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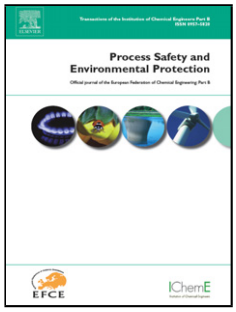

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Occurrence, seasonal variations and removal of Organic Micropollutants in 76 Wastewater Treatment Plants

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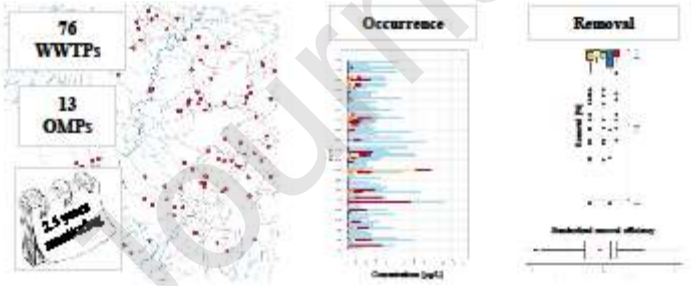
Graphical abstract



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Highlights:

- A monitoring campaign of 34 months was conducted on 76 Wastewater Treatment Plants.
- Influent and effluent concentrations of 13 Organic Micropollutants were determined.
- Some illicit drugs and Ketoprofen showed the highest concentrations.
- Steroid concentrations were in most cases under the detection limits.
- Secondary-tertiary treatment provided the best removal for most of the pollutants.

Abstract

The present study shows the results of an experimental survey conducted over 34 months on 76 full-scale Wastewater Treatment Plants located in central Italy with the aim to determine the influent and effluent concentrations of 13 Organic Micropollutants belonging to the class of illicit drugs, pharmaceuticals and steroids. The survey focused on a large set of plants differing for the main characteristics (e.g. treatment capacity, type of lay-out). Based on the values measured in the influent and effluent, removal efficiency of each contaminant in each plant was also determined, as well as the seasonal variation of the influent concentration. Among the monitored pollutants, some illicit drugs (i.e. Benzoylecgonine, 11-nor-carboxy-Δ9-tetrahydrocannabinol) and Ketoprofen showed the highest concentrations in the influent and were also the most frequently detected in the wastewater; nonetheless, the plants were capable of removing these pollutants at high extent (median removal value of 70%, 65% and 74%, respectively). On the other side, steroid concentrations were in most cases under the detection limits. About the type of lay-out, the comparison of the efficiency obtained by the different plants showed that combination of secondary and tertiary treatment provides the best removal for most of the target Organic Micropollutants.

Keywords: Endocrine Disrupting Compounds, Illicit drugs, Organic Emerging Micro-Pollutants, Pharmaceuticals, Removal, Wastewater

1 Introduction

Focus of studies in the field of wastewater treatments has recently moved from traditional contaminants, e.g. organic carbon and nitrogen, to a wide class of organic pollutants grouped together under the generic names of Organic Emerging Micro-Pollutants (OMPs) or Contaminants of Emerging Concern (CECs). A very high number of chemicals, such as disinfection by-products, pharmaceuticals, personal care products and illicit drugs, has been so far classified as OMPs. All of them are characterized by the very low concentrations in the environment (e.g. from ng/L to µg/L in wastewater); furthermore, in the past they have not been subjected to routine monitoring because of the uncertainties and difficulties in their analytical determination. As a consequence of the recent progress in instrumentations and techniques, it has been possible to start measuring different types of OMPs with sufficient reliability; therefore, it is expected that the list of compounds classified as OMPs will increase further in the near future (Rodriguez-Narvaez et al., 2017). Sources of OMPs are widespread, most of which are anthropogenic such as industrial activity, personal care, houses, hospitals, livestock, and agriculture. Releases from these sources are often collected into the sewage networks, which then transfer OMPs to Wastewater Treatment Plants (WWTPs). Here the removal is usually very limited since the plants are not specifically designed to address these contaminants. Therefore, the final effluent still contains the OMPs or their transformation products, which are consequently released into the aquatic environment. It has been demonstrated that many OMPs are toxic for living organisms or can cause adverse effects (Gogoi et al., 2018). At the moment, due to the lack of a comprehensive knowledge on the presence, fate and effects, limits on the maximum concentration for the effluent from WWTPs have not been fixed yet for most of OMPs. The proposal for regulation of the European Parliament and the Council on minimum requirements for water reuse, however, includes a clause that considers the possibility of implementing additional requirements for water quality on substances of emerging concern, such as pharmaceuticals and pesticides, based on technical and scientific progresses (COM(2018) 337 final).

According to several studies, the removal efficiency of OMPs is strongly dependent on the technology implemented in the WWTP (Loos et al., 2013). In Italy, most of the plants apply the secondary treatment

process, with or without the primary sedimentation; tertiary treatments are so far less commonly diffused (Patrolecco et al., 2015). Primary treatments usually do not contribute significantly to OMPs removal because they operate only a physical separation of solid particles and sometimes colloids, while OMPs are usually present as dissolved in the liquid phase. Several studies proved that biological treatments are effective for the removal of biodegradable OMPs, such as illicit drugs (Chiavola et al., 2019). In contrast, they are unable to remove recalcitrant OMPs such as perfluoroalkyl substances (Chiavola et al., 2020). Some researches refer that tertiary treatments can be more effective for this purpose (Garcia-Rodríguez et al., 2014; Morlay et al., 2018).

This study presents the results of an experimental survey conducted over 34 months on 76 full-scale WWTPs in central Italy with the aim to determine the influent and effluent concentrations of 13 OMPs belonging to the class of illicit drugs, pharmaceuticals and steroids. The target OMPs investigated in the survey were selected being representative of some of the most diffused classes of OMPs in the domestic wastewater (illicit drugs, pharmaceuticals and steroids), as referred by the scientific literature. Furthermore, for other classes of OMPs the analytical methods still present some uncertainties, whereas for the selected pollutants the research group has been able through previous studies to assess reliable detection methods (Boni et al., 2018a; Chiavola et al., 2019, 2017, 2016). Particularly, within the class of illicit drugs and their metabolites, the following compounds were investigated: Benzoylecgonine (BE), 11-nor-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), Amphetamine (AM), Methamphetamine (MET). The examined pharmaceuticals included: Ketoprofen (KTP), Sulfamethoxazole (SMX), Carbamazepine (CBZ), Trimethoprim (TMT), Lincomycin (LCN). Steroids included: Progesterone (P4), Estrone (E1), 17β Estradiol (E2), 17α Ethynylestradiol (EE2). Some of the selected compounds, such as E1, E2, EE2 are included in the list of substances for European Union-wide monitoring updated in Decision 2018/840/EU (European Commission, 2018).

For instance, illicit drugs are found in the sewage as originated from human excretion or waste, and their concentrations are correlated with human consumption; BE is the major human metabolite of cocaine, THC-COOH is the main secondary metabolite of tetrahydrocannabinol (THC), AM and MET are central nervous system stimulants. Pharmaceutical compounds and hormones concentrations are also related to human consumption as well as industrial waste; KTP is a nonsteroidal anti-inflammatory drugs, SMX is a

sulphonamide bacteriostatic antibiotic, TMT and LCN are antibiotics, and CBZ is an anticonvulsant or anti-epileptic drug. P4 is an endogenous steroid and progestogen sex hormone, E1 is a steroid, a weak estrogen and a minor female sex hormone. E2 is an estrogen steroid hormone and the major female sex hormone. Beside the wide diffusion in the environment, the high bioreactivity and low biodegradability make them to represent a potential ecological risk to aquatic environments and the more sensitive living species (Bradley et al., 2017). Because of these issues, some of the target compounds are under the attention of the European community and it is likely that in the near future some regulations or control strategies will be issued on them.

The aim of the study was to assess the occurrence in the influent and effluent of the target OMPs in a wide set of WWTPs; the overall efficiency of the plants depending on the treatment level (i.e. primary, secondary and tertiary treatments) was also estimated. Additionally, the seasonal variation of the influent and effluent concentrations was evaluated.

The study provides fundamental data for the enhancement of the current knowledge about the presence and fate of OMPs in the WWTPs, and also on the effect of the level of treatment adopted. Despite the increasing number of studies on the presence of OMPs in wastewater, very few considered so many different plants and correlated the efficiency to the specific-lay-out (Cosenza et al., 2018; Meffe and de Bustamante, 2014; Patrolecco et al., 2015; Zuccato et al., 2006, 2005). Furthermore, the data also allows to fill the gap of knowledge about the seasonal variability of OMPs concentrations in the influent to and effluent from WWTPs, which was highlighted by several studies (Ebele et al., 2017; Qi et al., 2014). With respect to the previous literature, the present study investigates a very high number of full-scale plants, whereas past studies considered only a few cases; furthermore, the present monitoring campaign lasted much longer than those reported by other studies, thus making the results obtained more significant.

2 Materials and Methods

2.1 Chemicals

Standard solutions of the analysed OMPs (BE, CBZ, THC-COOH, AM, MET, KTP, SMX, TMT, LCN, P4, E1, E2, EE2) and of the internal standard Carbamazepine-d10 were purchased from Sigma-Aldrich Company

(Gillingham, UK), each one at a concentration of 100 µg/mL in methanol. Main chemical-physical characteristics of the contaminants are reported in Table 1 (Williams et al., 2017).

Table 1.

Main chemical-physical characteristics of the target OMPs: CAS n. = CAS number; Formula= Chemical formula; MW=Molecular Weight; pK_a=-log of acid dissociation constant; Log K_{ow}=log of octanol-water partition coefficient; K_H=Henry's law constant; Log K_{oc}=log of organic carbon-water partition coefficient; S=water solubility.

OMPs	**CAS n.**	**Formula**	**MW**	**pK_a**	**LogK_{ow}**	**K_H**	**Log K_{OC}**	**S @25°C**
	/	**/**	**[g/mol]**	**/**	**/**	**[atm×m³/mol]**	**[L/kg]**	**[mg/L]**
BE	519-09-5	$C_{16}H_{19}NO_4$	289.326	3.15	-1.32	$1.03\ 10^{-13}$	2.548	$1.60\ 10^{3}$
THC-COOH	56354 -06-4	$C_{21}H_{28}O_4$	52857.365	4.21	1.74	$3.87\ 10^{-12}$	2.794	711.9
AM	300-62-9	$C_9H_{13}N$	135.206	10.1	1.76	$1.08\ 10^{-6}$	3.045	$2.81\ 10^{4}$
MET	537-46-2	$C_{10}H_{15}N$	149.237	9.87	2.07	$2.37\ 10^{-6}$	3.207	$1.33\ 10^{4}$
KTP	22071-15-4	$C_{16}H_{14}O_3$	254.285	4.5	3.12	$2.12\ 10^{-11}$	2.459	51 (22°C)
SMX	723-46-6	$C_{10}H_{11}N_3O_3S$	253.276	6.16	0.89	$9.56\ 10^{-13}$	3.185	$3.94\ 10^{3}$
CBZ	298-46-4	$C_{15}H_{12}N_2O$	236.2686	13.9	2.45	$1.08\ 10^{-10}$	3.588	17.7
TMT	738-70-5	$C_{14}H_{18}N_4O_3$	290.318	7.12	0.91	$2.39\ 10^{-14}$	2.957	$2.33\ 10^{3}$
LCN	154-21-2	$C_{18}H_{34}N_2O_6S$	406.538	7.6	0.2	$3.00\ 10^{-23}$	1.768	927
P4	57-83-0	$C_{21}H_{30}O_2$	314.462	18.47	3.87	$6.49\ 10^{-8}$	3.902	8.81
E1	53-16-7	$C_{18}H_{22}O_2$	270.366	10.33	3.13	$3.80\ 10^{-10}$	3.13	30
E2	50-28-2	$C_{18}H_{24}O_2$	272.388	10.33	3.13	$1.41\ 10^{-12}$	4.205	81.97
EE2	57-63-6	$C_{20}H_{24}O_2$	296.41	10.33	3.67	$7.94\ 10^{-12}$	4.678	11.3

2.2 Sample collection at the WWTPs

Influent and effluent samples were collected from 76 different WWTPs located in central Italy. The study was conducted over about 2.5 years (from March 2017 to December 2019). Because of the long hydraulic retention times of the WWTPs, it was decided to perform wastewater collection through grab sampling; the

data were then statistically analysed in order to obtain representative results. During the first year, the monitoring campaign included a total number of 728 measurements and focused on selected illicit drugs and steroids. Starting from the second year, the list of monitored compounds was enriched by adding also pharmaceuticals to illicit drugs and steroids; the total number of measurements increased to 1858. The total number of sampling days varied from 1 to 12 depending on the WWTP. Supplementary Materials (S.M.) report the main lay-out of each WWTP and the number of samples collected. The WWTPs were grouped into 4 classes based on the increasing complexity of the treatment level: 1) only secondary treatment (ST) which includes the biological reactor followed by the secondary settlement tank; 2) primary treatment (primary settlement tank) followed by the secondary treatment (PT+ST); 3) secondary and tertiary treatments (ST+TT); 4) primary, secondary and tertiary treatments (PT+ST+TT). The tertiary treatments considered in the present study are those providing the effluent of the biological reactor + secondary settlement with a further treatment stage; specifically, they consisted of microfiltration, ultrafiltration or their combination (see S.M.). For the purpose of grouping the treatments based on their relative complexity, MBR was considered equivalent to the ST+TT lay-out, since it combines the biological treatment with the membrane separation process (microfiltration or ultrafiltration).

The disinfection unit was not considered as a tertiary treatment because in all the investigated 76 WWTPs it is not operated continuously over the entire year, but only for a few months (usually from April to September which corresponds to the bathing season).

Table 2 shows a summary of the classes of plants considered in the study, along with the corresponding number of WWTPs (n.) belonging to each class and the number of measurements carried out for each group of contaminants.

Table 2.

Summary of the entire data set about WWTPs.

Treatments	**WWTPs**	**Illicit drugs**	**Pharmaceuticals**	**Steroids**
	[n.]	**[n. measurements]**	**[n. measurements]**	**[n. measurements]**

ST	29	270	206	144
PT+ST	6	24	0	24
ST+TT	39	404	216	296
PS+ST+TT	2	440	186	376
Total	76	1138	608	840

2.3 Analytical methods

The analytical technique chosen for the quantitative analysis of the OMPs in the samples was the Ultra-Performance Liquid Chromatography coupled to tandem Mass Spectrometry (UPLC–MS/MS). The analytical method is based on EPA 538 and previous studies of the same research group (Boni et al., 2018b; Chiavola et al., 2019; U.S. Environmental Protection Agency, 2009). For calibration and quantification, the internal standard (IS) approach was followed with Carbamazepine-d10 being used as the IS. The liquid sample pre-treatment consisted only of a filtration step by using a 0.2 μm membrane filter of regenerated cellulose.

Each contaminant was quantified by MRM (Multiple Reaction Monitoring ratio) using the two most abundant precursor/product ion transitions of the two analytes and the IS.

According to the method, filtration is followed by a direct injection in the UPLC-MS/MS system with the instrumental conditions reported below:

1) UPLC: Ultimate 3000 RS Thermo, with two pumps, degasser, column oven compartment and auto sampler; Chromatography column was Phenomenex Kinetex 2.6μm Biphenyl 100A, 100x2.1 mm with security-guard column at 30°C. Mobile phase A: 100 % Milli-Q water acidified with 0.1% formic acid; mobile phase B: 100 % LC-MS methanol acidified with 0.1% formic acid. The gradient elution conditions were from 95% A and 5% B to 0% A and 100% B in 8 min. Flow was fixed at 0.3-0.4 mL/min, whereas the injected volume was 50 μL.

2) Mass spectrometer: 5500 AB Sciex Q-Trap with Atlas Copco FS2 compressor, FX1 dryer, 270 L tank and nitrogen generator Zephyr Zero 16 LC-MS. The applied UPLC-MS/MS parameters are reported in S.M..

The overall response time for each liquid sample was below 30 min.

Limits of detection (LOD) were determined using signal/noise ratios of 10, for 7 replicates. Furthermore, Minimum Reporting Level (MRL) was defined as the LOD rounded to the second decimal, according to EPA method (U.S. Environmental Protection Agency, 2009). MRL values of each OMP are reported in supplementary materials; they also correspond to the minimum values of the concentration detected in the experimental samples (Table 3).

The quality assurance and quality control were checked within each series of measurement with the following criteria: the linearity coefficient (R2) and relative standard deviation (RSD%) of the calibration curves were up to 0.990 and 10%, respectively. The bias was lower than 30%. The repeatability of the measurements in the samples matrix (wastewater) was lower than 20%. The expanded uncertainty (UEXP) of the analytical method was lower than 39% with a confidence level of 95%.

2.4 Calculation methods

The frequency of detection (F_D) was calculated as outlined by Equation 1:

$$F_D[\%] = \frac{n}{N} \cdot 100 \quad (1)$$

where N is the total number of samples and n is the number of samples with a concentration above the MRL concentration, for a given contaminant.

All the boxplot graphs presented in this study were built using R software and they display different statistical elements, as reported in the right side of Fig. 2. For instance, the box shows the interquartile range (IQR), which represents the difference between the upper (Q3, the 75th percentile) and lower quartiles (Q1, the 25th percentile). The bar inside the box indicates the median value (the 50th percentile). The dots represent the outliers. The whisker (extreme lines) are the maximum and minimum values in the data which cannot be considered outliers and they are defined as (Wickham, 2016):

Q3+ 1.5×IQR for maximum (2)

Q1- 1.5×IQR for minimum (3)

The percentage removal efficiency (R) was calculated as indicated by below:

$$R\ [\%] = \frac{C_{in} - C_{ef}}{C_{in}} \cdot 100 \quad (4)$$

where Cin and Cef stand for the influent and effluent concentrations for each contaminant.

The removal was not calculated if the influent and effluent concentration were both equal to the MRL.

Statistical analysis of the performance of the different classes of WWTPs was carried out by using the one-way ANOVA test, with the R package "stats" (R Core Team, 2019).

The standardized removal efficiency (SRE) was calculated by following the equation below:

$$SRE = \frac{x - \mu}{\sigma} \cdot \frac{n}{N} \quad (5)$$

where x represents each individual removal efficiency for a given contaminant in a specific WWTP and sampling day, μ is the average removal efficiency for the contaminant over all WWTPs, σ is the standard deviation of the removal efficiencies for a contaminant over all WWTPs, n is the number of measurements for the contaminant in the WWTPs class which the plant belongs to and N is the number of measurements across all WWTPs (Ben et al., 2018).

The seasonal variation was calculated as follow:

$$Season\ [\%] = \frac{C_{se} - C_{av}}{C_{av}} \cdot 100 \quad (6)$$

where Cse and Cav stand for the influent average concentrations measured over each season and over the entire period of monitoring, respectively. Each season was considered three months-long and defined as: Winter (December, January and February), Spring (March, April and May), Summer (June, July and August) and Autumn (September, October and November).

3 Results and discussions

3.1 OMPs occurrence in the influent and effluent

Fig. 1 shows the average concentration of the target OMPs measured in the influent and effluent of each WWTP over the entire monitoring period. In the plot, each bar represents the cumulated concentration of all the target OMPs in one WWTP. Although OMPs concentration varied among the plants, a reduction from the influent to the effluent can be observed in most of the plants.

BE was the most abundant compound found in the influent, followed by KTP and the other pharmaceuticals.

Concentration of steroids and AM, MET were found under the detection limits in most of the influent and effluent samples. THC-COOH represented 2.4% of the cumulated median influent concentrations therefore, its presence can be considered of no concern as compared to other investigated compounds.

The difference in the concentration profiles has to be ascribed to the different characteristics of the plants such as the use of the population served by the plant and the lay-outs, as well as to the sampling season during the year and the environmental conditions which can affect stability of the OMPs in the sewage network (Castiglioni et al., 2013; Couto et al., 2019). It is important to notice that, considering the long duration of the monitoring campaign and the wide number of OMPs and of WWTPs, the sampling was conducted randomly during the week and day. Therefore, temporal variability could be a reason of variations between concentration profiles across the monitored WWTPs. Indeed, several studies have identified temporal and spatial variability in different plants. Besides, chemical stability of the investigated OMPs is also considered a significant factor that affects different profiles of OMPs in the influent wastewater (Petrie et al., 2015). Coutu et al. (2013) investigated intra-day variation of antibiotics concentrations in the influent of one WWTP and observed peak concentrations between hours 07:00 and 09:00. These results are explained by the accumulation of consumed drugs in urine during sleep, and can be extended to the pharmaceutical,

illicit drugs and administered hormones considered in this study (Coutu et al., 2013). Furthermore, inter-day variability of illicit drugs concentrations in influent wastewater has been identified by other authors; in an extensive study including 19 European countries higher benzoylecgonine and MDMA concentrations were measured on Saturdays and Sundays, due to higher consumption during the weekend (Thomas et al., 2012). Spatial distribution of contaminant concentrations in wastewater is often described in scientific literature as a notoriously complex factor to assess; it may be caused from dilutions (due to industrial influent, rainfall), size of population served by WWTPs, or chemical degradation into the sewage network which is itself a complex source of data variability (Petrie et al., 2014).

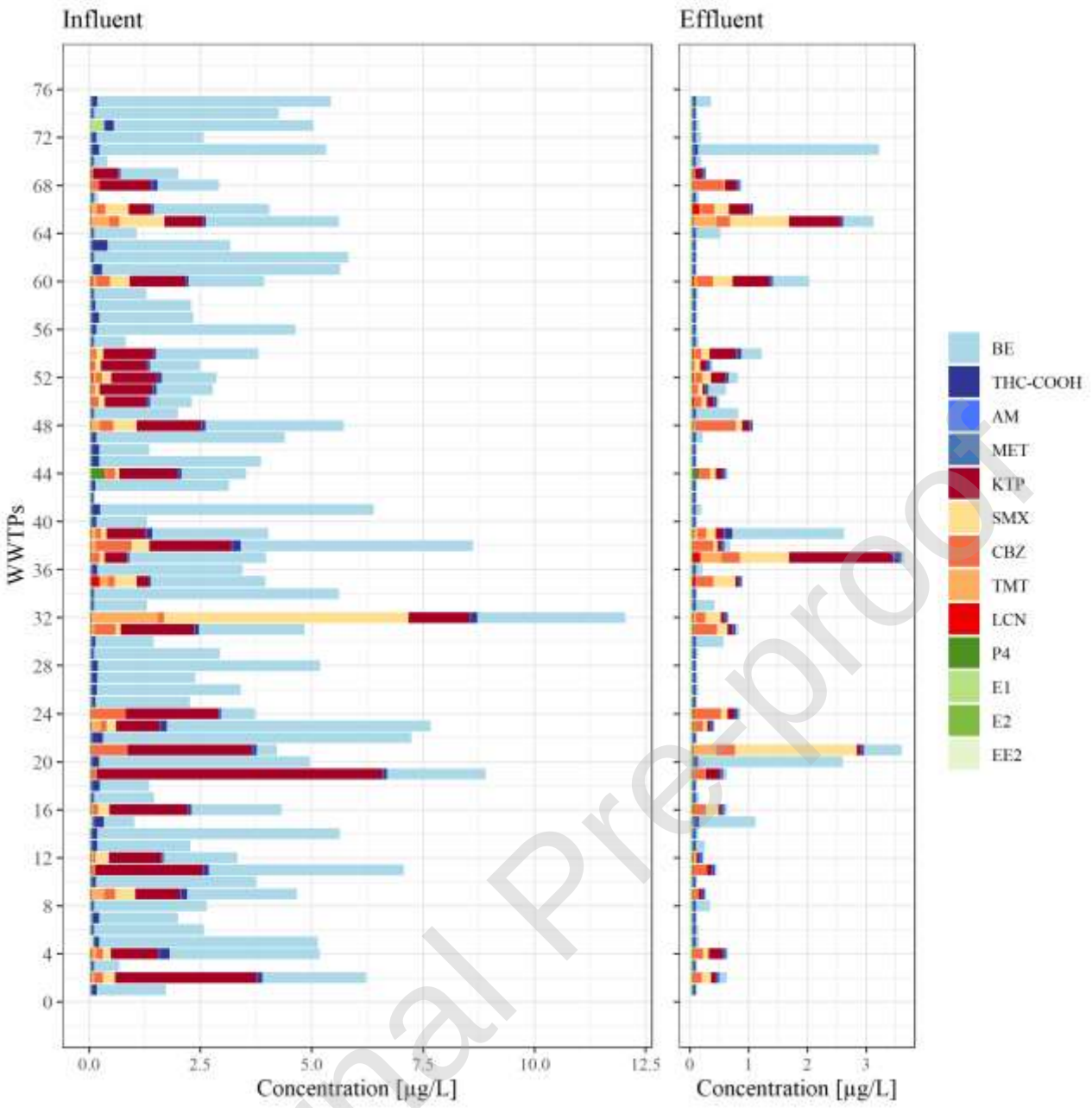

Fig. 1. Average concentrations of the target OMPs in the influent and effluent of each WWTP measured over the entire monitoring period (each bar represents one WWTP).

Table 3 summarises data of the frequency of detection (F_D) of the 13 OMPs in the influent (IN) and effluent (OUT) of the 76 WWTPs, along with the maximum, minimum and average concentrations measured in all the plants. Frequency of detection indicates the percentage of measurements that provided a value above the MRL (Eq. 1), which represents the minimum concentration that can be reported as a quantitated value for the analysed sample (U.S. Environmental protection Agency, EPA, 2009). The contaminants found with the

highest frequency of detection in the influent were: BE (96%), LCN (98%), KTP (98%) and CBZ (96%). The compounds found most frequently in the effluent samples were: BE (58%), KTP (81%), CBZ (91%), along with SMX (71%). It is noteworthy that SMX, KTP and CBZ showed very similar influent and effluent F_D values.

On the other hand, steroids, AM and MET were the contaminants found with the lowest F_D.

The two illicit drugs were never detected in influent and effluent of the WWTPs, which is in accordance with other studies (S. Castiglioni et al., 2006; Repice et al., 2013). The last Italian drug report refers also a decreasing trend of the amphetamines use, which represented in 2017 the 0.3% of the total drugs consumption in the country among young adults (which represent the main illicit drugs users) (European Monitoring Centre for Drugs and Drug Addiction, 2019). It is noteworthy that the range of the detected concentrations of steroids in wastewater was few ng/L in several scientific studies, as reported in Table 5; therefore, the low frequency of detection highlighted for this class of compounds in the present study might result higher by adopting a different analytical method. Indeed, as reported in the Materials and Methods section, the limits of detection assumed in the present study for these compounds were equal to the corresponding MLR; however, these values are above the maximum acceptable detection limits of the analytical methods as indicated by the 2015/495/UE decision (which are 0.035 ng/L for EE2 and 0.4 ng/L for E1 and E2) (Barbosa et al., 2016; European Commission, 2015). Lowering the MLR would have been also possible in the present case, provided that a complex pre-treatment of the samples was adopted. However, since the aim of the study was to conduct a monitoring campaign with a routine-based approach, it was preferred to analyse a higher number of samples to provide reliable data than to adopt the more expensive pre-treatment.

Table 3.

Minimum, maximum and average concentrations of each OMPs in the influent (IN) and effluent (OUT) of the 76 WWTPs monitored. F_D=frequency of detection.

Compound	Minimum	Maximum	Average	F_D

	IN [µg/L]	OUT [µg/L]	IN [µg/L]	OUT [µg/L]	IN [µg/L]	OUT [µg/L]	IN [%]	OUT [%]
BE	<0.010	<0.010	10.553	3.080	2.382	0.175	96	58
THC-COOH	<0.025	<0.025	0.410	0.280	0.075	0.029	50	5
AM	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	0	0
MET	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0	0
KTP	<0.010	<0.010	6.414	1.770	1.429	0.204	98	81
SMX	<0.010	<0.010	5.490	2.070	0.286	0.182	68	71
CBZ	<0.010	<0.010	1.381	0.890	0.209	0.193	96	91
TMT	<0.010	<0.010	1.490	0.400	0.080	0.037	45	35
LCN	<0.005	<0.005	0.190	0.130	0.017	0.017	98	24
P4	<0.010	<0.010	1.150	0.390	0.018	0.013	1	1
E1	<0.010	<0.010	0.300	<0.010	0.013	<0.010	5	0
E2	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0	0
EE2	<0.020	<0.020	0.080	<0.020	0.021	<0.020	4	0

Fig. 2 depicts the statistical variation around the median value of the influent and effluent concentrations of each OMP in the 76 WWTPs.

Fig. 2 highlights that the highest influent concentration was 10.55 µg/L of BE, which is the major metabolite of cocaine; this contaminant was also the one showing the widest variability among the WWTPs. The two second highest influent concentrations were 6.41 µg/L of KTP and 5.49 µg/L of SMX, both belonging to the class of pharmaceuticals. Among the investigated compounds, P4, E1, E2, EE2, AM and MET were found at

the lowest concentrations (similar to the MRL). In general, illicit drugs and pharmaceuticals showed higher concentrations than steroids.

As seen in Fig. 2, similarly to the findings for the influent, the OMPs detected at the highest concentrations in the effluent samples were BE, SMX and KTP, with average concentrations equal to 3.08 µg/L, 2.07 µg/L and 1.77 µg/L, respectively.

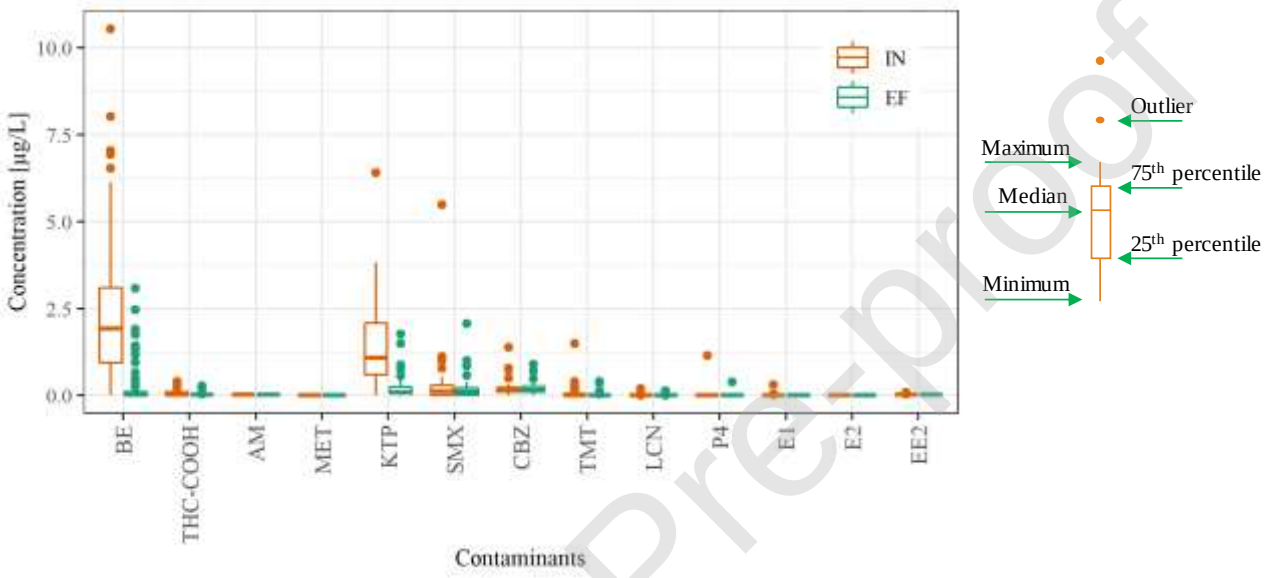

Fig. 2. Statistical variation around the median value of influent (IN) and effluent (OUT) concentrations of each OMP in the 76 WWTPs.

3.2 Removal efficiencies

Fig. 3 illustrates the removal efficiencies (R) calculated using Eq. 4, for each contaminant and for each class of WWTP as listed in Table 2. The legend of the plot shows the classes of WWTPs and in brackets the number of plants belonging to each class. On the right side of the plot, the number of samples considered for each plant lay-out is also reported.

As mentioned above, when the influent and effluent concentrations were both equal to the MRL, the removal was not calculated. Particularly, the values of removal efficiency obtained for AM, MET, P4, E1, E2 and EE2 were not considered relevant due to the very low F_D ($F_D \leq 5$) both in the influent and effluent samples.

Nevertheless, some values of the removal percentages for E1 and EE2 were calculated to be above 50% (see Fig. 3).

As highlighted by Fig. 3, CBZ exhibited a very low removal efficiency (below 50%) for all the levels of treatments. Particularly, the lowest efficiency resulted when the sole secondary treatment is applied, whereas a slight improvement can be observed when primary and/or tertiary treatment are present. Some negative values of the removal efficiency were also measured, which represent an increase of the concentration during the treatment. Since CBZ measurements provided significant F_D values, this gives a statistical relevancy to the results. These findings can be ascribed to the characteristics of the compound which is recalcitrant to biological treatment and has a low capacity to be adsorbed on the sewage sludge (Couto et al., 2019; Fernandez-Fontaina et al., 2016). Therefore, it might be accumulated and later released giving rise to higher concentrations in the effluent (Luo et al., 2014).

TMT showed similar removal efficiency (at around 50%) for both ST and PT+ST levels of treatment, whereas an appreciable improvement was observed when the tertiary treatment was present. Similarly, SMX exhibited removal efficiency below 50% when the lay-out consisted only of ST and PT+ST, while in the presence of TT performance increased up to about 80%. TMT was better removed compared to SMX, which is usually co-administered, probably because it can be biodegraded by nitrifying activated sludge bacteria contrary to SMX which was found to be more persistent to biodegradation (Oh et al., 2018).

In most of the cases, the ST and PT+ST lay-outs provided similar performances, while an improvement was obtained when the tertiary treatment was implemented. Particularly, for CBZ, SMX and TMT, the presence of tertiary treatments in the WWTPs lay-out seems to be responsible for a better removal. This positive effect could be ascribed to the good efficiency of the filtration processes implemented in the tertiary treatment in the removal of polar compounds such as these OMPs (Rodriguez-Narvaez et al., 2017).

No removal was observed for LCN. This finding is in agreement with previous studies which refer LCN to be recalcitrant and not expected to be adsorbed onto sludge; instead it can more easily dissociate in the aqueous phase (LogKow < 3 and pKa = 7.6) (Tran et al., 2018, 2016). It is also noteworthy that due to the very low concentration in the influent, it is unlikely that LCN could support microorganisms growth as energy and carbon source.

For the other OMPs (BE, THC-COOH and KTP), the removal values were found equal or greater than 50%. Particularly, the best removals were observed for BE (media removal > 70%) and KTP (media removal > 75%), with a homogeneous distribution of the data as highlighted by the dimensions of the boxes in Fig. 3. THC-COOH was removed with a median efficiency of 65%. For these OMPs, performance between the different classes of WWTPs did not change significantly, according to the results of the one-way ANOVA test (p-value > 0.05).

MET and AM concentrations were detected under MRL, and therefore the removal was not calculated.

Aside from the low efficiency observed for LCN and CBZ, the other investigated OMPs (i.e. BE, THC-COOH, KTP, E2, E1) were removed for values equal or above 50%. These results must be evaluated considering that so far domestic sewage WWTPs, such as those of the present study, have not been designed to treat a wastewater containing these type of pollutants; therefore the observed removals took place along with or as a consequence of those of the target compounds (e.g. COD, BOD, solids, nutrients). Further studies should assess if, giving also the small influent concentrations, these removals are high enough to produce a final effluent which does not pose a risk for the environment and human health.

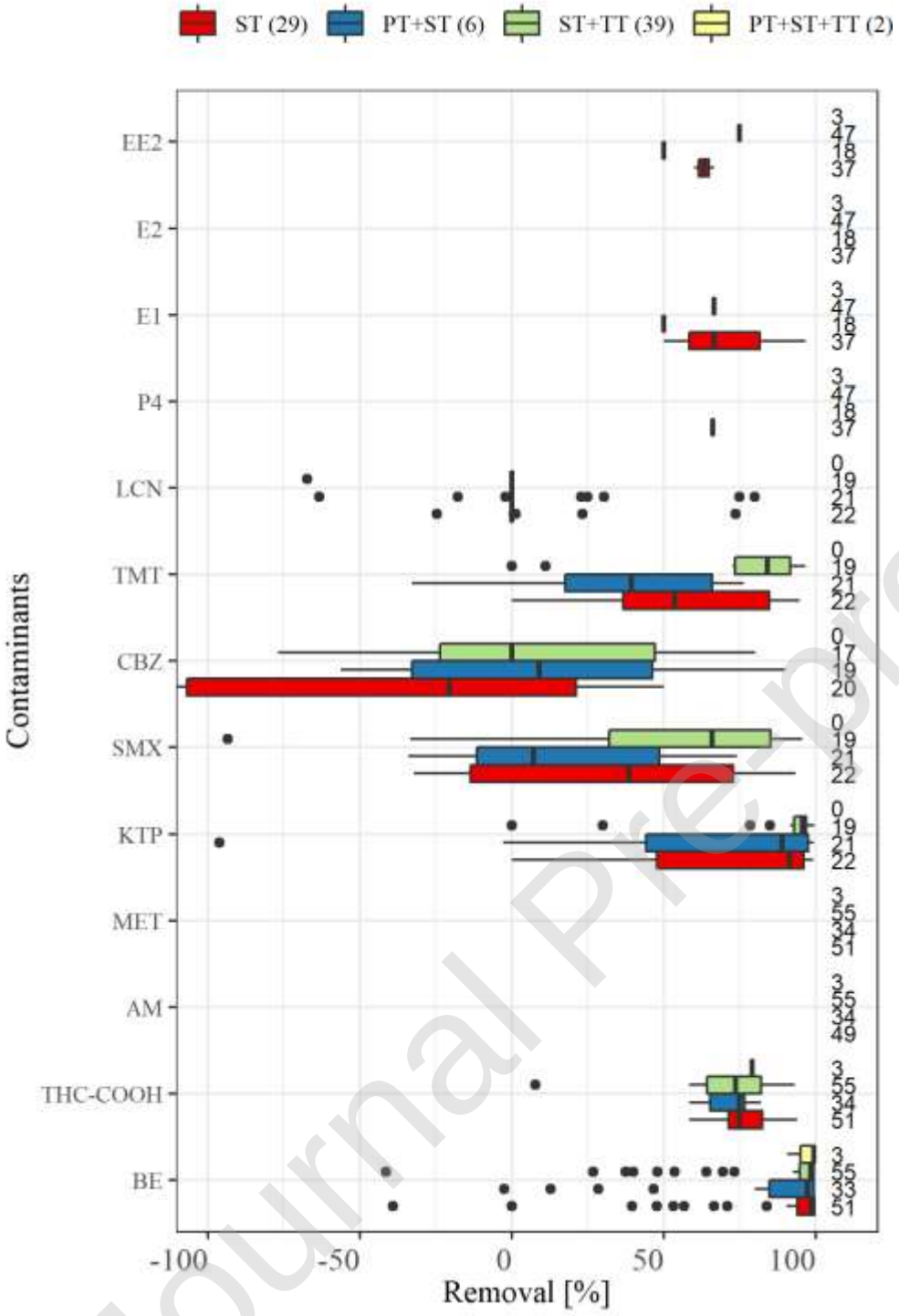

Fig. 3. Removal efficiency of each OMP for the different classes of WWTPs.

3.3 Standardized removal efficiencies

For a better evaluation of the influence of the different classes of treatment on the removal efficiencies, the Standardized Removal Efficiency (SRE) was calculated for each contaminant by following Eq.5. The SRE

weighs the removal efficiencies, R, per treatment class considering the overall average and the standard deviation per OMP and the number of measurements available per treatment class. The distributions of the SRE values, for all the OMPs together (Fig.4) and for each specific OMPs (Fig. 5) were represented as box plots and the red circles indicate the average values of SREs. The position of this symbol indicates the overall removal achieved by a class of WWTPs with respect to the average removal of the entire dataset: the right side of the graph indicates a better removal, while the left side lower removal.

Fig.4 shows the SRE distribution for all the OMPs by class of wastewater treatment. This representation allows to evaluate the overall performance of each class of WWTP with respect to the mix of contaminants monitored, in view of the possible cumulative risk assessment related to the potential synergistic effects. On the right side of the plot, the number of measurements used to build each box is reported, while in the left side the number of plants is indicated in brackets.

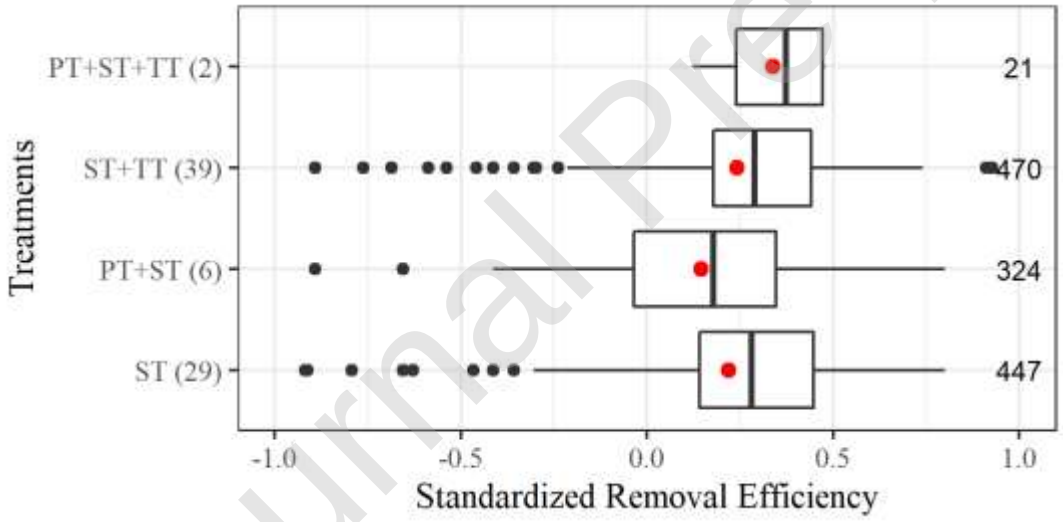

Fig. 4. Standardized removal efficiency of the different classes of WWTPs related to all the OMPs together.

Based on the most probable SRE values (which are those falling within 25th-75th percentile and graphically represented by the box) and the average (red dot inside the box) and median (black vertical line inside the box) values, the PT+ST+TT lay-out seems to be capable of the best removal. However, this treatment lay-out was the less commonly found among the investigated WWTPs; therefore, the number of measurements was

very low (21) and not enough to consider this result statistically representative (as described in Eq.5). The ST+TT class also showed a relatively high value of the SRE: since this result was obtained using a high number of measurements, it can be considered statistically significant. The plants with the secondary and primary treatments showed the lowest standardized removal and more widely dispersed results. Performance of the plants with ST only were slightly lower than those of ST+TT, although the data were more dispersed.

Based on these results, it can be assessed that for the investigated OMPs showing to be removed by the WWTPs for domestic sewage, the addition of the tertiary treatment to the secondary process (i.e. ST+TT lay-out) allows improved plant performance. This result is in accordance with several scientific studies (Morlay et al., 2018; Rizzo et al., 2019; Yadav et al., 2019). About the primary treatment, it is known that pollutant removal occurs due to settling of solids containing adsorbed contaminants. The results obtained in the present study demonstrated that this is not a relevant mechanism in the case of the investigated OMPs. This finding can be considered also of interest since very few studies have focused in the past on the role of the primary treatment (Couto et al., 2019).

A detailed focus on each OMP was provided in Fig. 5, considering only the contaminants detected with $F_D >$ 5%. It can be observed that BE, KTP and CBZ were similarly removed by all the classes of WWTPs as demonstrated by the correspondence between the median values (PT+ST+TT was not considered in this evaluation due to the insufficient number of available data, as explained before). However, in the presence of primary treatment the SRE values of BE and KTP were more dispersed as compared to the other treatment layouts. Instead, in the case of CBZ the presence of PT caused a reduction in the variability of the results whereas the ST+TT determined a slightly positive effect on data distribution. THC-COOH showed the same behaviour between the treatment classes based on the median values; however, there was a higher variability of the data as compared to BE and KTP, particularly in the ST+TT configuration. SMX and TMT were characterized by similar pattern: the best performance belonged to ST+TT, followed by ST and PT+ST, although SMX values were more scattered and the difference between the three layouts was more evident. The comparable behaviour of the two compounds can be related to their combined presence within the same antibiotic drug where they act synergistically (Straub, 2013). No relevant differences were found in terms of SRE for LCN between the treatments.

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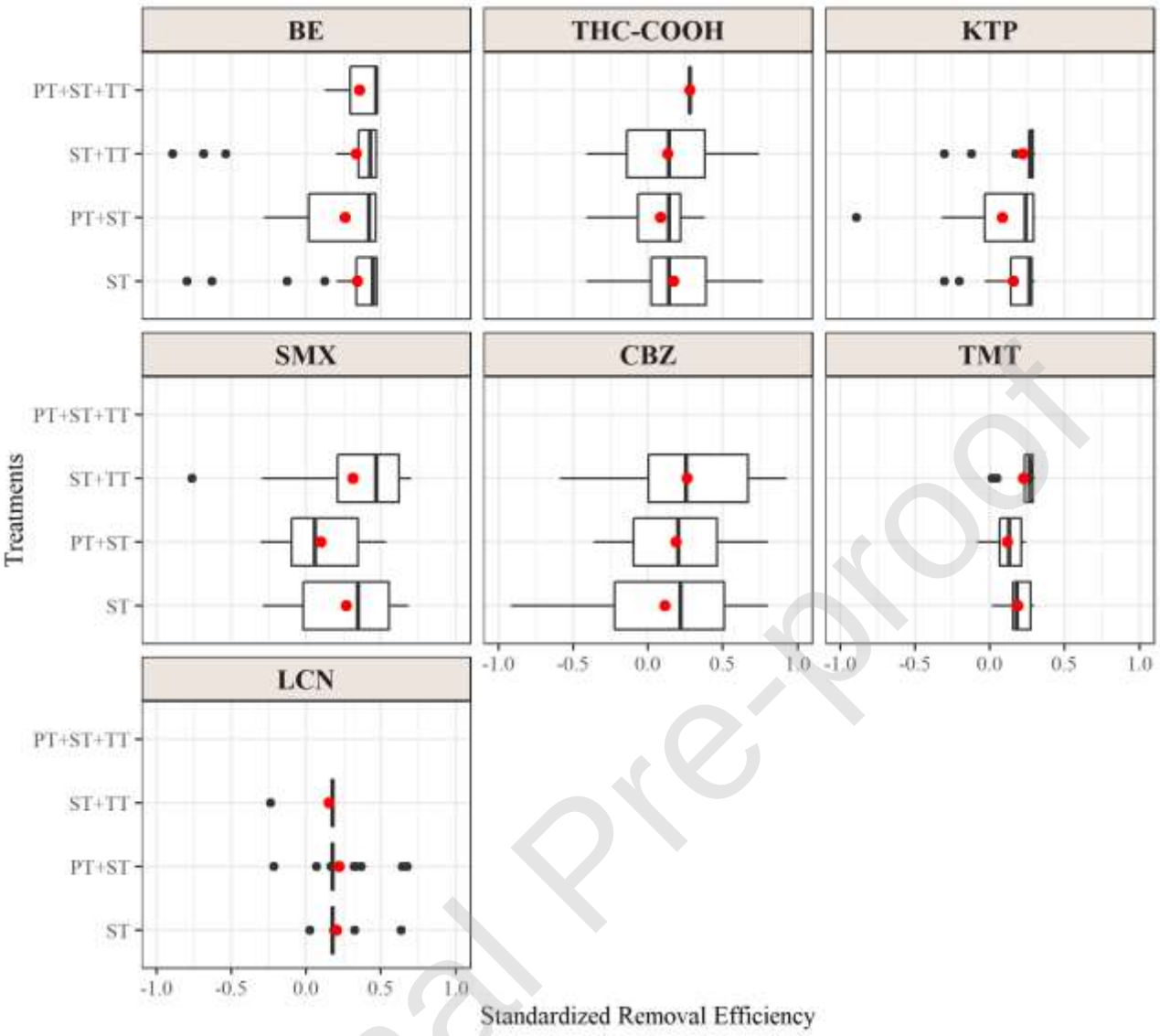

Fig. 5. Standardized removal efficiency of the different classes of WWTPs related to each OMP with F_D > 5%.

3.4 Seasonal variations of concentration profiles

It is likely that warmer seasons favour more social human activities outside, while lower temperatures may cause more illness and restrict the time used for socialising. Since illicit drugs and pharmaceuticals concentrations in the sewage networks are linked to human consumption and behaviour and to environmental conditions, there might be a correlation between seasonal changes and the discharged contaminant loads (Couto et al., 2019). Table 4 shows the average influent concentrations for each OMP measured over the

entire period of monitoring and the percentage difference with the average concentration of a specific season (Eq. 6).

Table 4.

Seasonal variations of the influent and effluent concentrations of the OMPs with F_D > 5%.

	BE	**SMX**	**TMT**	**THC-COOH**	**KTP**	**LCN**	**CBZ**
Influent							
Average [µg/L]	2.382	0.286	0.080	0.075	1.429	0.017	0.209
Winter [%]	-16	6	-14	-43	44	13	118
Spring [%]	-10	-72	-88	6	0	1	-39
Summer [%]	25	75	111	24	5	10	38
Autumn [%]	-2	-17	-38	-26	-18	-17	-57
Effluent							
Average [µg/L]	0.175	0.182	0.037	0.029	0.204	0.017	0.193
Winter [%]	6	3	-53	-15	33	11	59
Spring [%]	-13	-60	-73	-11	-44	-26	-35
Summer [%]	40	40	54	-2	-32	-2	47
Autumn [%]	-23	12	25	44	72	24	-54

The grey coloured cells indicate the highest percentage difference per OMP. When the F_D < 5%, the seasonal variation was considered not appreciable and is therefore not reported in Table 4. This condition was satisfied for AM, MET, P4, E1, E2 and EE2 influent and effluent concentration.

For the influent concentrations, the highest seasonal percentage increase was found for CBZ (118%) in winter (average temperature 7°C), followed by TMT (111%) in summer (average temperature 25°C). TMT is mostly used in combination with SMX to treat urinary tract infections; indeed, also SMX showed a similar

seasonal pattern. This is explained by an estimated seasonal peak in occurrence of urinary tract infections during summer (Rossignol et al., 2013). Therefore, looking at the OMPs found at the highest average concentrations (i.e. BE and KTP), their variations accounted for 25% and 44%, in summer and winter, respectively. It is likely that winter conditions weaken the immune system and favour illness, giving rise to higher antibiotic and anti-inflammatory consumption (KTP and LCN). It is noted LCN exhibits a less significant seasonal percent variation, explained by its low overall average concentration value (0.017 µg/L). In contrast, for CBZ, the highest concentrations were found in both winter and summer, without a specific explanation related to the consumption rates since it is an anticonvulsant drug whose use should thus be evenly spread over the entire year. However, for most of compounds, the influent concentration was higher in summer as compared to the other seasons. Generally, winter and summer were the seasons most affecting the contaminant presence in the influent, by increasing the concentrations with respect to the overall averages. The observed changes might be also due to the effects of the environmental conditions on the physical-chemical behaviour of the contaminants in water as reported by many authors (Baker and Kasprzyk-Hordern, 2013; Paíga et al., 2019; Sousa et al., 2017): for instance, solubility, chemical stability and chemical state of the compounds are affected by many parameters such as temperature, pH and composition of the liquid matrix, with all changing during the year in the WWTPs (Castiglioni et al., 2013).

For the effluent concentrations, the highest positive variations were found for KTP (72%) in autumn, CBZ (59%) in winter and TMT (54%) in summer. The greatest seasonal increases for CBZ, TMT, BE, and SMX in the effluent are consistent with the variations observed in the influent reported above: this suggests that for these contaminants, a prediction of the effluent concentration during the year can be made based on the influent load and the removal through the treatment units. Only KTP and LCN effluent seasonal variations did not match with the influent pattern. During autumn the average effluent concentration exhibited an increase for both OMPs, whereas the influent decreased (by -18% for KTP and -17% for LCN, on average basis). During summer and spring times, influent values did not show a significant variation with respect to the average, whereas the effluent concentrations decreased appreciably. This interesting observation common to both LCN and KTP might be explained as follows: the higher temperatures of spring and summer seasons combined with the longer retention time in the WWTP (due to the decrease of the influent flow rate in the dry season) could favour chemical degradation of these OMPs, thus determining a lower concentration in the

effluent (Castiglioni et al., 2013). Regarding THC-COOH, thus the effluent seasonal variability was not considered of interest because the compound was detected only in 5% of the samples.

3.5 Comparison with literature data

With the aim of validating the data obtained by the present study, a group of scientific papers was selected as a comparison within the wide and continuously increasing number of publications on the topic. The criteria used for the selection were the number and reliability of data provided and the similarities in terms of characteristics and conditions of the plants. As general observation it can be assessed that most of the values found in the present study for influent and effluent concentrations fall within the ranges reported by the selected scientific literature results; however, it is worth noting that these ranges are quite broad because of the differences among the investigated plants. Table 5 provides an overview of the main data reported by the specialized literature regarding the influent and effluent concentrations of the target OMPs measured in WWTPs. Some specific comments can be made about the different OMPs. Particularly, with respect to the scientific literature data, the present study measured higher inlet concentrations for BE: for instance, the maximum influent concentration was 10.55 µg/L while Table 5 lists a value of 4.75 µg/L at the top of the range. The value was measured at the middle of August 2019 (corresponding to the high holiday season in Italy) in a WWTPs that serves a costal municipality, which is more crowded during this period. Therefore the particular condition can explain the high value of cocaine's metabolite concentrations found in the wastewater, that is also similar to the value detected by Bijlsma et al. (2014) during music event (i.e. above 10 µg/L). About the BE in effluent, the concentrations measured in the present study fall within the range reported by the literature. Bijlsma et al. (2012) found for BE similar F_D values equal to 100% and 75% in the influent and effluent of Dutch WWTPs, respectively. Influent concentrations of THC-COOH fall within the reported range, although the highest value was higher than the top level of the range. Similar observations can be done for the effluent values. The removals achieved for this compounds belong to the range reported by Chiavola et al. (2019) and Cosenza et al. (2018). Differently, the values of the illicit drugs AM and MET (always below the MRL in influent and effluent samples) are similar to the lowest limits of the range reported by scientific literature. MET results are strictly in accordance with the study performed by Cosenza et al. (2018) regarding WWTPs in Italy.

About the class of pharmaceuticals, similar frequencies of detection were reported by Loos et al. (2013) for CBZ (90%) and TMT (93%) in the effluents of 90 WWTPs in Europe. By contrast, in the same study SMX was detected more frequently (83%), while LCN and KTP less frequently (37% and 47%, respectively) as compared to the results obtained in the present study. For SMX, the influent concentrations were similar, whereas the effluent values exceeded the highest level of the range reported in scientific literature selection. The values of the removal efficiency of SMX are very broad: for instance D'Alessio et al. (2018) and Spataro et al. (2019) reported removals values ranging from 0-75 % and 6-46 %, respectively. Another study conducted in Italian WWTPs found removal of this compound of 45-78 %, which are in the upper range of the values obtained through the present monitoring campaign (Castiglioni et al., 2006). TMT showed higher influent values than those listed in Table 5, and comparable values of the removal with Tran et al. (2016) (R% ranging from 23.8% to 42.2%) in the presence of primary and secondary treatments. Both influent and effluent concentrations of LCN and CBZ measured in the present study were far below the upper level of the ranges indicated by others scientific studies. Their removals were very low or negligible, according to other studies where a poor or negative removal was also highlighted in WWTPs using activated sludge processes (Behera et al., 2011; Castiglioni et al., 2018; Couto et al., 2019; Fernandez-Fontaina et al., 2016; Rodriguez-Narvaez et al., 2017). The KTP influent concentrations reported in Table 5 were higher than the values found in the present study, i.e. 11.24 µg/L as compared to 6.41 µg/L. Conversely, the highest effluent concentration was lower than the maximum value observed in the present study. Furthermore, a better removal efficiency was registered with respect to the values reported in the review by Grandclément et al. (2017) which ranged between 50-65 % with activated sludge treatments. The range of CBZ and KTP concentrations detected in the study of Patrolecco et al. (2015), performed on some of the WWTPs considered in the present study, are comparable to the average and minimum values observed during the present monitoring campaign; by contrast, the maximum contamination levels were much lower for KTP (0.198±0.034 µg/L) but similar for CBZ (1.519±0.114), indicating a change in the population consumptions and released through the years or to plant specific conditions.

Ranges of all steroid concentrations found in the selected literature are of similar low magnitude as those measured in the present study. Particularly, Patrolecco et al. (2015) performed a study about pharmaceuticals

and steroids in four WWTPs in Rome and found similar results about steroids (the concentrations were mainly below detection limits).

The seasonal variability of the influent concentrations described above is in agreement with other studies, where higher levels of some OMPs (i.e. pharmaceuticals) were found in warmer seasons as compared to the cold period of the year (Patrolecco et al., 2015; Pereira et al., 2015). This result can be ascribed to the differences in both consumption and excretion patterns of the population, and also to the reduced frequency of rainfall, which is one of the causes associated with the fluctuation of OMPs concentrations in the sewage network (Couto et al., 2019; Patrolecco et al., 2015; Pereira et al., 2015; van Nuijs et al., 2009).

Table 5.

Scientific literature overview of the influent (IN) and effluent (OUT) concentrations in WWTPs.

OMPs	**IN [µg/L]**	**OUT [µg/L]**	**References**
BE	0.005 – 4.75	0.0008 – 3.42	(Balakrishna et al., 2017; Bijlsma et al., 2014; S. Castiglioni et al., 2006; Cosenza et al., 2018; Pal et al., 2013; Petrie et al., 2014; Senta et al., 2013; Skees et al., 2018)
THC-COOH	0.015 – 0.10	0.001 – 0.044	(Cosenza et al., 2018; Senta et al., 2013)
AM	0.002 – 4.72	0.0006 – 2.24	(Balakrishna et al., 2017; Petrie et al., 2014; Senta et al., 2013)
MET	0.001 – 0.39	0.0002 – 0.50	(Balakrishna et al., 2017; Cosenza et al., 2018; D'Alessio et al., 2018; Petrie et al., 2014; Senta et al., 2013)
KTP	0.00013 – 11.24	0.00034 – 0.146	(Behera et al., 2011; Couto et al., 2019; Deblonde et al., 2011; Lishman et al., 2006)
SMX	0.00029 – 4.97	0.02 – 0.45	(Balakrishna et al., 2017; Behera et al., 2011; D'Alessio et al., 2018; Deblonde et al., 2011; Petrie et al., 2014)
CBZ	0.043 – 2.59	0.00037 – 3.117	(Balakrishna et al., 2017; Behera et al., 2011; D'Alessio et al., 2018; Deblonde et al., 2011; Krzeminski et al., 2019; Petrie et al., 2014; Tran and Gin, 2017)

TMT	0.033 – 0.29	0.013 – 1.152	(Balakrishna et al., 2017; Behera et al., 2011; D'Alessio et al., 2018; Deblonde et al., 2011; Petrie et al., 2014)
LCN	0.015 – 19.40	0.043 – 9.089	(Balakrishna et al., 2017; Behera et al., 2011; Verlicchi et al., 2012)
P4	0.009	0.0004	(Couto et al., 2019; D'Alessio et al., 2018)
E1	0.0032 – 0.005	0.0003 – 0.024	(Barbosa et al., 2016; Behera et al., 2011; Petrie et al., 2014; Zhang and Fent, 2018)
E2	0 – 0.005	0 – 0.0001	(Barbosa et al., 2016; Behera et al., 2011; Petrie et al., 2014; Zhang and Fent, 2018)
EE2	0 – 0.022	0.00005 – 0.0062	(Krzeminski et al., 2019; Petrie et al., 2014; Zhang and Fent, 2018)

4 Conclusions

The present study aimed at investigating the occurrence, removal and seasonal variation of 13 OMPs in 76 WWTPs located in the central Italy.

Based on the results obtained in the different plants, it is possible to draw the following general conclusions:

- the contaminants belonging to the class of steroids were mostly present at concentrations under the MRL;
- within the class of illicit drugs, AM and MET were detected at concentrations below MRL, BE was the contaminant found at the highest concentration (2.4 µg/L as average influent concentration) and also the one removed at to the highest extent (the median removal value of 100%), whereas THC-COOH showed an average concentration slightly above the MRL (0.08 versus 0.03 µg/L) and removal higher than 60% in most of the investigated WWTPs;
- in the class of pharmaceuticals, the highest concentration was found for KTP with an average influent value of 1.4 µg/L and median removal between 75% and 99% in most of the plants; CBZ showed the most heterogeneous distribution of the removal efficiency, also with negative values; TMT and SMX were detected in the influent at average concentrations of 0.08 µg/L and 0.3 µg/L, respectively, and removed in the ranges 45%-80% and 7%-70%, respectively; LCN was detected at average influent concentration slightly below 0.02 µg/L and it was the only compound not removed in most of the plants;

- the WWTPs with the secondary and tertiary treatments showed the best removal efficiency for most of the target OMPs;
- the most relevant increases of the influent concentrations due to season change were observed for TMT and SMX in summer and for CBZ and KTP in winter.

With respect to previous literature, the present study investigates a very high number of full-scale plants, whereas the past studies considered only a few cases; furthermore, the present monitoring campaign lasted much longer than those reported by other studies, thus making the results obtained more significant.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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