Illicit Drugs and Metabolites in the Llobregat River Basin

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Abstract Recently, the study of illicit drugs and metabolites in the aquatic environment has become a matter of scientific interest. An increasing number of studies have been carried out worldwide in this area of research in the last years. The Llobregat River basin has been one of the investigated areas in Spain. Its water quality has been shown to be affected by the presence of this type of emerging contaminants, often to a larger extent than other rivers, due to its marked Mediterranean character and urban and industrial pressures. This chapter reviews the occurrence of illicit drugs and their metabolites in both wastewaters and surface waters along the Llobregat River basin, and the analytical methodologies developed for their determination. Measured levels of these substances in the Llobregat River basin are compared with the levels found in other Spanish and European areas. Since treated wastewaters constitute the main source of illicit drugs and metabolites to the natural receiving waters, and surface waters are used for water supply purposes, the reported removal of these substances in wastewater treatment plants and drinking water treatment plants along the basin is also reviewed. Finally, the use of influent wastewater levels to estimate illicit drug use in riverine populations is also discussed.

Keywords Illicit drugs, Llobregat River, Psychoactive substances, Surface water, Wastewater

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Abbreviations

6ACM	6-Acetyl-morphine
ACN	Acetonitrile
AM	1-Phenylpropan-2-amine or amphetamine
BE	Benzoylecgonine
CAS	Conventional activated sludge
CE	Cocaethylene
COC	Cocaine
CODE	Codeine
DUI	Drug use indicator
DWTP	Drinking water treatment plant
EDDP	2-Ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine
EPH	Ephedrine
ESI	Electrospray
FENTA	Fentanyl
HER	Heroin
IS	Internal standard
KETA	Ketamine
LC	Liquid chromatography
LOQ	Limit of quantification
LSD	Lysergic acid diethylamide
MA	*N*-Methyl-1-phenylpropan-2-amine or methamphetamine
MDA	3,4-Methylenedioxyamphetamine
MDEA	3,4-Methylenedioxyethamphetamine
MDMA	3,4-Methylenedioxymethamphetamine or ecstasy
MeOH	Methanol
METH	Methadone
MOR	Morphine
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry

nor-CODE	Nor-codeine
nor-LSD	Nor-LSD and nor-iso-LSD
nor-MOR	Nor-morphine
OH-THC	11-Hydroxy-Δ^9-tetrahydrocannabinol
O-OH-LSD	2-Oxo-3-hydroxy LSD
PCP	Phencyclidine
QqLIT	Hybrid quadrupole-linear ion trap
QqQ	Triple quadrupole
RO	Reverse osmosis
RSD	Relative standard deviations
SPE	Solid phase extraction
SRM	Selective reaction monitoring
SW	Surface water
THC	Δ^9-Tetrahydrocannabinol
THC-COOH	11-Nor-9-carboxy- Δ^9-tetrahydrocannabinol
UF	Ultrafiltration
UPLC	Ultra-performance liquid chromatography
WW	Wastewater
WWTP	Wastewater treatment plant

1 Introduction

Illicit drugs and their metabolites have been recently recognized as emerging environmental contaminants of concern. Since 2004, year in which Jones-Lepp and coworkers [1] reported for the first time the presence of this type of compounds in waters, an increasing number of studies that confirm the presence of these substances in water matrices [2–4] and also in other environmental matrices, such as atmospheric particles [5–11], and sludge and river sediments [12, 13] have been published. The analysis of illicit drugs and their metabolites in environmental matrices requires the use of highly sensitive and selective reliable techniques capable of detecting the low levels at which these compounds are present in the environment. To this end, the determination of these compounds in concentrated extracts has been mainly carried out by means of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), since the use of gas chromatographic techniques requires the derivatization of the analytes in order to increase their volatility.

The main source of these substances to the environment is their consumption and production. Unlike pharmaceuticals, direct deposition of illicit drugs is less likely. After consumption, different amounts of the consumed drug and its metabolization products are excreted via urine and feces. Under the best case scenario, these substances undergo physical–chemical and/or biological transformation in wastewater treatment plants (WWTPs). However, there is already enough evidence about their incomplete removal during wastewater treatment [14], and the

proportion of biotransformation and mineralization that they experience is still unknown. Yet, wastewater treatment is an important process to reduce the levels of these substances before their release into the aquatic environment. This, together with the dilution that treated wastewaters experience when discharged into natural water masses, helps to attenuate the potential negative effects that these substances may pose to aquatic ecosystems and also to reduce their presence in drinking water sources. In this respect, Mediterranean river basins are more vulnerable to chemical pollution than other European catchments, because they experience drought periods. Water scarcity is directly related to an increase of the surface water levels of polar micropollutants present in discharged treated wastewaters (e.g., pharmaceuticals, illicit drugs, and metabolites), which are the main component of the river discharge in catchments with high industrial and urban pressures, and population densities, as it is the case of the Llobregat River basin. The study of illicit drugs and their metabolites in the Llobregat River basin is also justified because surface waters of the main river constitute the main apportionment source of water supply for the city of Barcelona and surrounding urban areas, thus, the presence of these substances represents a potential threat to public health. To date, various studies have been performed in this line [15–21], in some cases with a double objective: (1) to evaluate the occurrence of these compounds in wastewaters and surface waters of the Llobregat River basin and (2) to use environmental levels to estimate illicit drug use in the investigated areas as proposed by Daughton in 2001 [22], and implemented for the first time by Zuccato and coworkers [23] in 2005.

In this context, the objectives of the present chapter are to review the analytical techniques developed to analyze illicit drugs and metabolites in the Llobregat River basin and the levels reported in wastewaters and surface waters in this area, to compare these levels with those observed in other Spanish and European river basins, to assess the efficiency of removal of these compounds during wastewater treatment and drinking water production, and to examine the drug use estimations derived for various populations from the corresponding raw wastewater concentrations of drugs.

2 Analysis of Illicit Drugs and Metabolites in Waters

All analytical methodologies developed to investigate the presence of illicit drugs and metabolites in waters from the Llobregat River basin are based on solid phase extraction (SPE) of the target analytes present in the samples and further detection with LC-MS/MS [17, 19, 21]. They all cover illicit drugs and metabolites belonging to different chemical classes and their main features are summarized in Table 1.

Prior to analysis, water samples were filtered to remove suspended solids, and further spiked with mixtures of the deuterated analogues at known concentrations, which allows accurate quantification of the target analytes. In order to increase

Table 1 Review of analytical methods used for determination of illicit drugs and metabolites in waters from the Llobregat River basin

Ref.	Target analytes[a]	Sample pretreatment[b]	Liquid chromatography[c]		Mass spectrometry[d] Ionization- MS detector (acquisition mode)	Accuracy method recovery	Sensitivity[e] method LOQ (ng/L)	Precision RSD (%)
			Chromatographic column	Mobile phase				
[17]	MOR, 6ACM, nor-MOR, HER, METH, EDDP, CODE, nor-CODE, FENTA, THC, THC-COOH	200 mL (WW, SW) Filtration (1.6 μm) IS addition (50 ng/L) SPE (Oasis HLB 200 mg)	Acquity BEH C_{18} (100 × 2.1 mm, 1.7 μm)	400 μL/min (2 runs × 9 min) MeOH/H_2O (50 mM NH_4HCO_2) (pH 3.8)	(+) ESI-QqQ (2–3 SRM/analyte)	44–99 (SW) 42–96 (WW)	0.1–12.5 (SW) 0.3–25.0 (WW)	2–7 (SW) 2–8 (WW)
[19]	COC, BE, AM, MA, MDA, MDEA, MDMA, LSD, KETA, PCP, FENTA	100 mL (WW, SW) Filtration (1.6 μm) IS addition (12.5 ng/L) SPE (Oasis HLB 200 mg)	Acquity BEH C_{18} (100 × 2.1 mm, 1.7 μm)	500 μL/min (1 run × 6.5 min) ACN/H_2O (30 mM NH_4HCO_2) (pH 3.5)	(+) ESI-QqQ (2 SRM/analyte)	75–99 (SW) 70–101 (WW)	0.1–0.9 (SW) 0.2–2.1 (WW)	4–11 (SW) 6–11 (WW)
[21]	COC, BE, CE, AM, MA, MDMA, EPH, LSD, nor-LSD, O-OH-LSD, MOR, 6ACM, HER, THC, THC-COOH, OH-THC	2 × 5 mL (WW) Filtration (1 μm + 0.45 μm) IS addition (20 ng/L) *On-line* SPE (PLRPs and Oasis HLB – 10 × 2 mm)	Purospher star RP-18 (125 × 2.0 mm, 5 μm)	300 μL/min (2 runs × 35 min) ACN/H_2O	(+/−) ESI-QqLIT (2 SRM/analyte)	5–59 (WW)	0.7–6.0 (WW)	2–14 (WW)

[a] *6ACM* 6-acetyl morphine, *AM* 1-phenylpropan-2-amine (amphetamine), *BE* benzoylecgonine, *CE* cocaethylene, *COC* cocaine, *CODE* codeine, *EDDP* 2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine, *EPH* ephedrine, *FENTA* fentanyl, *HER* heroin, *KETA* ketamine, *LSD* lysergic acid diethylamide, *MA* N-methyl-1-phenylpropan-2-amine (methamphetamine), *MDA* 3,4-methylenedioxyamphetamine, *MDEA* 3,4-methylenedioxyethamphetamine, *MDMA* 3,4-methylenedioxymethamphetamine, *METH* methadone, *MOR* morphine, *nor-CODE* nor-codeine, *nor-LSD* nor-LSD and nor-iso-LSD, *nor-MOR* nor-morphine, *O-OH-LSD* 2-oxo-3-hydroxy LSD, *OH-THC* 11-hydroxy- Δ^9-tetrahydrocannabinol, *PCP* phencyclidine, *THC* Δ^9-tetrahydrocannabinol, *THC-COOH* 11-nor-carboxy-Δ^9-tetrahydrocannabinol

[b] *WW* wastewater, *SW* surface water, *IS* internal standard, *SPE* solid phase extraction

[c] *ACN* acetonitrile, *MeOH* methanol

[d] *ESI* electrospray, *QqQ* triple quadrupole, *QqLIT* quadrupole-linear ion trap, *SRM* selective reaction monitoring transition

[e] *LOQ* limit of quantification

[f] *RSD* relative standard deviation

method sensitivity and reduce matrix interferences, samples were preconcentrated onto polymeric SPE sorbents, such as Oasis HLB [17, 19, 21] and PLRPs [21] cartridges. Both *off-line* [17, 19, 21] and *on-line* SPE processes have been described [21]. The use of *on-line* SPE resulted in a fully automated methodology capable of achieving very low detection limits (in the pg or low ng/L level) with very low sample volumes (2 × 5 mL), and with minimal sample handling, high throughput, and time and labor saving as other clear advantages over *off-line* approaches. Conversely, the main drawbacks as compared to the *off-line* procedures are that no extract remains for further analysis and that the matrix may potentially interfere to a higher extent in the analysis, since the selection of washing and eluting solvents is somewhat less flexible than in *off-line* protocols.

Chromatographic separation was satisfactorily achieved with both LC in its classical version [21] and ultra-performance liquid chromatography (UPLC) [17, 19], by means of C_{18} columns (see Table 1). However, the use of UPLC columns (1.7 μm of particle size) allowed shortening the analytical chromatographic time and hence the consumption of solvents. Analytes were eluted from the column with a binary mobile phase consisting of acetonitrile [21] or methanol [17, 19] and water, by applying an organic solvent gradient at a constant flow rate. The mobile phase was in some cases buffered to an acidic pH (3.5–3.8) with ammonium formate/formic acid (see Table 1), in order to reduce chromatographic peak tailing and to improve ionization of the analytes determined in the positive mode [17, 19].

The investigated illicit drugs and metabolites were exclusively ionized by means of electrospray (ESI). With the exception of cannabinoids, which provide a better MS response under the negative ionization mode [24], all target analytes preferably produce positive ions in ESI (see Table 1). This atmospheric pressure ionization source, though highly versatile, is strongly affected by matrix interferences, which may negatively affect analyte recoveries in water samples, being more noticeable in highly polluted waters or with high organic matter content, e.g., influent wastewaters. The addition of isotopic-labeled analogues at the beginning of the analytical process allows correcting for matrix effects and also for potential analyte losses that may take place during the analysis due to evaporation, degradation, etc.

Tandem mass spectrometric determination was performed with triple quadrupole (QqQ) [17, 19] and hybrid quadrupole-linear ion trap (QqLIT) [21] instruments in the selective reaction monitoring (SRM) mode, by acquiring at least two transitions per target compound (see Table 1), which provides the best sensitivity and selectivity and allows obtaining four identification points [25]. On the contrary, just one SRM was registered to determine the isotope-stable internal standards used in the quantification process, since these compounds do not occur naturally in the environment.

3 Occurrence of Illicit Drugs and Metabolites in Wastewaters

The above presented methodologies were applied to investigate the levels of selected illicit drugs and metabolites in influent and effluent wastewaters of various WWTPs located along the Llobregat River basin [15, 18, 21]. Wastewater treatment in all studied WWTPs is based at least on a preliminary clarification step, which is followed by a biological treatment, which in most cases consists of conventional activated sludge processes (CAS), and a secondary clarification step. The studied WWTPs present different sizes, giving service to populations between 400 and 1,300,000 inhabitants. Their size and location, as well as the origin of the wastewaters treated, are shown in Fig. 1 [15, 18, 21]. Most of them were sampled only once, whereas five of them (see WWTPs 8, 10–13 in Fig. 1)

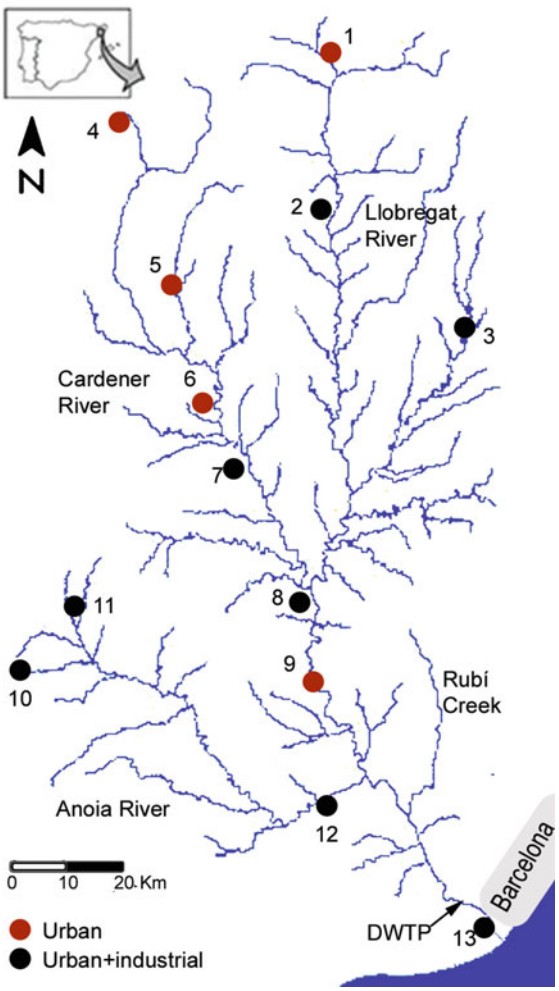

Fig. 1 Location of the WWTPs investigated in the Llobregat River basin (WWTP size: 1: 2,300 inhabitants, 2: 20,900 inh., 3: 5,700 inh., 4: 400 inh., 5: 16,500 inh., 6: 9,200 inh. 7: 20,200 inh., 8: 53,000 inh., 9: 3,200 inh., 10: 64,000 inh., 11: 151,500 inh., 12: 317,400 inh., 13: 1,300,000 inh.) [15, 18, 21]

were sampled in several occasions, in order to study removal efficiencies of illicit drugs and metabolites and/or the daily variation of the levels observed throughout the week.

3.1 Levels in Influent Wastewaters

Influent wastewaters, as expected, presented the highest levels of illicit drugs and metabolites in the investigated water matrices. Based on the reported data, the most ubiquitous and abundant compound in this matrix is the main cocaine metabolite, benzoylecgonine (BE), which is usually present at levels in the μg/L range (maximum concentration detected: 6 μg/L in WWTP 13 [21]). Cocaine (COC) and its metabolic product cocaethylene (CE), the amphetamine like compounds ephedrine (EPH), ecstasy (MDMA) and amphetamine (AM), the opioids morphine (MOR) and codeine (CODE), and the synthetic opioid used to treat heroin addiction, methadone (METH), and its main metabolite 2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) were also frequently detected in the investigated samples but at comparatively lower levels than BE. Only COC presented occasionally levels above 1 μg/L (maximum concentration: 1,236 ng/L in WWTP 11 [18]). In terms of abundance, COC is followed by EPH, MOR, and CODE, with maximum concentrations of 725 ng/L, 356 ng/L, and 314 ng/L, respectively, in WWTP 13 [15, 21], (which are mainly attributed to their therapeutic use), and MDMA, whose maximum concentration raised up to 302 ng/L in WWTP 12 [18]. The other most frequently detected compounds, i.e., CE, AM, EDDP, and METH were usually detected at levels below 100 ng/L in influent wastewaters.

Among the investigated substances, 3,4-methylenedioxyethamphetamine (MDEA), heroin (HER), and fentanyl (FENTA) were not detected in any sample, and the remaining analytes, i.e., Δ^9-tetrahydrocannabinol (THC) and its metabolites –11-hydroxy- Δ^9-tetrahydrocannabinol (OH-THC) and 11-nor-9-carboxy- Δ^9-tetrahydrocannabinol (THC-COOH)– , LSD and its metabolites – nor-LSD, nor-iso-LSD (nor-LSD), and 2-oxo-3-hydroxy LSD (O-OH-LSD) – , the heroin metabolite 6-acetyl-morphine (6ACM), ketamine (KETA) and the amphetamine like-compounds methamphetamine (MA), and 3,4-methylenedioxyamphetamine (MDA), were rarely observed, and presented levels usually below 10 ng/L.

Levels of illicit drugs and metabolites measured in influent wastewaters of WWTPs of the Llobregat River basin are in-line with those reported for these substances in the peer-reviewed literature. Figure 2 summarizes the concentrations found in influent and effluent wastewaters of the Llobregat River basin of those substances for which more data are available, and compares them with the levels measured in other Spanish areas, e.g., the Eastern Coast [21, 26], North Western [27, 28], the Ebro River basin [29], Catalonia [18] and South Eastern [30], and other European countries, like Switzerland [31], Ireland [32], Belgium [33–36], Italy [23, 37, 38], Germany [39], Croatia [40], France [41], and United Kingdom [42, 43]. As it can be observed in Fig. 2, levels of these compounds in influent

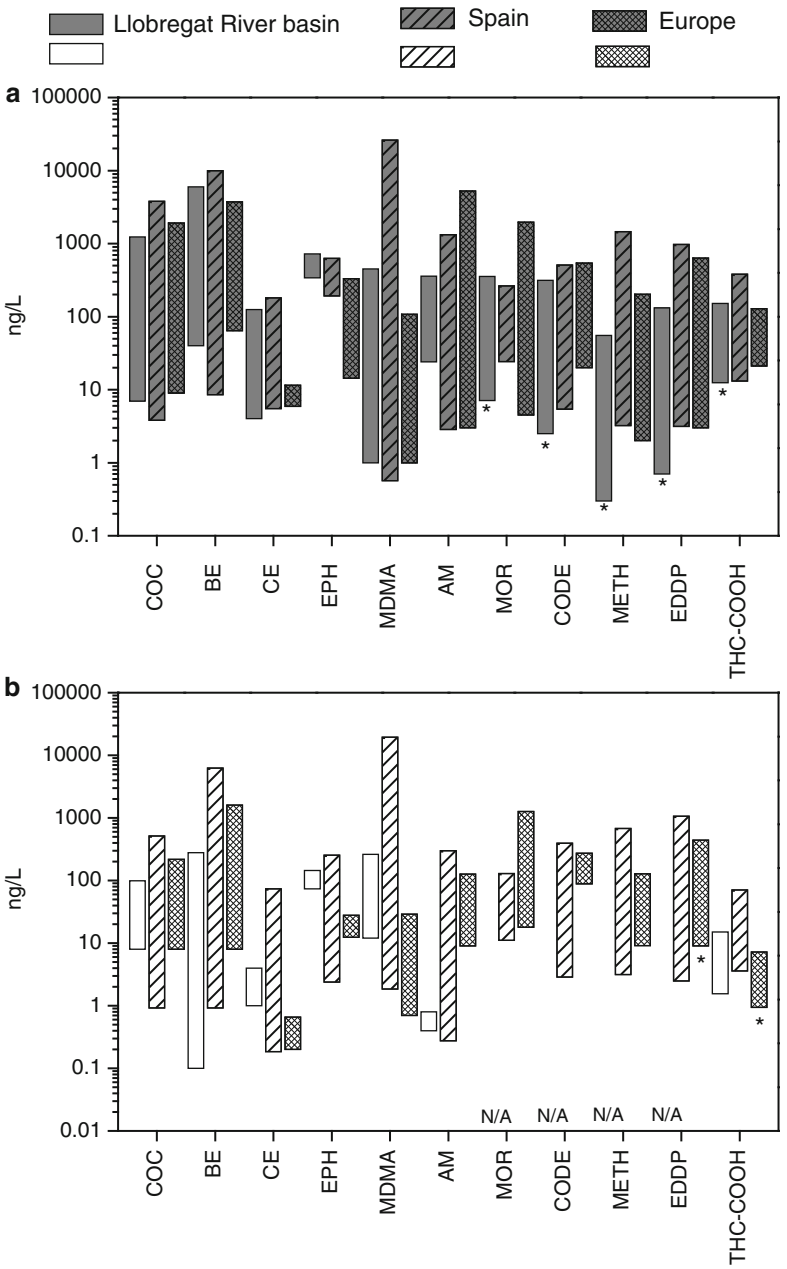

Fig. 2 Concentration ranges (expressed in ng/L in logarithmic scale) of the most investigated illicit drugs and metabolites in (a) influent and (b) effluent wastewaters from the Llobregat River basin and other Spanish and European areas (*N/A*: data not available, *asterisk*: just one value reported, minimum value is the method LOQ)

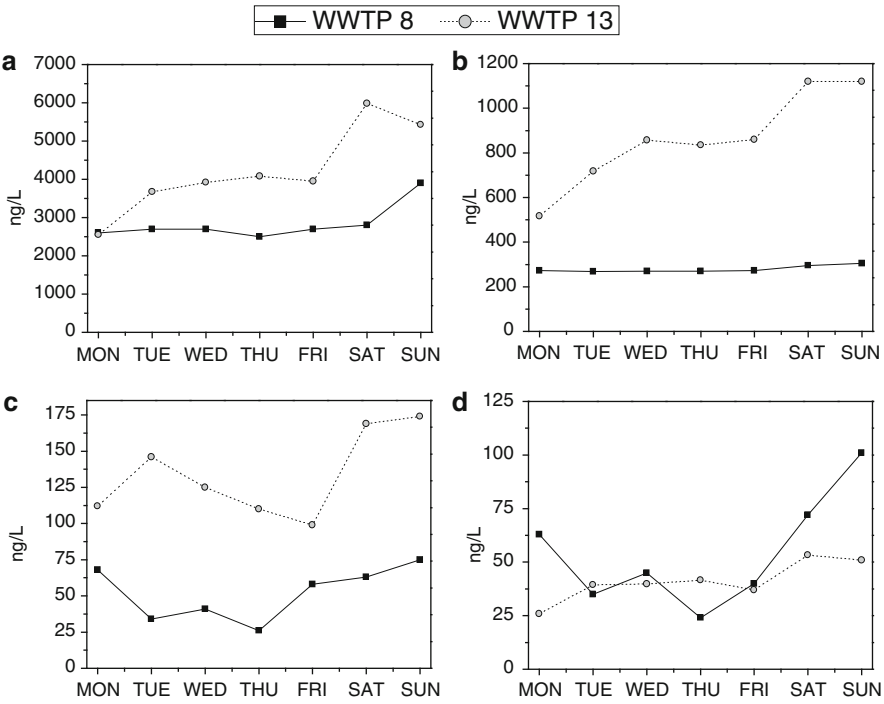

Fig. 3 Daily variation of (a) BE, (b) COC, (c) MDMA, and (d) AM levels in influent wastewaters of WWTP 8 and WWTP 13 (see location in Fig. 1)

wastewaters are similar to those reported in other Spanish areas, but for MDMA, AM, METH, EDDP, and THC-COOH, for which comparatively lower levels were reported in the Llobregat River basin. On the contrary, CE, EPH, and MDMA levels found in influent wastewaters of the Llobregat River basin are higher than those measured in influent wastewaters of other European WWTPs.

Huerta-Fontela et al. [18] and Postigo et al. [21] investigated the day-to-day variation of illicit drug levels throughout the week in influent and effluent wastewaters from WWTPs 8 and 13, respectively (see Fig. 1). Figure 3 shows the week profile of the concentrations of those compounds that were positively identified on a daily basis in both WWTPs, that is, COC and its main metabolite BE and the amphetamine-like compounds AM and MDMA. As it is shown, these substances presented higher levels at the weekend (Saturday and Sunday) than during the week (Monday to Friday), which may indicate their recreational use. Larger differences were observed for COC and BE levels in the largest WWTP (labeled as 13 in Fig. 1), being the average levels measured during the weekend 1.5 and 1.6 times larger, respectively, than the average levels observed during the working days. Other cocaine metabolite, CE, investigated only in WWTP 13, showed the same increasing trend, with average concentrations during the working days and the weekend of 59 ng/L and 125 ng/L, respectively. Regarding MDMA

and AM, larger differences in their concentrations were observed in WWTP 8, with average levels 1.5 and 2.1 times higher during the weekend than during the working days for MDMA and AM, respectively. MA levels were above the method LOD in waters from WWTP 8 only during the weekend; however, levels observed in WWTP 13 throughout the week do not show relevant variations. In the same way, levels of EPH and the opioid MOR, compounds for which the week profile was only studied in WWTP 13, remained constant during the week, which may be indicative of their therapeutic use.

3.2 Levels in Effluent Wastewaters

The most abundant and ubiquitous illicit drugs and metabolites in effluent wastewaters of the Llobregat River basin were COC and its main metabolite BE and the amphetamine-like compounds MDMA and EPH, which were also among the most abundant and ubiquitous substances in influent wastewaters (see Fig. 2). However, comparatively lower levels, usually below 100 ng/L, were found, due to their partial removal during wastewater treatment processes [18, 21]. The range of concentrations measured for these compounds in this matrix in the Llobregat River basin and in other wastewater treatment facilities located in other areas of Spain and in other European countries is summarized in Fig. 2. As it can be observed in the figure, levels of illicit drugs and metabolites in effluent wastewaters of the Llobregat River basin are in general comparatively lower than those measured in other Spanish and European areas; only EPH and MDMA show distinctly lower levels in Europe.

HER, MOR, FENTA, and THC were not detected in any effluent wastewater sample [15, 21], and data to assess the presence of CODE, METH, and EDDP in this matrix have not been specifically reported for the Llobregat River basin [15, 17]. The remaining investigated compounds were occasionally detected at a few ng/L or even in the pg/L range.

Contrary to what it was observed in influent wastewaters, the levels of illicit drugs and metabolites in effluent wastewaters remained constant throughout the week [18, 21]. Additionally, the highest concentrations measured at the WWTP outlet did not correspond with the highest levels observed at the WWTP inlet, and therefore, effluent levels seem to be determined by diverse working parameters, other than the contaminant load entering the WWTP.

3.3 Removal During Wastewater Treatment Processes

Huerta-Fontela et al. [18] and Postigo et al. [21] calculated the removal efficiencies of illicit drugs and metabolites from waters in several WWTPs of the Llobregat River basin (plants 8, 10, 11, 12, and 13 in Fig. 1), based on the levels of these

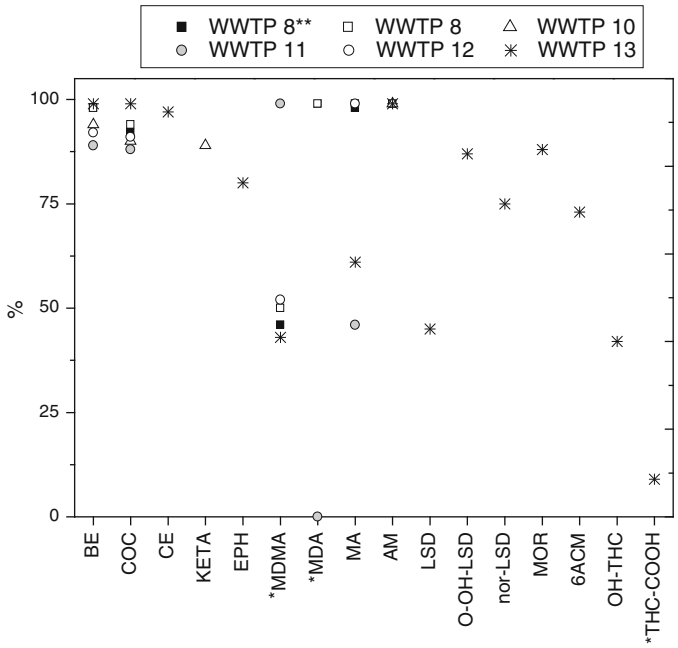

Fig. 4 Reported removals of illicit drugs and metabolites in WWTPs located along the Llobregat River basin. *WWTP 8***: average value of 7 consecutive days; *WWTP 8, 10–12*: average value of 4 days, *WWTP 13*: average value of 11 days. *Compounds occasionally observed at higher levels in effluent compared to influent

substances measured in influent and effluent composite samples, which were collected taking into account the hydraulic retention time of each WWTP. All investigated WWTPs use conventional activated sludge as secondary biological treatment. Figure 4 summarizes the reported data. As it can be observed, COC and its metabolites BE and CE, KETA, the amphetamine-like compounds EPH and AM, O-OH-LSD, and MOR showed the best elimination rates (above 80% on average) in all investigated WWTPs [18, 21]. Additionally, the day-to-day variation of the removal efficiencies of these compounds in WWTP 13 ($n = 11$) presented relative standard deviations (RSD) values below 3% for BE, COC, CE, AM, and MOR, and of 6% and 9% for EPH and O-OH-LSD, respectively.

Removal efficiencies observed for the remaining amphetamine-like compounds, i.e., MDMA, MDA, and MA, were observed to vary among the different investigated WWTPs [18] and within the same WWTP. For instance, MA and MDMA elimination rates presented RSD of 23% and 42%, respectively, in WWTP5. MDA and MDMA were determined occasionally at higher concentrations in effluent than in influent waters. In the case of MDA, this was attributed to N-demethylation of MDMA during wastewater treatment processes [18], whereas for MDMA, the process that leads to an increase of the effluent levels, which was also observed in WWTPs from the Ebro River basin [29], has not been elucidated yet.

Regarding the removal efficiency of the investigated opioids, other than MOR, in the Llobregat River basin, only elimination rates for 6ACM could be assessed, being 73% on average in WWTP 13. Boleda et al. [15] reported poor removal of METH and its metabolite EDDP, nor-morphine (nor-MOR), CODE, and nor-codeine (nor-CODE) in different WWTPs of Catalonia, two of them located in the Llobregat River basin, and occasionally higher concentrations in the effluents than in the influents. Persistence of EDDP and METH has been also reported in WWTPs of other European countries [32, 37]. In the case of nor-MOR and nor-CODE, N-demethylation of MOR and CODE, respectively, has been proposed as the mechanism that contributes to increase their levels during wastewater treatment [15].

Among the investigated cannabinoids, THC-COOH is removed comparatively worse than THC and OH-THC. This compound has also been measured occasionally at higher concentration in effluents than in influent wastewaters, which could be attributed to the cleavage of conjugated forms during treatment; however, it has not been investigated yet [15, 17, 21].

Overall, WWTPs based on CAS treatments, like the ones investigated in the Llobregat River basin, provide better elimination rates for illicit drugs and metabolites than wastewater treatment facilities that operate with only primary treatment [44] or with a secondary biological treatment based on biological filters [29, 41, 43, 45]. However, removal efficiency of illicit drugs and metabolites from wastewaters may be improved by incorporating advanced tertiary treatments to the existing wastewater treatment facilities. In this respect, reverse osmosis membranes have been observed to efficiently remove amphetamine-like compounds from municipal secondary treatment effluent wastewaters [46], and photocatalytic processes, such as photo-Fenton and heterogeneous photocatalysis with TiO_2, were able to mineralize cocaine, methadone, and their corresponding major metabolites in simulated municipal effluent wastewaters [47, 48].

3.4 Estimation of Illicit Drug Use

Back-calculation of illicit drug use at the community level from the levels of illicit drugs and metabolites measured in influent wastewaters is straightforward. To estimate illicit drug use by means of what is so-called the sewage epidemiology approach, concentrations of a drug use indicator (DUI) have to be normalized across the water volume entering the plant, the people served by the WWTP, and corrected by a factor that takes into account the molar mass ratio between the drug itself and the DUI, and the average excretion rate of the DUI.

Levels found throughout the week in wastewaters entering WWTP 13 were used to estimate illicit drug use in the area served by this facility, which covers about 1,300,000 people [21]. DUIs selected were the drug itself in the case of amphetamine-like compounds (AM, MA, and MDMA), and a drug metabolite in the case of cocaine (BE), heroin (6ACM), and cannabis (OH-THC). Results

Table 2 Estimations of illicit drug use, expressed in mg/day/1,000 people, in the Llobregat River basin and in other Spanish and European areas obtained with the sewage epidemiology approach

Studied area	Ref.	COC	MDMA	AM	MA	THC	HER
WWTP 13 – Llobregat River basin (Spain)	[21]	1,303–3,060	83–147	19–37	5–14	71–123	184–362
Catalonia (NE Spain)	[15, 18]	1,400	200	n/a	n/a	3,466[a]	138[a]
Ebro River basin (Spain)	[29]	1793[a]	60[b]	460[a]	2[b]	680[a]	24[a]
River Po Basin (Italy)	[23]	700	n.i.	n.i.	n.i.	n.i.	n.i.
Milano (Italy)	[44]	909	6	9	10	3,040	70
Lugano (Switzerland)	[44]	622	11	n.d.	n.d.	6,536	100
London (UK)	[44]	690	5	79	6	7,600	210
South Wales (UK)	[45]	900	n.i.	2,500	n.i.	n.i.	n.i.
Belgium	[33–35]	40–1,289	n.i	n.i	n.i	n.i	n.i
Zagreb (Croatia)	[40]	166	3.6	9.7	n.i.	3,690	262
Paris (France)	[41]	110–979[b]	1.6–15.4[b]	n.d.	n.i.	n.i.	n.i.

n.i. not investigated, *n.d.* compound not detected in influent wastewaters, *n/a* not reported data
[a]mg/day/1,000 adult people (15–64 years)
[b]mg/day/1,000 young people (15–34 or 15–44 years)

obtained, expressed in mg/day/1,000 people, are summarized in Table 2 and compared with illicit drug use figures obtained by applying the sewage epidemiology method in other investigated areas [15, 18, 23, 29, 33–35, 40, 41, 49, 50]. Note that figures shown are not fully comparable, since variations may exist in the DUI used in the back-calculations, e.g., MOR vs. 6ACM for heroin; THC-COOH vs. OH-THC for cannabis, or in the average excretion rate of the DUI considered. Moreover, different segments of the population have been occasionally considered in the peer-reviewed studies.

According to the figures obtained in the WWTP located in the lower part of the Llobregat River basin, the most consumed drug in this area is cocaine (2,200 mg/day/1,000 people on average), followed by heroin (244 mg/day/1,000 people on average), ecstasy (113 mg/day/1,000 people on average), cannabis (88 mg/day/1,000 people on average), amphetamine (30 mg/day/1,000 people on average), and methamphetamine (9 mg/day/1,000 people on average). These figures slightly differ from illicit drug use official estimates, which point out to cannabis as the most consumed drug followed by cocaine, ecstasy, amphetamine and methamphetamine, and heroin [51]. There are three explanations to these differences: (1) the existence of a local pattern of illicit drug use different to the national one, (2) the presence of biases in the sewage epidemiology approach applied, and (3) the presence of biases in the official methods.

The sewage epidemiology approach represents a very useful tool for illicit drug use estimation; however, it is still in full development and is subject to some limitations, which have been critically reviewed by Van Nuijs et al. [52]. For instance, in the case of cannabis, underestimation may occur because only the levels of the cannabis consumption indicator in the aqueous phase are considered, and these substances, due to their physical–chemical properties (log $K_{ow} > 5$) are likely to adsorb onto the wastewater suspended solids. On the other hand, THC is

highly metabolized, and therefore, its main excretion products are excreted at a very low rate (<2%), which may lead to a high uncertainty of the figures obtained. On the other hand, the estimation of the consumption of amphetamine-like compounds requires the analysis of their enantiomeric forms to discern between their licit and illicit use, which has only been addressed in one of the peer-reviewed methodologies developed to determine these compounds in water [53]. Regarding estimation of heroin use, if MOR is used as DUI, it is necessary to correct for the amount of MOR excreted into the sewage system due to its therapeutic use. If 6ACM is used as DUI, its low stability and low excretion rate, may lead to underestimation of heroin real use.

Overall, uncertainty of the estimation figures obtained can be reduced by further research on metabolization patterns of illicit drugs, accurate measurements of the water flow entering the wastewater facilities and the development of methodologies that allow calculating the real number of people served by the WWTP.

4 Occurrence of Illicit Drugs and Metabolites in Surface Waters

Incomplete elimination of illicit drugs and metabolites during wastewater treatment leads to their continuous release into receiving surface waters. The seasonal and spatial variation of the levels of these substances in the Llobregat River basin has been evaluated in the main river, which also constitutes one of the main water sources for drinking water production, its two main tributaries, the Anoia River and the Cardener River (see Fig. 1), and one additional stream, the Rubí Creek. [15, 20]. Sampling points were selected in order to evaluate industrial and urban pressures on the chemical quality of the natural waters of the Llobregat River basin; this is, to assess the effects of WWTPs discharges and river flow diversion after heavily populated and industrialized areas on illicit drug levels in surface waters. The most investigated point in the Llobregat River is located at the entrance of a drinking water treatment plant (DWTP) that is located in the lower part of the basin and supplies drinking water to part of Barcelona and its metropolitan area [15, 16, 20].

4.1 Levels in the Llobregat River and Its Tributaries

The most abundant and ubiquitous compound in surface waters of the Llobregat River basin was one of the two major COC metabolites, BE, which is present in almost all analyzed samples and measured at a maximum level of 1,350 ng/L at the intake of the DWTP [20]. COC, MDMA, and AM were also frequently detected in surface waters, and their maximum concentrations (120, 190, and 90 ng/L, respectively) were also measured at the intake of the DWTP [20].

According to the results obtained from various monitoring studies carried out at the intake of this DWTP, levels of COC and amphetamine-like compounds increase

in the summer period, which could be attributed to a lower river discharge. However, the highest concentrations were observed in winter, being 4-fold, 6-fold, and 13-fold higher for BE, COC, and MDMA, respectively, than in fall, which could be related, as suggested by Huerta-Fontela et al. [20], to a low degradation rate under the environmental conditions prevailing in winter. Regarding daily variation of the levels of these compounds throughout the week, the highest levels were observed during the weekend, which may be indicative of their recreational use, as aforementioned for influent wastewaters [20].

Among the most ubiquitous and abundant opioids in surface waters of the Llobregat River basin were CODE, EDDP, and METH, whose levels raise up to 251, 61, and 18 ng/L, respectively. MOR presented a lower frequency of detection and was quantified at a maximum concentration of 31 ng/L. The levels of CODE and EDDP in surface waters were also observed to increase in winter compared to spring and fall, whereas no relevant variations were observed for the levels of METH and MOR [15].

The THC metabolite, THC-COOH, was determined also in most of the analyzed samples and presented a maximum concentration of 80 ng/L [15]. Regarding its seasonal variation, levels of this compound were only measurable in winter, which may be attributed to a lower river flow [15].

LSD, phencyclidine (PCP), nor-MOR, and HER were always below the method limit of detection (12.5 ng/L for nor-MOR and 1.5 ng/L for the other compounds). The remaining investigated compounds were sporadically (KETA, 6ACM, FENTA, nor-CODE, and THC) or with a low frequency (MA, MDA) detected, presenting in all cases levels below 14 ng/L [15, 16, 20].

Figure 5 compares the levels of the most frequently detected compounds in surface waters of the Llobregat River basin with those determined in other river catchments from Spain and Europe. Overall, measured levels of illicit drugs and metabolites in surface waters of the Llobregat River basin are in agreement with those determined in other river water catchments from Spain [27, 29, 30, 54] and Europe [23, 31, 32, 35, 39, 42, 43, 55]. As it can be observed in Fig. 5, only the amphetamine-like compounds MDMA, MA, and AM and the cannabinoid THC-COOH presented distinctly higher levels in surface waters from the Llobregat River basin than in other rivers investigated in Europe.

The chemical pressure regarding the presence of illicit drugs and metabolites was observed to increase downstream the main river, which is likely related to the increase in population density and WWTP discharges. This behavior was not observed for the detected cannabinoid, THC-COOH, which presented the highest levels in the sampling points located upstream in the Llobregat and Cardener rivers. A relevant increase in the concentrations of cocaine-related and amphetamine-like compounds was detected in the Llobregat River waters collected after the mouths of the Cardener and Anoia tributaries (63% and 31% of increase in the total load of illicit drugs and metabolites detected, respectively), which may be directly related to the discharges of these rivers into the main stream. In this respect, it is important to remark that 90% of the Anoia River discharge is diverted due to its high pollution levels. Relevant loads of these substances were also found in the Rubí Creek (89 g/day

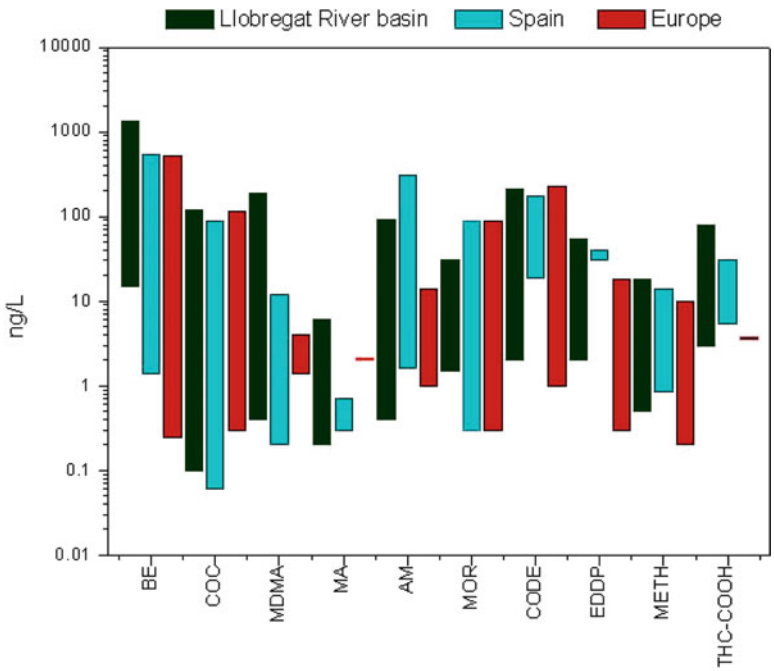

Fig. 5 Concentration range (expressed in ng/L) of the most frequently detected illicit drugs and metabolites in surface waters from the Llobregat River Basin and other Spanish and European water catchments

of cocaine-related and amphetamine-like compounds and 687 ng/L of opioids and cannabinoids), which flows through a densely populated and highly industrialized area. However, these concentrations will not end up into the main river, because the flow of the Rubí creek is fully diverted in order to prevent the dumping of high contaminant loads into the Llobregat River.

4.2 Removal During Drinking Water Treatment

The Llobregat River basin constitutes an important source for drinking water production in the Barcelona province. Thus, the occurrence of illicit drugs and metabolites may potentially have public health implications if they are still present in the finished water. The elimination of illicit drugs and metabolites in a DWTP located in the lowest part of the main river, which supplies drinking water to about 1 million people living in Barcelona and its metropolitan area, was evaluated by Boleda et al. [15, 16] and Huerta-Fontela et al. [20].

Water treatment in this DWTP consists of a conventional pre-oxidation step with chlorine until the break-point is achieved, followed by coagulation with alum-

coagulants, e.g., $Al_2(SO_4)_3$, Al_xCl_3 or Al_2O_3, and flocculation. The clarified water is then sand filtered and afterward diluted with groundwater in variable proportions to improve its quality. Water is further treated with ozone, granular activated carbon filtration, and chlorine, the latter in order to ensure a chlorine residual concentration through the distribution system.

Unlike MDMA, COC, BE, METH, and EDDP, compounds that showed poor removal during the first steps (prechlorination, coagulation, flocculation, and sand filtration) of the drinking water treatment (23%, 13%, 9%, 54%, and 28%, respectively), the amphetamine-like compounds MA, AM, and MDA, and the opioids MOR, CODE, and nor-CODE were almost completely eliminated (above 90% on average) already after sand filtration. The subsequent ozonation process was found to entirely remove MOR, MA, AM, and MDA, whereas all other compounds remained to a higher or lower extent in the water (the elimination rates achieved by ozonation were lower than 56%). Filtration of the ozonated water through granular activated carbon further removed CODE and nor-CODE and an important proportion of other drugs (>99% COC, 72% BE, and 88% MDMA). However, only between 52 and 59% of METH and EDDP was removed during this treatment step. These substances, together with BE, were in fact still detected at the very end of the process in the finished drinking water. The average global removals observed for METH, EDDP, and BE were 91%, 88%, and 89%, respectively [15]. Despite these high elimination rates, final concentrations of these compounds in treated water reached up to 130 ng/L in the case of BE and were below 3 ng/L in the case of METH and EDDP.

The target cannabinoids and FENTA were also eliminated completely through the drinking water treatment process; however, this assessment is based on only one observation, since these compounds were present in only one raw water sample.

Groundwaters used to dilute river waters during drinking water production contained only traces of METH and EDDP, with maximum concentrations of 0.5 ng/L and 2.3 ng/L, respectively. Therefore, the contribution of this water to the illicit drug loads observed in finished waters could be considered negligible.

Boleda et al. [16] compared elimination efficiencies of illicit drugs and metabolites obtained with a conventional drinking water treatment process, such as the one aforementioned, and an advanced treatment. The latter consisted of ultrafiltration (UF) followed by ultraviolet disinfection, reverse osmosis (RO), and remineralization with calcite. According to this study, the compounds determined in raw waters, i.e., COC, BE, nor-BE, CODE, nor-CODE, METH, EDDP, MDMA, and KETA, presented satisfactory elimination rates (above 89%) with the conventional treatment. However, removal of these compounds was slightly improved with the treatment based on UF/RO (above 97%). In this respect, major elimination rates were observed after RO. In fact, UF and UV disinfection were not very effective in compound removal.

The main problem related to conventional drinking water treatment is the formation of disinfection by-products when the natural organic matter present in the water and potential reactive contaminants react with disinfection agents, such as chlorine, chloramine, and chlorine dioxide. The disinfection by-products generated when illicit drugs and metabolites react with chlorine or ozone still needs

investigation. In this respect, the amphetamine-like compounds present amine moieties that are capable of reacting with chlorine, generating the potential human carcinogens *N*-nitrosodimethylamine-related compounds [56].

5 Conclusions

The application of several analytical methodologies to determine illicit drugs and metabolites in aqueous matrices to wastewaters and surface waters of the Llobregat River basin revealed the occurrence of this group of emerging contaminants in this area. The highest levels of these compounds were measured in influent wastewaters, whereas their concentrations were between one and two orders of magnitude lower in effluent wastewaters and surface waters. Potential ecotoxicity effects of environmental levels have not been investigated yet. The most frequently detected compounds in all investigated aqueous matrices were cocaine and its metabolite BE, the amphetamine-like compound MDMA, the opioids CODE and METH, and the metabolite of the latter, EDDP.

Levels found in influent wastewaters have been used to estimate illicit drug use in the investigated area by means of the sewage epidemiology approach. Despite the fact that estimations performed with this methodology are subject to uncertainty, this approach represents a very useful tool to evaluate drug use trends and detect possible hot-spots, which can help to allocate and develop more effective drug prevention programs.

BE, METH, and EDDP were also detected in finished drinking waters derived from the Llobregat River. Despite the fact that the levels found are too low to produce psychoactive effects through water ingestion, other health effects associated to these substances or the disinfection by-products formed during the drinking water process (halogenated derivatives) cannot be discarded and deserve investigation.

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