasible and reproducible at field-relevant concentrations, making it possible for numerous laboratories to practice this type of screening. Among the selected PACs, the majority exhibited detection frequencies of 100 %. However, the pollutants with the highest concentrations in influents are not necessarily the most problematic after treatment. ATE, TRA and DIC are respectively the three PACs with the highest concentrations in effluents and should be closely monitored to track pollution in the natural environment.

Significant variations in removal efficiencies were found throughout the tracking. Three groups can be distinguished among the selected PACs: highly removed (ACM, GEM, SCA), moderately removed (ATE, MET, DOX, COD, IBU, NAP, KET and TRA) and poorly removed (OXA, CBZ and DIC) PACs. These groups are in good agreement with other studies, showing that the removal capacity of current treatment plants is limited, whatever the plant studied. The removal efficiencies of some specific compounds are higher than those reported in previous studies, e.g. for CBZ with a median removal of 30 %. However, for poorly removed compounds, some negative removal efficiencies are also recorded. The chosen season (i.e. summer) could have an impact on the yield of biological degradation processes. Summer is considered to be favourable to a high biological activity, correlated with the removal of several PACs except ACM and SCA. Moreover, we propose that the removal efficiency of various PACs can be assessed by tracking the removal of nitrogen and/or BOD_5, as their removal is statistically correlated with the removal efficiency of various PACs.

Finally, the impact and the behaviour of the population were assessed from the back-calculation of DML and consumption. The experimentally calculated consumption was generally on the same order of magnitude as the theoretical one especially for year-long medication (psychotropic drugs, β-blockers). Conversely for some therapeutic classes (such as anti-inflammatory drugs) that are highly season-dependent, the experimental consumption was significantly lower than the theoretical one.

Lastly, this work has demonstrated that significant variations in removal efficiency occur within the same season, indicating that the seasonal effect is not a satisfactory explanation to assess removal variations throughout the year.

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