OCCURRENCE AND TRANSFORMATION OF ILLICIT DRUGS IN WASTEWATER TREATMENT PLANTS

A. COSENZA*, G. VIVIANI*, S. FANARA***, S. INDELICATO***, D. PISCIONIERI***, F. DI GAUDIO***, C.M. MAIDA**

**Dipartimento di Ingegneria Civile, Ambientale, Aerospaziale, dei Materiali (DICAM), Scuola Politecnica, Università di Palermo, Viale delle Scienze, 90128, Palermo, Italy*

*** Dipartimento di Scienze per la Promozione della Salute e Materno Infantile, Scuola di Medicina e Chirurgia, Università di Palermo, Via del Vespro 13, 90127, Palermo, Italy*

**** Mass Spectrometry Laboratory for Clinical Risk and Quality Control, A.O.U.P. "P. Giaccone", Palermo, Italy.*

Keywords: Drug behavior; water treatment; contaminants of emerging concern; drugs

Abstract *In this study the occurrence and behaviour of illicit drugs and their metabolites has been investigated for two Sicilian WWTPs (namely, WWTP-1 and WWTP-2). Samples were analyzed for metham-phetamine, 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). Results have shown that for both the monitored WWTPs BEG ,COC, MOR and THC-COOH were found at the highest concentration. This compounds are directly related to the use of cocaine and cannabis of the population. In terms of removal efficiency results showed that inside the WWTP1 very poor efficiency occurred and sometimes negative efficiencies took placed. For the WWTP2 average removal efficiency of 86 %, 94%, 90 % and 65 % were found for BEG ,COC, MOR and THC-COOH, respectively.*

1. Introduction

During the last twenty years there has been an increasing concern on the occurrence of emerging pollutants (EPs) in the environment; EPs have been defined as new chemicals with unknown impact on the environment and human health (Deblonde et al., 2011). A wide range of substances are defined as EPs: personal care products, endocrine disruptors, illicit drugs (IDs), surfactants, pharmaceuticals, gasoline additives and radionuclides (Loos et al., 2013). IDs and their metabolites have been recently recognized as a new group of water EPs with potent psychoactive properties and unknown effects to the aquatic environment (Pal et al., 2013). The recent interest towards the amount of IDs discharged into the environment is mainly related to: i. the increased use of IDs (UNODC, 2014); ii. the improvements of the analytic methods to detect IDs in the environment compounds (mainly water) has enabled the detection of low-levels concentrations (Santana-Viera et al., 2016). Indeed, thanks to the development of advanced analytic methods, IDs and some of their

metabolites have been detected in the water cycle (Li et al., 2016). Further, 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.

IDs are excreted via urine and feces and arrive at wastewater treatment plants (WWTPs) where can reach ppb levels (Castiglioni et al., 2006, Furhacker, 2008). As 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 al., 2015). Hence, over the few last years, IDs concentrations in raw and treated urban wastewater (WW) have been extensively monitored (Dong et al., 2016).

The role of the conventional biological processes in WWTPs on the IDs transformation is still poorly understood in literature. Indeed, several studies have demonstrated the difficulties on discriminating the key processes affecting the IDs and their metabolites transformation inside the WWTPs (Evgenidou et al., 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 al., 2015). Some authors have demonstrated that the WWTPs have a very poor effect on IDs removal (Evgenidou et al., 2015). Thus, they are discharged into water bodies through the treated effluent (Postigo et al., 2011). Therefore, monitoring the IDs concentration in WWTPs can have several advantages: 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 al., 2014).

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 total suspended solids (TSS), 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).

2. Materials and methods

2.1. Monitored wastewater treatment plants and sampling campaign

Two WWTPs (namely, WWTP-1 and WWTP-2) located at the north-western Sicilian coast have been monitored for 5 months. More precisely the water line of each WWTP has been monitored. The two 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 (Figure 1).The two plants mainly differ for their potentiality. Indeed, the average daily flow expressed as m^3d^{-1} for WWTP-1 and WWTP-2 was equal to 153,600 and 19,704, respectively.

For the both WWTPs samples were collected from the sampling locations as reported in Figure 1 one time per week. Specifically, grab and composite samples were withdrawn for the influent WW (sampling section 0) and for the effluent (sampling section 3). Furthermore, only grab samples were also withdrawn for the mixed liquor (sampling section 1) and the returned sludge (sampling section 2). 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.

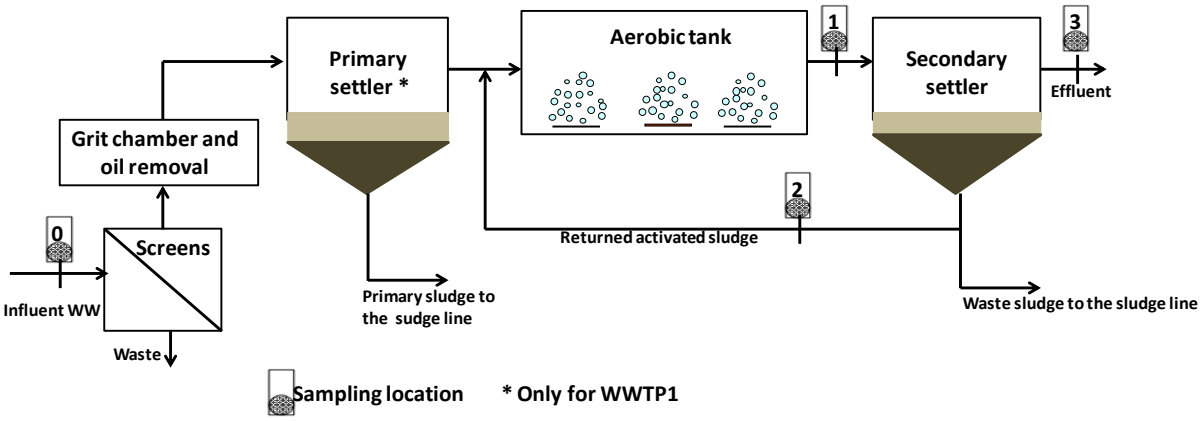

Figure 1. *Lay out of each investigated WWTP*

2.2. Analytic methods

The collected samples were maintained at 4° C in dark during transport. Upon arrival to the laboratory, samples were stored at -20 °C until the analysis. 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. chromatographic analysis. During the first phase samples were vacuum filtered through 1-11 μm cellulose filter paper (Whatman) filters and then through cellulose nitrate filter (Sartorius Stedim Biotech GmbH). Subsequently, HCl at 37% has been added to 500 mL of filtered sample to achieve the pH value of 2. Further, 500 μL of a solution of deuterated standards at the concentration of 1 mg/L was added. In order to perform the second phase Bond Elut Plexa PCX cartridges (60 mg, 3 mL - Agilent Technologies) were adopted. The cartridges were first conditioned by using 6 mL of CH_3OH, 3 mL of H_2O Milli Q Unit (Millipore, Bedford USA) and 3 mL of HCl 0.01M. Thus, during the second phase, samples were preconcentrated onto the cartridges at the flow rate of 5 mL/min, using a vacuum system. After sample preconcentration, cartridges were rinsed with 5 mL of HPLC grade water and vacuum dried for 15–20 min to remove excess water. Elution of target compounds was performed with 2 × 4 mL pure methanol. Instrumental analysis was performed by high performance liquid

chromatography coupled to a quadrupole – linear ion trap mass spectrometry HPLC Ultimate 3000 (Thermo Scientific®) and mass spectrometer Q-Exactive (Thermo Scientific®),

3. Results and discussion

In the following sections the results in terms of measured concentration for each ID and metabolite and WWTP investigated will be presented and discussed. The comparison analysis has been performed by comparing only the result 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.

3.1. Grab versus composite sample

In Figure 2 the measured values of each IDs and metabolite for WWTP2 are reported both for grab (a) and composite samples (b) withdrawn from the location 0 (influent wastewater). By analyzing data reported in Figure 2 one may observe that as often occurs for the macropollutants, a substantial differences between the grab and composite pollutant concentrations occurred. 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 al., 2006). With this regards, Roberts and Thomas (2006) have proposed of using composite samples over a period longer than plant HRT in order to improve the comparability between influent and effluent.

3.2. WWTP 1

Table 1 summarizes the average measured value of each analyzed sample. By analyzing Table 1 one can observe that the compounds that have been found with the highest concentration are: BEG (cocaine's' metabolite), COC, MOR and THC-COOH (cannabis metabolite); all the other compounds, excepting for METH and MEAMPH, have been often found at the concentration lower than 0.21 ng L^{-1}. The high value of the influent concentration for BEG (cocaine's' metabolite), COC, MOR and THC-COOH (cannabis metabolite) are mainly excreted by human. Indeed, once ID are metabolized their excreted metabolites and unalterated parent compounds arrive in the WWTP where can be subjected to further modification due to biological, chemical and physical processes (Deblonde et al., 2011; Fatta-Kassinos et al., 2011). Indeed, by analyzing data reported in Table 1 a very low removal occurred for the IDs and metabolites analyzed. In some cases the amount (in terms of concentration) of the analyzed compound increased from the influent (as composite sample) to the effluent, thus obtaining negative removal. Such a result, can be ascribable either to the presence of deconjugates interfering with biological transformation of the deconjugated compounds or to the release of IDs and metabolites sorbed onto the particulate dissolving after the biological treatment (Al Aukid, 2011). Indeed, as reported in Table 1, inside the aerobic tank the composite effluent concentration of seveval compounds increase.

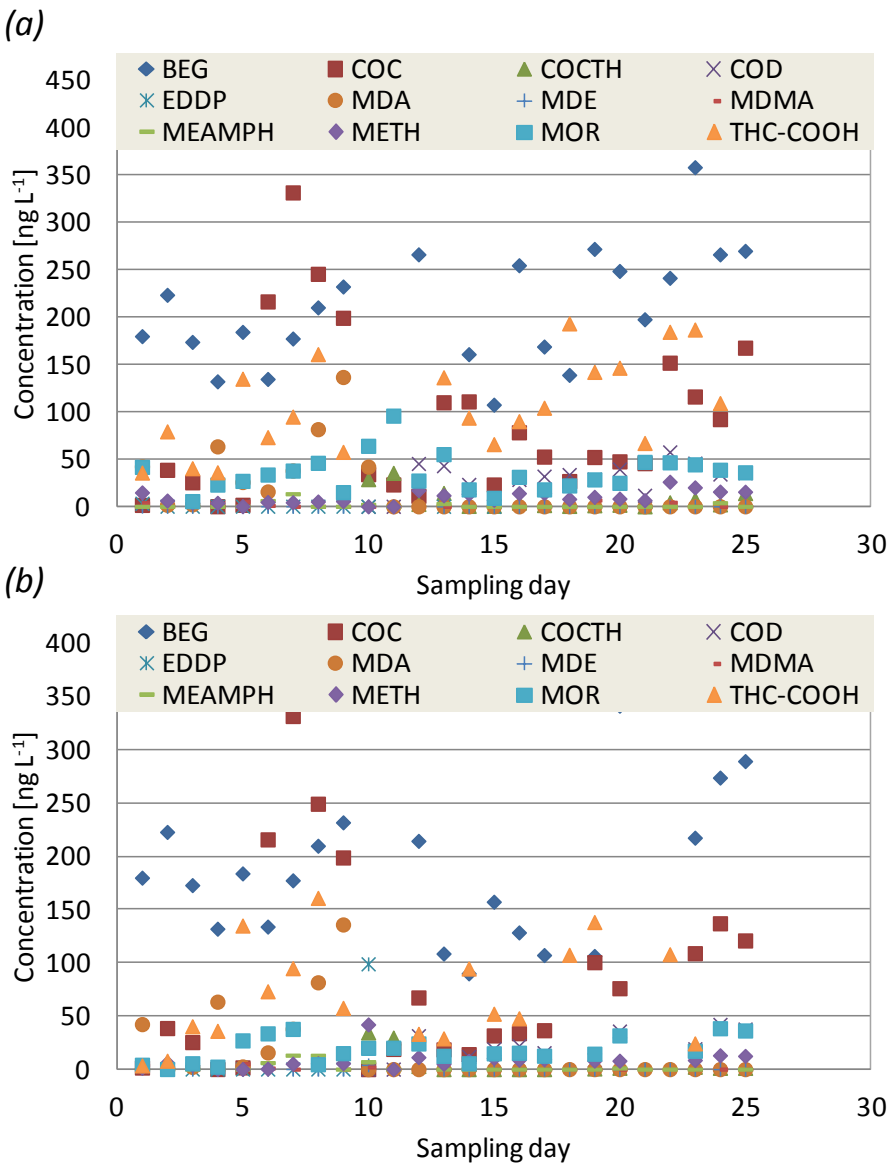

Figure 2. *Trend of each IDs and metabolite concentrations for the grab and composite samples withdrawn from sampling location 0 (influent wastewater)*

Table 1. *Average concentration measured for each ID and metabolite in the WWTP1*

	Average Concentration [ng L^{-1}]											
SAMPLING SECTION	BEG	COC	COCTH	COD	EDDP	MDA	MDE	MDMA	MEAMPH	METH	MOR	THC-COOH
0 - grab	301.25	92.59	5.20	20.16	0.19	21.02	0.04	0.94	1.26	10.49	40.44	98.28
3 - grab	25.13	2.81	0.87	0.81	1.45	0.46	0.04	12.22	1.22	42.66	20.27	36.49
0 - composite	2.60	0.84	0.70	1.67	0.59	0.06	14.67	1.75	54.61	17.84	54.73	0.00
3 - composite	41.31	4.92	0.03	3.99	0.05	0.46	0.09	0.16	0.29	5.94	6.49	19.52
1- gab	16.07	1.41	0.05	6.87	3.07	0.00	0.00	0.00	0.13	5.44	2.68	17.12
2 - grab	20.54	303.72	0.07	8.85	3.50	0.02	0.64	0.08	2.53	7.92	5.78	22.23

3.3. WWTP 2

In terms of predominant concentration of BEG (cocaine's' metabolite), COC, MOR and THC-COOH (cannabis metabolite) the same result of WWTP1 were found for the WWTP2 (Table 2) However, for these compounds in WWTP a very high average removal efficiency was obtained. In Figure 3 the trend of the removal efficiency for BEG (cocaine's' metabolite), COC, MOR and THC-COOH is shown. In terms of removal efficiency the WWTP2 showed quite high performances with an average value close to 86 %, 94%, 90 % and 65 %, for BEG ,COC, MOR and THC-COOH, respectively (Figure 3). This result is in good agreement with literature where a sufficient removal efficiency of THC-COOH was found (Evgenidou et al., 2015). This is likely due to the high adsorption coefficients which favors their mobility through the sludge.

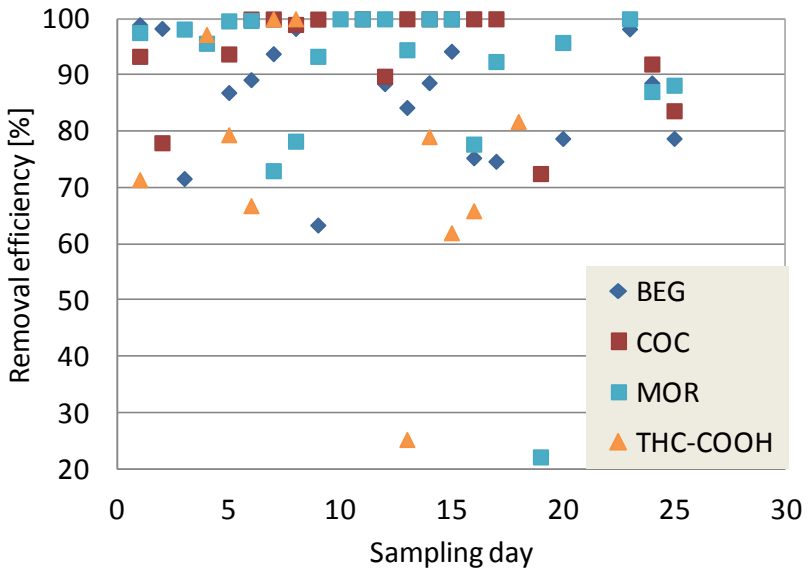

Figure 3. *Trend of the removal efficiency of WWTP2 for BEG, COC, MOR and THC-COOH*

Table 2. *Average concentration measured for each ID and metabolite in the WWTP2*

	Average Concentration [ng L^{-1}]											
SAMPLING SECTION	BEG	COC	COCTH	COD	EDDP	MDA	MDE	MDMA	MEAMPH	METH	MOR	THC-COOH
0 - grab	218.29	88.18	7.38	30.54	0.33	17.96	0.04	0.87	1.17	9.37	34.82	106.36
3 - grab	35.74	3.71	0.01	9.93	2.33	0.35	0.04	0.11	0.04	7.01	2.26	33.05
0 - composite	184.06	83.10	5.66	19.59	10.30	15.32	0.24	0.55	1.75	7.84	17.88	69.11
3 - composite	46.94	5.59	0.07	7.23	0.10	0.37	0.08	0.13	0.21	6.42	5.15	22.59
1- gab	33.19	2.47	0.10	11.49	59.00	0.00	0.00	0.00	0.14	9.51	3.44	35.66
2 - grab	34.05	469.33	0.06	12.73	59.00	0.00	0.00	0.00	0.14	10.65	3.54	35.82

4. Conclusion

The occurrence and the behaviour of IDs and their metabolites inside WWTPs have been analysed. In terms of sampling, as different concentration have been found for IDs and metabolites between grab and composite samples it is suggested to adopt composite sample to obtain an accurate analysis. Results highlighted that a great amount of BEG, COC, MOR and THC-COOH were found in the monitored plants. This compounds are directly related to the use of cocaine and cannabis of the population. In terms of removal efficiency results showed that inside the WWTP1 very poor efficiency occurred and sometimes negative efficiencies took placed. This result could be ascribable either to the presence of deconjugates interfering with biological transformation of the deconjugated compounds or to the release of IDs and metabolites sorbed onto the particulate dissolving after the biological treatment. For the WWTP2 average removal efficiency of 86 %, 94%, 90 % and 65 % were found for BEG ,COC, MOR and THC-COOH, respectively.

Acknowledgements

This study was financially supported by the Italian Ministry of Education, University and Research with the Research project of national interest PRIN 2010–2011 (D.M. 1152/ric 27/12/2011, prot. 2010 WLNFYZ) entitled 'Emerging contaminants in air, soil, and water: from source to the marine environment'.

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