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ILLICIT DRUGS IN WASTEWATER TREATMENT PLANTS: OCCURRENCE AND BEHAVIOUR

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EXTENDED ABSTRACT

The recent interest towards the amount of illicit drugs discharged into the environment is mainly related to the increased use of illicit drugs and to their potential negative environmental impact (towards flora and fauna). The monitoring of illicit drugs concentration within wastewater treatment plants (WWTPs) could allow to better understand the processes for their degradation, indirectly estimate the community level consumption or identify the optimal plant operating conditions for increasing their degradation.

In this study two full-scale wastewater treatment plants (WWTPs) (namely, WWTP-1 and WWTP-2) located in the South of Italy (Sicily) have been monitored in view of analyzing the occurrence and the behavior of illicit drugs. Both plants have been monitored for four months. During the monitoring campaign, grab and composite samples of influent wastewater and treated effluent were withdrawn. Furthermore, grab samples were also withdrawn for the mixed liquor and the returned sludge. In view of analyzing the behavior of illicit drugs inside the WWTPs the following compounds have been selected and investigated: methamphetamine, cocaine (COC), 3,4-methylenedioxymethamphetamine (MDMA), methadone (METH), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), 3,4-methylenedioxy amphetamine (MDA); 3,4-methylenedioxy ethylamphetamine (MDEA), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) and Benzoylecgonine (BEG).

The adopted analytical methodology is divided into three phases: i. sample preparation; ii. solid phase extraction; iii. instrumental analysis.

Samples were first filtered by using first an 8 mm cellulose filter (Whatman, Kent, UK) and then a 0.45 mm nitrate cellulose filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany). Excepting for THC-COOH. Excepting for THC-COOH, all the analyzed illicit drugs and their metabolites were extracted by using the solid phase extraction (SPE) procedure. The eluates obtained after the SPE procedure were analyzed by adopting an high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) with a TSQ Quantiva triple-stage quadrupole mass spectrometer system interfaced to UHPLC Ultimate 3000 (Thermo Fisher Scientific Inc., Waltham, USA). Only for the THC-COOH measurement the Turbo-FlowTM online sample preparation technology was adopted.

The monitoring campaign of both WWTPs has revealed the presence of residues of illicit drugs in the influents wastewater. Different concentrations have been found for IDs and metabolites between grab and composite samples. Therefore, it is suggested to adopt composite sample to obtain an accurate analysis.

In particular, for the influent of WWTP-1 results showed high concentration of BEG (193.7 ng L^{-1} and 180.84 ng L^{-1} for grab and composite samples, respectively), COC (52.36 ng L^{-1} and 51.74 ng L^{-1} for grab and composite samples, respectively), CODEINE (82.05 ng L^{-1} and 62.47 ng L^{-1} for grab and composite samples, respectively) and THC-COOH cannabis metabolite) (104.98 ng L^{-1} and 87.91 ng L^{-1} for grab and composite samples, respectively). These compounds are directly related to the use of cocaine and cannabis of the population. All the other compounds have a negligible concentration in the influent wastewater of WWTP-1. The effluent illicit drugs and metabolite concentrations in were for WWTP-1 one order of magnitude lower than that of the influent samples (referring to the influent and effluent grab samples). However, a negative removal efficiency occurred for EDDP in WWTP-1. This result could be ascribable either to the presence of de-conjugates interfering with biological transformation of the de-conjugated compounds.

In WWTP-2 high influent concentration of some illicit drugs were found: BEG (cocaine's' metabolite) (235.11 ng L^{-1} and 183.53 ng L^{-1} for grab and composite samples, respectively), COC (81.14 ng L^{-1} and 74.03 ng L^{-1} for grab and composite samples, respectively), CODEINE (32.14 ng L^{-1} and 21.85 ng L^{-1} for grab and composite samples, respectively), MOR (33.80 ng L^{-1} and 19.42 ng L^{-1} for grab and composite samples, respectively) and THC-COOH (cannabis metabolite) (104.65 ng L^{-1} and 68.91 ng L^{-1} for grab and composite samples, respectively). A quite high average removal efficiency were found for BEG (77.85%), COC (92.34%), CODEINE (64.75%), MOR (90.16%) and THC-COOH (68.64%). Especially for WWTP-2 a substantial difference between the average value of grab and composite concentration for each compound were found. This result corroborate the literature findings which have demonstrated that for illicit drugs, where considerable temporal variability exists, the best sampling strategy is the composite daily sampling (ideally 24-h composites).

ABSTRACT

The occurrence of illicit drugs and related metabolites in two wastewater treatment plants (WWTPs) (namely, WWTP-1 and WWTP-2) located in Sicily (island in the South of Italy) is here discussed. The following illicit drugs have been investigated: methamphetamine, cocaine (COC), 3,4-methylenedioxymethamphetamine (MDMA), methadone (METH), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), 3,4-methylenedioxy amphetamine (MDA); 3,4-methylenedioxy ethylamphetamine (MDEA), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) and Benzoylecgonine (BEG). BEG, COC, MOR and THC-COOH have the highest concentration for both WWTPs. Inside WWTP-1 negative removal efficiencies were sometimes obtained. For WWTP-2 found for BEG (77.85%), COC (92.34%), CODEINE (64.75%), MOR (90.16%) and THC-COOH (68.64%).

Keywords: *drug behavior, water treatment, contaminants of emerging concern, illicit drugs*

INTRODUCTION

The interest towards the occurrence of emerging pollutants (EPs) in the environment has strongly increased during the last years. Several substances are defined as EPs: personal care products, endocrine disruptors surfactants, pharmaceuticals, gasoline additives and radionuclides (Loos *et alii*, 2013; Cosenza *et alii*, 2015). Illicit drugs (IDs) and their metabolites also represent a new group of water Eps (Pal *et alii*, 2013; Evgenidou *et alii*, 2015). IDs include cocaine, cannabis, amphetamine-type stimulants, ecstasy, heroin and other opioids (Degenhardt *et alii*, 2004).

The recent interest towards the amount of IDs discharged into the environment is mainly related to a twofold reason: i. the increased use of IDs (UNODC, 2014; UNODC, 2016); ii. their potential environmental impact (Li *et alii*, 2016; Santana-Viera *et alii*, 2016; Mastroianni *et alii*, 2017). Regarding the use of IDs, global production and consumption of illicit drugs has increased notably in recent decades. The UNODC (2016) revealed that 5% of the world population ranging between 15 and 64 years consumed an illicit drug in 2014. Since the IDs are often non-prescribed drugs and their use is prohibited by national drug control laws, it is difficult to quantify their amount discharged into environment. With this regards, several authors have recently established a new methodology (Wastewater-Based Epidemiology -WBE) to back-calculate the population drug use on the basis of the amount of a drug target residue concentration in the wastewater (among others, Maida *et alii*, 2017; Causanilles *et alii*, 2017). Indeed, IDs are excreted via urine and feces and arrive at wastewater treatment plants (WWTPs) where can reach ppb levels (Mohapatra *et alii*, 2016; Furhacker, 2008).

IDs can have serious potential effect on the environment due to their feature of being persistent. Indeed, the IDs' metabolites preserve the same active action of the original IDs, thus generating toxicological effects on non-target microorganisms (Causanilles *et alii*, 2017). The WWTPs represent a vehicle through IDs are discharged into the water bodies and environment. However, since no legal requirements have been set for the IDs discharge into the water bodies, the interest towards the influence of the WWTP on the IDs transformation and/or removal has progressively increased in view of protecting both the environment and the human health. Indeed, the main transport pathways of these compounds into the environment are via WWTPs where they may be only partially eliminated (Evgenidou *et alii*, 2015). Hence, over the few last years, IDs concentrations in raw and treated urban wastewater (WW) have been extensively monitored (Dong *et alii*, 2016). However, the role of the conventional biological processes in WWTPs on the IDs transformation is still poorly understood in literature. Some authors have demonstrated that the WWTPs have a very poor effect on IDs removal (Evgenidou *et alii*, 2015). A number of illicit drugs have been detected in the treated wastewater effluent due to the inability of being removed by conventional WWTP processes (Zuccato *et alii*, 2008, 2011; Bartelt-Hunt *et alii*, 2009). Thus, they are discharged into water bodies through the treated effluent (Postigo *et alii*, 2011). Several studies have demonstrated the difficulties on discriminating the key processes affecting the IDs and their metabolites transformation inside the WWTPs (Evgenidou *et alii*, 2015). Among these processes one can found: i. degradation to lower molecular weight compounds; ii. physical removal by solids and sludge waste; iii. transformation into conjugates compounds that can be hydrolyzed inside the WWTP and consequently released as parent compounds (Evgenidou *et alii*, 2015). Therefore, monitoring the IDs concentration in WWTPs can have several advantages (Yadav *et alii*, 2017): i. increase the knowledge on the amount of IDs discharged in the environment; ii. estimate the IDs effect on the water environment; iii. indirectly estimate the community level consumption; iv. identify the key plant operating factors mainly affecting the IDs transformation inside the WWTP (Senta *et alii*, 2014).

However, very few studies have been conducted in South of Italy (Zuccato *et alii*, 2016) and only some studies have been published for Sicily (Cosenza *et alii*, 2016; Maida *et alii*, 2017; Cosenza *et alii*, 2018).

Bearing in mind the aforementioned considerations, the objective of this paper is to provide a comprehensive analysis of the occurrence and behavior of IDs and their metabolites in two Sicilian WWTPs. Specifically, two WWTPs (namely, WWTP-1 and WWTP-2) located at the north-western Sicilian coast have been monitored for 5 months (one sampling per week). Samples were analyzed for IDs and their metabolites: methamphetamine (MEAMPH), cocaine (COC), 3,4-methylenedioxymethamphetamine

(MDMA), methadone (METH), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), 3,4-methylenedioxy amphetamine (MDA), 3,4-methylenedioxy ethylamphetamine (MDE), 3,4-methylenedioxy ethylamphetamine (MDEA), morphine (MOR), codeine (COD), cocaethylene (COCTH), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) and Benzoylecgonine (BEG).

MATERIALS AND METHODS

Wastewater treatment plants

Two WWTPs (namely, WWTP-1 and WWTP-2) located at the north-western Sicilian coast (South of Italy) have been monitored. WWTP-1 was monitored for 4 months, while WWTP-2 for 5.5 months. The water line of each WWTP has been monitored. Both WWTPs have a conventional scheme as reported in Figure 1. More precisely, the influent wastewater (WW) is first subjected to the primary treatments (screening for solid separation, oil and grease removal); later the secondary treatments, such as activated sludge processes are employed. The primary settling is employed only for the WWTP1 (Fig. 1). The two plants mainly differ for their potentiality. Indeed, the design average daily flow expressed as m^3d^{-1} for WWTP-1 and WWTP-2 was equal to 152,064 (corresponding to 440,000 inhabitants) and 36,300 (corresponding to 105,000 inhabitants), respectively.

For both WWTPs, samples were collected one time per week from the sampling locations as reported in Figure 1.

Sample collection

During the monitoring campaign, grab and composite samples were withdrawn for the influent WW (sampling section 0) and for the effluent (sampling section 3) (Fig. 1). Furthermore, only grab samples were also withdrawn for the mixed liquor (sampling section 1) and the returned sludge (sampling section 2) (Fig. 1). For the sampling locations 0 and 3, 1.5 L of sample was withdrawn for each sampling. While, 2.5 L were collected for the sampling locations 1 and 2.

During the monitoring campaign the influent wastewater of each WWTP was also characterized in terms of influent flow rate (QIN), pH, temperature (T) and concentration of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), nitrogen (N), phosphorus (P) and ammonia (NH4). With this regards, the Standard Methods have been adopted (APHA, 2005). Table 1 summarizes the average features of the influent wastewater during the monitoring campaign and the standard deviation (SD).

Analytic methods

In terms of IDs, samples were analyzed for methamphetamine (MEAMPH), cocaine (COC), 3,4-methylenedioxymethamphetamine (MDMA), methadone (METH), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), 3,4-methylenedioxy amphetamine (MDA), 3,4-methylenedioxy ethylamphetamine (MDE), 3,4-methylenedioxy ethylamphetamine (MDEA), morphine (MOR), codeine (COD), cocaethylene (COCTH), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) and Benzoylecgonine (BEG).

The adopted analytical methodology is divided into three phases: i). sample preparation; ii). solid phase extraction; iii). instrumental analysis.

Sample preparation and solid phase extraction

The collected samples were maintained at 4° C in dark during transport. Upon arrival to the laboratory, samples were filtered and stored at -20°C and processed within 2 days. In particular, samples were filtered by using first an 8 μm cellulose filter (Whatman, Kent, UK) and then a 0.45 μm nitrate cellulose filter (Sartorius Stedim Biotech GmbH, Gttingen, Germany).

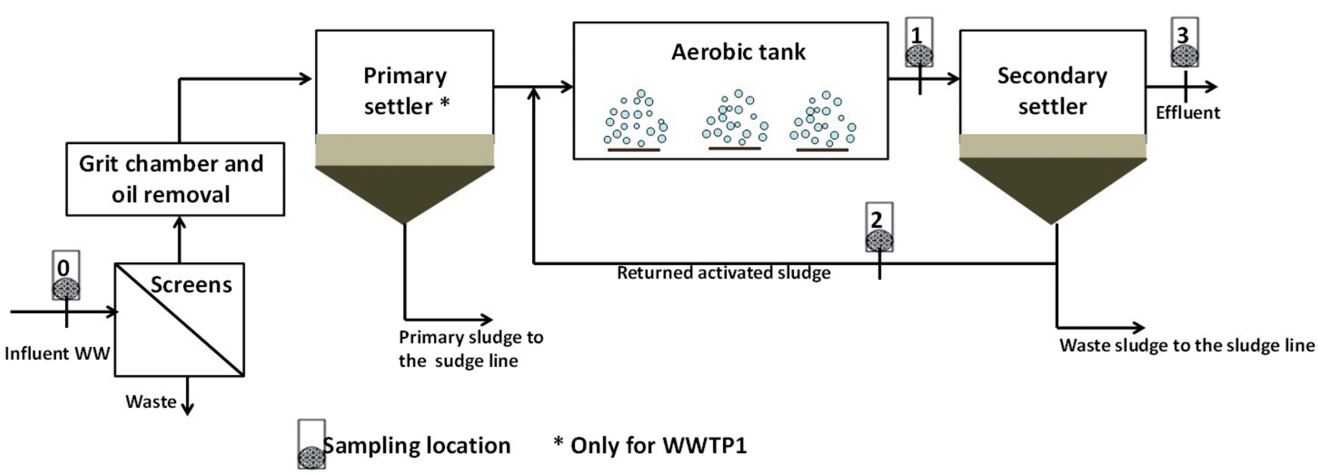

Fig. 1 - Lay out of each investigated WWTPs

			WWTP-1		WWTP-2	
Definition	**Symbol**	**Unit**	**Average value**	**SD**	**Average value**	**SD**
Population served	P	inh	340,000	-	45,000	-
Influent flow rate	Q_{IN}	$m^3\ d^{-1}$	102,450	2,000	17,149	832
pH	pH	-	7.4	0.1	7.5	0.15
Temperature	T	°C	20	4	19.8	3
Biochemical Oxygen Demand	BOD	$mg\ L^{-1}$	280	35	300	48
Chemical Oxygen Demand	COD	$mg\ L^{-1}$	410	35	390	42
Nitrogen	N	$mg\ L^{-1}$	59	8	65	6.9
Phosphorus	P	$mg\ L^{-1}$	8.1	0.5	6.9	0.9
Ammonia	NH_4	$mg\ L^{-1}$	48	4.5	55	6

Tab. 1 - Average features and standard deviation (SD) of the influent wastewater during the monitoring campaign for each WWTP

Excepting for THC-COOH, all the analyzed IDs and their metabolites were extracted by using the solid phase extraction (SPE) procedure as described by Castiglioni *et alii* (2006). With this regards, the mixed reversed-phase/cation-exchange cartridges were adopted (Bond Elut Plexa PCX Agilent, CA, USA). In details, samples (50 mL) were spiked with 50 µL of a mix solution of IS (1 µg/mL) and the pH was adjusted to 2.0 with 37% HCl. The SPE cartridges were first conditioned by means of 6 mL of methanol, 3 mL of Milli-Q water, and 3 mL of water acidified to pH 2. Samples were then passed through the cartridges under vacuum at a flow rate of 5 mL/min. Cartridges were vacuum-dried for 5 min, washed with 3 mL of methanol and eluted with 3 mL of a 2% ammonia solution in methanol. The eluates were dried using a vacuum rotary dryer (EZ-2-Plus Genevac, SP Scientific Industries, PA, USA) and added to 200 mL of water with 0.1% of acid formic, centrifuged at 1000 rpm for 10 min at 20°C and transferred into glass vials to be analyzed.

Instrumental analysis

The eluates were analyzed by adopting an high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) with a TSQ Quantiva triple-stage quadrupole mass spectrometer system interfaced to UHPLC Ultimate 3000 (Thermo Fisher Scientific Inc., Waltham, USA). Only for the THC-COOH measurement the Turbo-FlowTM online sample preparation technology was adopted. The recoveries, repeatability, instrumental limits of detection (LODs), and limits of quantification (LOQs) for the entire method were calculated in wastewater samples as described by Castiglioni *et alii* (2006) (data not shown). The following LOQs values were obtained: 3.0 ng/L for BEG, 10.0 ng/L for cocaine, 5.0 ng/L for amphetamines and 10.0 ng/L for THC-COOH.

RESULTS AND DISCUSSION

In the following sections, the results in terms of measured concentration for each ID, metabolite and WWTP will be presented and discussed. The discussion has been performed in terms of composite sample. Indeed, as discussed below not negligible difference have been evaluated for the IDs and metabolites concentration in the grab and composite influent.

Illicit drugs and metabolites concentration in WWTP-1

Table 2 summarizes the average measured values and SDs for each sample and analyzed compound. The compounds that have been found in the influent wastewater (section 0) with the highest concentration are: BEG (cocaine's' metabolite) (193.7 ng L^{-1} and 180.84 ng L^{-1} for grab and composite samples, respectively), COC (52.36 ng L^{-1} and 51.74 ng L^{-1} for grab and composite samples, respectively), CODEINE (82.05 ng L^{-1} and 62.47 ng L^{-1} for grab and composite samples, respectively) and THC-COOH (cannabis metabolite) (104.98 ng L^{-1} and 87.91 ng L^{-1} for grab and composite samples, respectively) (Tab. 2). All the other compounds, excepting for EDDP, MOR and METH, have been often found at the concentration lower than LOQ (Tab. 2). Moreover, high EDDP value was also obtained for grab sample of the inlet (25.37 ng L^{-1}) and outlet (38.99 ng L^{-1}) wastewater (sections 0 and 3 of Fig. 1). For the composite influent and effluent samples (sections 0 and 3 of Fig. 1) the value of EDDP was quite lower than that of the grab sample. A substantial difference between the grab and composite pollutant concentrations occurred even for the other compounds (Tab. 2). Therefore, as also suggested in literature the use of 24-h composite has to be considered in order to perform an adequate analysis and to consider the hourly EP load variation (among others, Castiglioni *et alii*, 2006).

The IDs and their metabolites found in the influent wastewater were also found in the effluent wastewater (section 3 of Figure 1).

Sample	***0 - grab***		***3 - grab***		***0 - composite***	
Compound	***Average [ng L^{-1}]***	***SD [ng L^{-1}]***	***Average [ng L^{-1}]***	***SD [ng L^{-1}]***	***Average [ng L^{-1}]***	***SD [ng L^{-1}]***
BEG	193.7	97.9	25.75	47.51	180.84	106.51
COC	52.36	72.15	6.17	19.01	51.75	65.5
COCTH	5.27	11.01	<LOQ	<LOQ	<LOQ	<LOQ
CODEINE	82.05	131.96	17.58	18.51	62.47	45.18
EDDP	25.37	40.32	38.99	43.27	<LOQ	1.88
MDA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MDMA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MEAMPH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
METH	7.49	4.68	8.99	3.96	8.38	5.1
MOR	32.65	16.9	4.1	7.61	33.65	18.62
THC-COOH	104.98	146.71	44.91	86.67	87.58	34.98
Sample	***3 - composite***		***1- grab***		***2 - grab***	
Compound	***Average [ng L^{-1}]***	***SD [ng L^{-1}]***	***Average [ng L^{-1}]***	***SD [ng L^{-1}]***	***Average [ng L^{-1}]***	***SD [ng L^{-1}]***
BEG	39.39	50.76	20.42	40.77	26.36	55.85
COC	6.51	10.63	9.4	19.95	6.14	20.06
COCTH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CODEINE	23.64	11.18	20.65	15.89	34.43	24.32
EDDP	5.88	6.7	26.17	32.65	13.68	13.77
MDA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MDMA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MEAMPH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
METH	7.31	4.47	10.24	13.74	8.62	4.49
MOR	<LOQ	9.35	<LOQ	7.28	6.67	12.91
THC-COOH	47.37	94.09	26.61	14.95	25.94	11.61

Tab. 2 - Average concentration measured in WWTP-1 for each ID, metabolite and sample

Generally, the IDs and their metabolite concentrations measured in wastewater effluent were lower than influent concentration (Tab. 2). Thus, revealing that a great part of these compounds are removed in the WWTP either due to biodegradation or adsorption to the solid phase.

For sake of completeness, in Figure 2 the trend of the influent and effluent concentration of some IDs and metabolites are reported. As reported in Figure 2, the effluent IDs and metabolite concentrations were one order of magnitude lower than that of the influent samples (referring to the influent and effluent grab samples) (Fig. 2). The removal efficiency (calculated on the basis of the influent and effluent concentration of the composite samples) ranges between 88% (obtained for COC) and 13% (obtained for METH).

However, for EDDP average treated effluent concentration (section 3), both for grab and composite samples, was higher than the influent concertation. Consequently, no removal took place for EDDP. This result is likely due to the presence of EDDP as a charged species in solution and concentrated in the aqueous phase during the treatment process. Similar results were also obtained by Bones *et alii* (2007). This result could be ascribable either to the presence of de-conjugates interfering with biological transformation of the de-conjugated compounds or to the release of IDs and metabolites adsorbed onto the

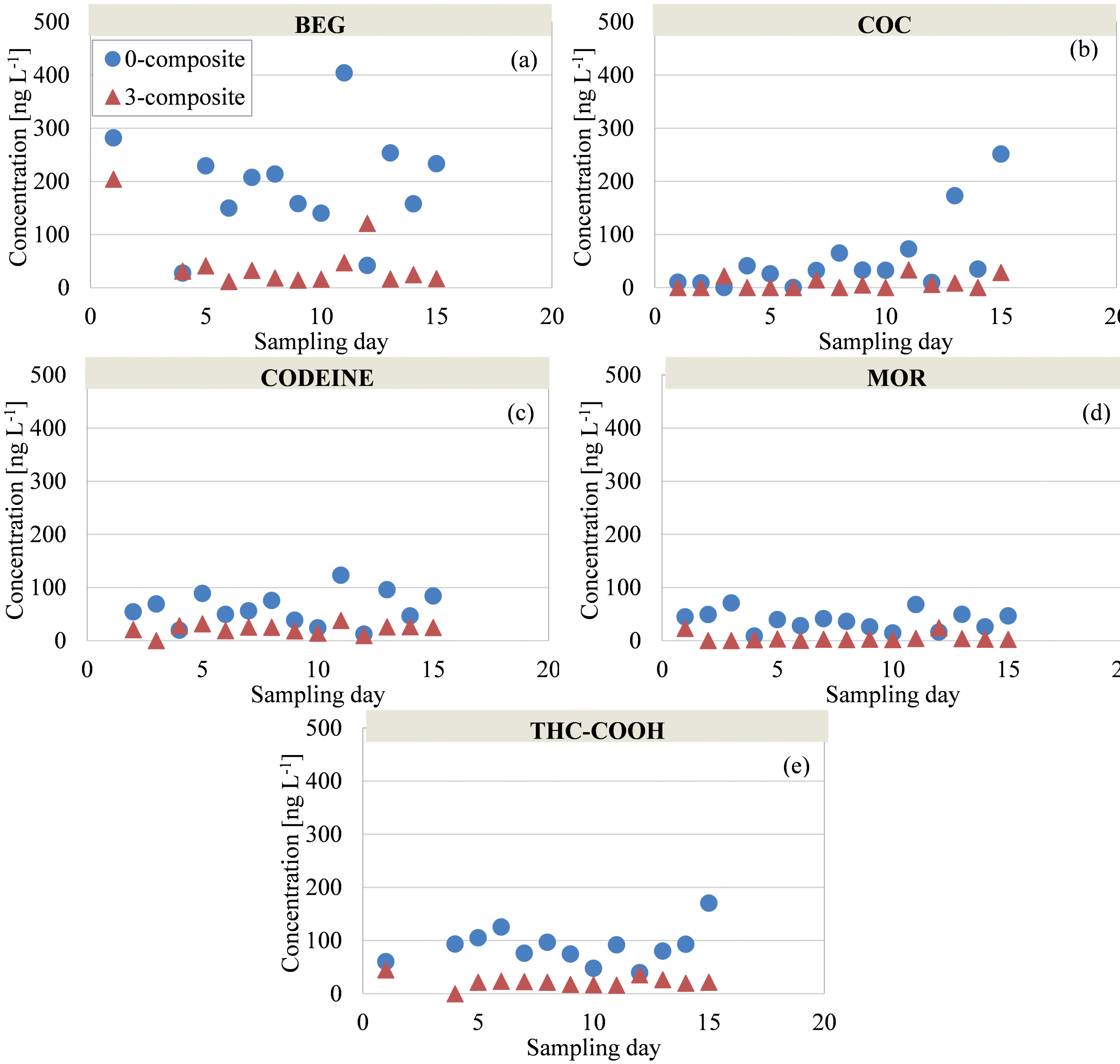

Fig. 2 - Trend of the influent and effluent composite concentration of BEG (a), COC (b), CODEINE (c), MOR (d) and THC-COOH (e) for WWTP-1

particulate dissolving after the biological treatment (Al Aukid, 2011). Indeed, as reported in Table 2, for the EDDP a quite high concentration inside the aerobic tank (section 1) and in the returned sludge (section 2) was found (26.17 and 13.68 ng L^{-1}, respectively) (Tab. 2). With regards to the negative efficiency some authors have recently suggested the use of "fractionated approach" to account for the influence of hydrodynamics in WWTPs and for estimating the removal of micropollutants (Baalbaki *et alii*, 2017). However, this aspect is out of the scope of this study.

Illicit drugs and metabolites concentration in WWTP-2

Table 3 summarizes the average measured values and SDs for each sample and analyzed compound. For WWTP-2 the same results of WWTP-1 were found in terms of predominant concentration of BEG (cocaine's' metabolite), COC, CODEINE and THC-COOH (cannabis metabolite) (Tab. 3). More specifically, the compounds with the highest concentration in the influent wastewater (section 0) samples are: BEG (cocaine's' metabolite) (235.11 ng L^{-1} and 183.53 ng L^{-1} for grab and composite samples, respectively), COC (81.14 ng L^{-1} and 74.03 ng L^{-1} for grab and

Sample	0 - grab		3 - grab		0 - composite	
Compound	Average [ng L-1]	SD [ng L-1]	Average [ng L-1]	SD [ng L-1]	Average [ng L-1]	SD [ng L-1]
BEG	235.11	91.97	41.14	32.76	183.53	88.12
COC	81.14	81.17	4.54	6.97	74.03	77.17
COCTH	5.1	9.06	<LOQ	<LOQ	2.71	7.68
CODEINE	32.14	14.82	10.93	9.68	21.85	13.14
EDDP	0.4	0.98	2.79	8.51	10.3	31.18
MDA	8.02	24.01	<LOQ	<LOQ	8.33	25.59
MDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MDMA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MEAMPH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
METH	10.78	6.46	8.01	<LOQ	8.33	7.14
MOR	33.8	19.34	3.15	4.14	19.42	11.7
THC-COOH	104.65	49.64	34.88	45.18	68.91	44.5
Sample	**3 - composite**		**1- grab**		**2 - grab**	
Compound	**Average [ng L-1]**	**SD [ng L-1]**	**Average [ng L-1]**	**SD [ng L-1]**	**Average [ng L-1]**	**SD [ng L-1]**
BEG	40.66	57.47	34.17	23.97	34.41	25.91
COC	5.67	11.24	3.09	8.83	2.11	5.66
COCTH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CODEINE	7.7	6.74	13.08	10.67	15.13	12.56
EDDP	0.1	0.03	59	5	13	1
MDA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MDMA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MEAMPH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
METH	7.05	3.35	9.01	4.17	8.95	4.03
MOR	<LOQ	3.34	3.98	4.26	4.14	4.43
THC-COOH	21.61	14.17	35.66	17.61	39.4	20.09

Tab. 3 - Average concentration measured in WWTP-2 for each ID, metabolite and sample

composite samples, respectively), CODEINE (32.14 ng L^{-1} and 21.85 ng L^{-1} for grab and composite samples, respectively), MOR (33.80 ng L^{-1} and 19.42 ng L^{-1} for grab and composite samples, respectively) and THC-COOH (cannabis metabolite) (104.65 ng L^{-1} and 68.91 ng L^{-1} for grab and composite samples, respectively) (Tab. 3). In terms of removal efficiency the WWTP2 showed quite high performances with an average value (evaluated on the basis of the composite samples) close to 77.85%, 92.34%, 64.75%, 90.16% and 68.64% for BEG, COC, CODEINE, MOR and THC-COOH, respectively.

By analyzing data of Table 3 one can observe that even for WWTP-2 a substantial difference between the average value of grab and composite concentration for each compound occurred. This results corroborate the literature findings. Indeed, Daelman *et alii* (2013) have demonstrated that for illicit drugs, where considerable temporal variability exists, the best sampling strategy is the composite daily sampling (ideally 24-h composites).

Differently to WWTP-1, in WWTP-2 the removal efficiency was high for all compounds. For all compounds, the average treated effluent concentration (section 3), both for grab and composite samples, were lower than the influent concentration (Tab. 3).

CONCLUSION

Chemical analysis of wastewater performed in the present study has revealed the presence of residues of illicit drugs in the influents of two WWTPs in South of Italy. In terms of sampling, different concentration have been found for IDs and metabolites between grab and composite samples. Therefore, it is suggested to adopt composite sample to obtain an accurate analysis. Results showed high concentration of BEG, COC, CODEINE and THC-COOH. These compounds are directly related to the use of cocaine and cannabis of the population. In terms of removal efficiency results showed that inside the WWTP-1 negative efficiencies for EDDP took placed. This result could be ascribable either to the presence of de-conjugates interfering with biological transformation of the de-conjugated compounds. For the WWTP-2 quite high average removal efficiency were found for BEG (77.85%), COC (92.34%), CODEINE (64.75%), MOR (90.16%) and THC-COOH (68.64%).

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REFERENCES

Al Aukidy M. (2011) - *Environmental risk of pharmaceuticals in waters: investigation on their occurrence and removal in conventional treatment plants.* Ph.D Thesis, Università degli Studi di Ferrara.

Andrés-Costa M.J., Rubio-Lòpez N., Morales Suarez-Varela M. & Pico Y. (2014) - *Occurrence and removal of drugs of abuse in wastewater treatment plants of Valencia (Spain).* Environmental Pollution, **194**: 152-162.

APHA (2005) - *Standard methods for the examination of water and wastewater.* APHA, AWWA and WPCF, Washington DC, USA.

Baalbaki Z., Sultana T., Metcalfe C. & Yargeau V. (2017) - *Estimating removals of contaminants of emerging concern from wastewater treatment plants: The critical role of wastewater hydrodynamics.* Chemosphere, **178**: 439-448.

Bartelt-Hunt S.L., Snow D.D., Damon T., Shockley J. & Hoagland K. (2009) - *The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska.* Environ. Pollut., **157**: 786-791.

Bones J., Thomas K.V. & Paull B. (2007) - *Using environmental analytical data to estimate levels of community consumption of illicit drugs and abused pharmaceuticals.* J. Environ. Monit., **9**: 701-707.

Castiglioni S., Zuccato E., Crisci E., Chiabrando C., Fanelli R. & Bagnati R. (2006) - *Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry.* Anal Chem, **78**: 8421-8429.

Causanilles A., Ruepert C., Ibáñez M., Emke E., Hernández F. & De Voogt P. (2017) - *Occurrence and fate of illicit drugs and pharmaceuticals in wastewater from two wastewater treatment plants in Costa Rica.* Science of the Total Environment 599–600, 98–107.

Cosenza A, Rizzo S, Sansone Santamaria A. & Viviani G. (2015) - *Radionuclides in wastewater treatment plants: monitoring of Sicilian plants.* Water Sci Technol, **71** (2): 252-258.

Cosenza A., Piscionieri D., Maida C.M., Papa G. & Viviani G. (2016) - *Il destino dei microinquinanti negli impianti di depurazione e nei corpi idrici.* In: Proceedings of XXV Convegno Nazionale di Idraulica e Costruzioni Idrauliche (IDRA 2016). Bologna, 14-16 settembre 2016. In Italian.

Cosenza A., Maida C.M., Piscionieri D., Fanara S., Di Gaudio F. & Viviani G. (2018) - *Occurrence of illicit drugs in two wastewater treatment plants in the South of Italy.* Chemosphere, **198**: 377-385.

Daelman M.R.J., De Baets B., Van Loosdrecht M.C.M. & Volcke E.I.P. (2013) - *Influence of sampling strategies on the estimated nitrous oxide emission from wastewater treatment plants.* Water Res., **47**: 3120-3130.

Degenhardt L., Hall W., Warner-Smith M. & Lynskey M. (2004) - *Illicit drug use. Comparative quantification of health risks: global and regional burden of disease attributable to selected major.* Risk Factors (1): 1109-1176.

Dong R., Yu G., Guan Y., Wang B., Huang J., Deng S. & Wang Y. (2016) - *Occurrence and discharge of pharmaceuticals and personal care products in dewatered sludge from WWTPs in Beijing and Shenzhen*. Emerging Contaminants, **2** (1): 1-6.

Evgenidou E.N., Konstantinou I.K. & Lambropoulou D.A. (2015) - *Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: a review.* Science of the Total Environment, **505**: 905-926

Furhacker M. (2008) - *The water framework directive. Can we reach the target?* Water Sci Technol, **57**: 9-17.

Li K., Peng D., Xu Z., Gao T. & Li X. (2016) - *Occurrence of illicit drugs in surface waters in China.* Environmental Pollution, **213**: 395-402.

Loos R., Carvalho R., Antonio D.C., Comero S., Locoro G., Tavazzi S., Paracchini B., Ghiani M., Lettieri T., Blaha L., Jarosova B., Voorspoels S., Servaes K., Haglund P., Fick J., Lindberg R.H., Schwesig D. & Gawlik B.M. (2013) - *EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents.* Water Research, **47**: 6475-6487.

Maida C.M., Di Gaudio F., Tramuto F., Mazzucco W., Piscionieri D., Cosenza A. & Viviani G. (2017) - *Illicit drugs consumption evaluation by wastewater-based epidemiology in the urban area of Palermo city (Italy)*. Ann 1st Super Sanità, **53** (3): 192-198.

Mastroianni N., López-García E., Postigo C., Barceló D. & López De Alda M. (2017) - *Five-year monitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends.* Science of the Total Environment, **609**: 916-926.

Mohapatra S. Huang C.-H., Mukherji S. & Padhye L.P. (2016) - *Occurrence and fate of pharmaceuticals in WWTPs in India and comparison with a similar study in the United States.* Chemosphere, **159**: 526-535.

Pal R., Megharaj M., Kirkbride K.P. & Naidu R. (2013) - *Illicit drugs and the environment: a review.* Science of the Total Environment, **463-464**: 1079-1092.

Postigo C., López De Alda M. & Barcelò D. (2011) - In: Castiglioni S., Zuccato E. & Fanelli R. (Eds.). *Illicit drugs in the environment: occurrence, analysis, and fate using mass spectrometry.* John Wiley & Sons, Inc., Hoboken, NJ, 115 pp.

Roberts P.H. & Thomas K.V. (2006) - *The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment.* Sci Total Environ, **356**: 143-153.

Santana-Viera S., Montesdeoca-Esponda S., Sosa-Ferrera Z. & Santana-Rodríguez J.J. (2016) - *Cytostatic drugs in environmental samples: an update on the extraction and determinationprocedures.* TrAC Trends in Analytical Chemistry, **80**: 373-386.

Senta I., Krizman I., Ahel M. & Terzic S. (2014) - *Assessment of stability of drug biomarkers in municipal wastewater as a factor influencing the estimation of drug consumption using sewage epidemiology.* Science of the Total Environment, **487**: 659-665.

United Nations Office on Drugs and Crime (UNODC) (2004) - *World Drug Report 2004.* Available from: www.unodc. org/pdf/WDR_2004/volume_1.pdf

United Nations Office on Drugs and Crime (UNODC) (2014) - *World Drug Report, 2014.* New York.

United Nations Office on Drugs and Crime (UNODC) (2016) - *World Drug Report, 2016.* Terminology and Information on Drugs, third ed., New York.

Yadav M.K., Short M.D., Arial R., Gerber C., Van Den Akker B. & Saint C.P. (2017) - *Occurrence of illicit drugs in water and wastewater and their removal during wastewater treatment.* Water Research, **124**: 713-727.

Zuccato E., Castiglioni S., Senta I., Borsotti A., Genetti B., Andreotti A., Pieretti G. & Serpelloni G. (2016) - *Population surveys compared with wastewater analysis for monitoring illicit drug consumption in Italy in 2010-2014.* Drug Alcohol Depend, **161**: 178-188.

Zuccato E., Castiglioni S., Bagnati R., Chiabrando C., Grassi P. & Fanelli R. (2008) - *Illicit drugs, a novel group of environmental contaminants.* Water Res., **42**: 961-968.

Zuccato E., Castiglioni S., Tettamanti M., Olandese R., Bagnati R., Melis M. & Fanelli R. (2011) - *Changes in illicit drug consumption patterns in 2009 detected by wastewater analysis.* Drug Alcohol Dependence, **118**: 464-469.

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