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Título artículo / Títol article:	Occurrence and behavior of illicit drugs and metabolites in sewagewater from the Spanish Mediterranean coast (Valencia region)
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Revista:	Science of the Total Environment, 2014, 487
Versión / Versió:	Preprint de l'autor
Cita bibliográfica / Cita bibliogràfica (ISO 690):	BIJLSMA, Lubertus, et al. Occurrence and behavior of illicit drugs and metabolites in sewage water from the Spanish Mediterranean coast (Valencia region). Science of the Total Environment, 2014, 487: 703-709.
url Repositori UJI:	http://hdl.handle.net/10234/124803

Occurrence and behavior of illicit drugs and metabolites in sewage water from the Spanish Mediterranean coast (Valencia region)

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Abstract

In this work, a study on the occurrence and behavior of illicit drugs and metabolites in sewage water systems has been made. A comprehensive dataset was obtained by analyzing illicit drugs daily in influent and effluent waters from three sewage treatment plants (STPs), over three different weeks. To complete this dataset, monitoring was conducted during an international pop/rock festival, an interesting facet within this study. The STPs selected were sited along the Spanish Mediterranean coast (Castellón province, Valencia region) and represent towns of different sizes, with appreciable variations in the population in the summer period. Illicit drug concentrations in the influents were low, except during the celebration of the music festival, when the levels of cocaine, benzoylecgonine, amphetamine, MDA and MDMA increased. Comparing the influent and effluent concentration data allowed the rough estimation of the removal of illicit drugs and metabolites by each STP. Removal efficiencies were estimated between 75 and 100% for most of the analytes under investigation. The loads discharged into the aquatic ecosystem were also calculated from effluent data. Weekly discharges of drugs and metabolites via effluent sewage waters presented values commonly below 10 g for each individual drug, with the exception of benzoylecgonine, which usually exceeded this level. The increase in population and drug consumption during the music event led to a notable increase in the weekly discharges, reaching values up to 406 g of MDMA and 122 g of benzoylecgonine.

1. Introduction

Illicit drugs enter the sewage system, unaltered or as metabolites, most commonly after their consumption and excretion, and also after illegal discharges as consequence of police interventions (Thomas et al. 2012). Studies on the occurrence of these compounds in influent waters from sewage treatment plants (STPs) provide useful information on drug use and consumption trends at local, national and international levels (Nefau et al. 2013; Thomas et al. 2012; van Nuijs et al. 2011a; Zuccato et al. 2008a). The possibility of performing daily sample analysis makes it feasible to have real-time and objective information on drug use of a community. Some recent studies report spatial and temporal variations in the occurrence of illicit drugs in wastewater, including holidays and "control" periods (Lai et al. 2013a), or performing analysis along one year (Harman et al. 2011; van Nuijs et al 2011a). Standard sampling devices are normally used to obtain 24-h composite samples, but polar organic chemical integrative samplers (POCIS) have also been used (Harman et al. 2011).

Some limitations of sewage water analysis such as the estimation of population sizes contributing to the samples in each catchment area, drug excretion and analytes stability in the sewage system have been discussed in the recent literature (Castiglioni et al. 2013; Thomas et al. 2012; Van Nuijs et al 2011b). Despite the limitations and uncertainties associated with sewage water analysis, interesting trends in drug use have been observed, with prominent increase related to particular celebrations (Harman et al. 2011; Lai et al. 2013a, 2013b; van Nuijs et al 2011a). However, the removal of these compounds by STPs during these high peaks of drug usage was not evaluated.

The efficiency of the treatment processes in the STPs can be estimated from drug concentrations in influent and the corresponding effluent, taking into account the residence time of water in the STP (Bijlsma et al. 2012; Postigo et al. 2010). According to the literature, illicit drugs and their metabolites may be released into aquatic environments due to their insufficient elimination in STPs. Thus, these compounds have been detected in natural surface waters (Baker and Kasprzyk-Hordern, 2011; Berset et al. 2010; van der Aa et al. 2013; Zuccato et al. 2008b), including Spanish rivers (Huerta-Fontela et al. 2008; Pedrouzo et al. 2011; Postigo et al. 2010). Concentrations found are generally low (sub ppb level) and therefore no short-term direct environmental or human health effects are expected. However, very little is known about aquatic ecotoxicology and the effects of illicit drugs on biota; therefore, long-term

(chronic) effects and possible effect of combined exposure are not ruled out (van der Aa et al. 2013). Large monitoring campaigns reporting concentration data for illicit drugs in environmental waters might give more and better insight on the environmental fate of these compounds. Therefore, it is important to evaluate the removal efficiency for illicit drugs in the STPs and the possible discharge of these compounds to the aquatic environment not only under normal conditions but also in particular situations, when increased concentrations of illicit drugs are expected, i.e. festivities, music events, festivals, etc.

In this work we report data on occurrence of 11 illicit drugs and metabolites in influent and effluent waters from three STPs, representing towns of different sizes, along the Spanish Mediterranean coast (province of Castellón; Valencia region). Three sampling periods of one week each were spread over one year (June, July, January and/or April), including one week which coincided with a big music festival. In total, 126 wastewater samples were taken, making the data set comprehensive and allowing us to set-up the following objectives: (i) to have information on the occurrence of illicit drugs in sewage waters in the catchment area, including some periods when tourism increases appreciably, (ii) to study the removal of each compound by three STPs serving different cities, (iii) to estimate the weekly load discharge towards the aquatic ecosystem through concentration data in the effluents, and (iv) to evaluate the influence of an important music event on the amount of illicit drugs in wastewaters and on the removal efficiency of the STP. The information obtained in this study reveals short-time changes in drug use at local level and provides useful information on the suitability of the treatment processes used by the STPs.

2. Material and Methods

2.1 Sample collection

  The three STPs studied were located along the Spanish Mediterranean coast and served the communities of Benicasim, Castellón and Burriana (see graphical abstract). The number of people connected to each STP was taken from census information procured in 2009, and corresponded to 170.600 (Castellón), 40.283 (Burriana) and 15.564 (Benicasim). It is worth noting that the population of Benicasim drastically increases in summer, particularly during the festival included in this investigation. Influent and effluent sewage water samples were collected from the three STPs daily during one week in summer (June 2008) and one week in winter (January 2009). In addition, samples were also collected from Benicasim STP during one week in July 2008 (coinciding with this important music event) and from Castellón and Burriana during one week in April 2009. Samples consisted on 24-h composite samples which were taken using a time-proportional sampling mode (1 L, every hour). They were collected at refrigerated conditions (4 °C) in high-density polyethylene (HDPE) bottles and 1 L homogenized 24-h sample was transported to the laboratory directly after taking the last aliquot. Upon reception in the laboratory (within 15 min), samples were immediately stored in the dark at -20 °C until analyses (within 1 week) to minimize degradation of analytes.

2.2 Target analytes

  Illicit drugs were selected as a function of their use in our area, focusing the research on amphetamines, cocaine and cannabis. Their most relevant metabolites were also selected for this study. The target analytes were the following: amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylene-dioxymethamphetamine (MDMA, or ecstasy), 3,4-methylenedioxyethylamphetamine (MDEA), cocaine, cocaethylene, benzoylecgonine, norbenzoylecgonine, norcocaine, and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), a relevant metabolite of Δ^9-tetrahydrocannabinol (cannabis). Isotope-labeled compounds were used as surrogate internal standards (ILIS) for quantification: amphetamine-d_6, methamphetamine-d_5 MDA-d_5, MDMA-d_5, MDEA-d_5, cocaine-d_3, cocaethylene-d_8, benzoylecgonine-d_3 and

THC-COOH-d_3. More details on chemicals and materials can be found elsewhere (Bijlsma et al., 2009).

2.3 Sewage water treatment

All STPs investigated are equipped with conventional activated sludge secondary treatment. The main differences between them refer to their water treatment capacity and additional treatment processes. STP Benicasim applied an extra tertiary nitrogen and phosphate removal, whereas STP Burriana applied a disinfection step by chlorination and STP Castellón had an additional physical treatment (incl. grit removal). The average daily flow of each STP was Castellón 47412 m^3; Burriana 12056 m^3; and Benicasim 5253 m^3. For the three STPs, the average residence time is between 12 and 24 h.

2.4 Analytical Methodology

Sample treatment and specific information on instrument operating conditions, both chromatographic and spectrometric, and on method validation can be found elsewhere (Bijlsma et al., 2009). Briefly, 50 mL of effluent or 50 mL of five-time diluted influent sewage water were spiked with a mixed ILIS solution and the pH was adjusted to 2.0 with formic acid. Solid phase extraction (SPE) was performed using Oasis MCX cartridges. After elution with 2% ammonia solution in methanol, the extracts were evaporated and reconstructed in 1 mL of 10% methanol aqueous solution. The final sample extract (20 μL) was injected directly into the UHPLC-MS/MS system. Chromatographic separation of the compounds was achieved using an Acquity UPLC BEH C_{18} column and an optimized gradient using methanol: water (5 mM ammonium acetate, 0.1% formic acid). A TQD triple quadrupole mass spectrometer was operated in positive ionization mode, where three SRM transitions for each target compound were acquired. All data were acquired and processed using MassLynx v 4.1 software (Waters, Manchester, UK).

2.5 Calculation of removal efficiencies

Removal efficiencies were estimated by comparing effluent concentrations (Ce) from day (x+1) with influent concentrations (Ci) from day (x), thus considering an average residence time of 24 h. Efficiencies (E) were calculated as E = (1 − (Ce

(x+1)/Ci (x)) x 100% (Bijlsma et al. 2012). For each STP and compound, average removal efficiencies were estimated from the daily values.

3. Results and Discussion

3.1 Sample analysis and quality assurance

The determination of illicit drugs in influent and effluent sewage waters requires advanced analytical methodologies to be able to provide accurate concentration data in these complex matrices. At present, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is the workhorse in this field (Baker and Kasprzyk-Hordern 2011; Bijlsma et al. 2009; Pedrouzo et al. 2011; Thomas et al. 2012), due to its excellent performance in terms of sensitivity, selectivity and robustness.

In this work, we applied the methodology described by Bijlsma et al. (2009). Data reported are the mean concentration of 2 measurements (replicates) based on 2 individual extractions. In every sequence of analysis, samples were injected in between two calibration curves prepared in solvent. Two quality control samples (QCs), consisting of water samples with the lowest concentrations expected (i.e. samples collected from Tuesday to Thursday), spiked at the limit of quantification (LOQ) and 10xLOQ levels, were also analyzed in every sequence of sample analysis. The sequence was considered satisfactory when recoveries ranged from 70 to120% for each analyte. Quantification criteria and confirmation parameters, such as the ion ratio between recorded transitions, retention time and the accepted deviation, were applied before reporting positive findings (2002/657/EC; SANCO/12495/2011).

The analyte-ILIS of each illicit drug, except for norcocaine and norbenzoylecgonine, was used to compensate for matrix effects and for possible errors related to sample treatment. In general, matrix effects resulted in ionization suppression and, as expected, were stronger for influent wastewater. Amphetamines and especially THC-COOH were the analytes more affected by matrix effects.

Although some concentration data obtained in this work were below the lowest level validated (Bijlsma et al. 2009), they could be reported as the signal-to-noise ratio was $\geq$ 10 (i.e. the statistical limit of quantification). Furthermore, the reliable quantification of THC-COOH in influent samples was problematic, and only few data could be reported due to the strong matrix effects observed (ionization suppression) for this analyte. On the contrary, in effluent samples, which were much cleaner and less affected by matrix effects, THC-COOH could be quantified in several occasions, even if it was present at concentrations lower than in influent.

3.2 Occurrence of illicit drugs in influent waters

The occurrence of illicit drugs and metabolites in influent waters from the three STPs (i.e. communities) under study is shown in **Figures 1 – 2**. In general, cocaine and its main metabolite benzoylecognine (BE) were the most abundant compounds in the three weeks of monitoring, suggesting a notable consumption of cocaine in the area under study. This is consistent with the annual report of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), which reported levels of cocaine use in Spain above the European average using statistical data of 2008 (EMCDDA, 2010). Cannabis is another drug with high prevalence in Spain (EMCDDA, 2010). This might be explained by the fact that cocaine and cannabis enter Europe largely through the Iberian Peninsula (EMCDDA, 2010). Thus, the availability of these drugs is probably higher, allowing easier access and thereby increasing consumption of these drugs rather than others. The average estimated consumption of these illicit drugs in Spain (data reported for Barcelona, Castellón, Valencia and Santiago de Compostela) through sewage water analysis is approximately 1000 mg cocaine/day/1000 inhabitants (based on BE loads) and 100 mg THC-COOH/day/1000 inhabitants (Thomas et al. 2012).

Figure 1 shows the occurrence of illicit drugs in sewage water from Benicasim. The STP commonly serves only this town, which has a population of 15,564 inhabitants. However, it is a known holiday destination, mainly for Spanish families and pensioners, and therefore its population increases drastically during summer. Furthermore, it hosts one of the largest Spanish and European annual pop, rock and electronica festivals, visited by approximately 40,000 young music fans. In 2008, the festival was held from July 17 to 20 and the STP also treated the wastewater obtained from the portable toilets used at the festival terrain. It is important to point out that the disposal of waste from these portable toilets occurred before the composite sampler.

In this work, sewage samples from Benicasim were collected during one whole week of June 2008, July 2008, and January 2009. Slightly higher concentrations of BE and amphetamine were found in June (summer) compared with January (winter), which would reflect the increase in the consumption of these drugs in the holiday period. These findings are in agreement with the works from Harman et al. (2011) and Lai et al. (2013a), who also reported increased use of amphetamine, methamphetamine, cocaine

and MDMA in the holiday period in Oslo region (Norway), and a vacation area in Queensland (Australia), respectively.

The significant increase in concentration of MDMA, THC-COOH, cocaine and BE observed during the week of July 2008, which coincided with the music festival is worth noticing (**Figure 1, top**). Other compounds such as amphetamine, MDA and the cocaine metabolites, norbenzoylecgonine and cocaethylene were also found at higher concentrations during this period, although their levels were by far below BE, the main metabolite of cocaine (**Figure 1, bottom**). In fact, THC-COOH could only be quantified in the influent samples from July (concentration range 918 - 1638 ng/L). Several factors may explain the non-detection of this compound in most influent samples. Obviously, the analytical limitations, mainly the poor sensitivity, play a key role which makes the quantification of THC-COOH troublesome, and also sorption to solids may be greater (Harman et al., 2011), due to its different physico-chemical properties (lower polarity) compared with other illicit drugs.

The most interesting finding was the extraordinary increase in the party drug MDMA (ecstasy) during the music festival. This drug was barely detected during the weekends of June and January (**Figure 1, top**). The fact that MDA was also found in the influent samples of July is interesting. MDA is a minor metabolite (7% of a dose) of MDMA (Castiglioni et al., 2008), and MDMA was found at high concentrations in the influents (above 27 µg/L in some of the samples; see **Figure 1**). This might explain the finding of MDA in influents. However, MDA is also available on the illicit market. Therefore, the presence of this compound might be due to consumption of MDA itself, or it might proceed from the consumption or transformation in the sewage system from MDMA. The analytical method applied to the analysis of the water samples did not allow distinguishing between the enantiomeric forms of MDA. Enantiomeric profiling of chiral drugs has proven to be very helpful. Thus, an enrichment of MDA with S(+)-enantiomer in urine and subsequently in wastewater would, for example, indicate that MDA is present due to MDMA abuse and not direct MDA use (Kasprzyk-Hordern and Baker, 2012).

As outlined above, data obtained on illicit drugs in influents during the festival is interesting. The prominent increases in occurrence of illicit drugs were in agreement with data reported regarding similar events. For example, high levels of MDMA and cocaine use were observed on New Year´s Eve (Lai et al. 2013a; van Nuijs et al.

2011a). Similarly, higher MDMA concentrations could be linked to graduation festivities (Harman et al. 2011), a big dance party (van der Aa et al. 2013), and an annual Australian music festival (Lai et al. 2013b) celebrated within the catchment areas. And although methamphetamine was slightly lower, Gerrity et al. 2011 reported data suggesting an elevated use of cocaine during the Super Bowl weekend compared to an average weekend.

Weekly profiles for illicit drug concentrations in STP sited at the other two towns, Castellón and Burriana, are shown in **Figure 2** for three periods of the year (summer, winter and spring). The populations of Castellón and Burriana are relatively constant, although there are some fluctuations as many families move to the beach area (e.g. Benicasim) in summer. This means that the number of inhabitants in July and August is lower than the rest of the year. As previously mentioned, BE was the most abundant compound in all samples studied, maintaining a rather constant relative abundance with cocaine during all studied period. The average cocaine/BE ratio in all sewage waters included in this work was 0.30 ± 0.14, which is higher than the ratio ≤ 0.1 reported for human metabolism, but in agreement with ratios $(0.1 - 0.7)$ obtained from wastewater analysis of 21 STPs (Castiglioni et al 2013). Moreover, the highest concentrations of these compounds were commonly found on weekends, as illustrated by the data from samples collected from Saturday to Monday. The increased cocaine consumption during the weekend compared to weekdays was also observed by others (Thomas et al. 2012; van Nuijs et al 2011a). Generally, concentrations of illicit drugs in influent waters from Castellón were higher than that from Burriana. The population of Castellón is 5-times higher than that of Burriana and this fact seems to lead to higher concentrations of illicit drugs and metabolites in the influent waters. This is in accordance with other studies, where higher consumption was related to larger cities (van Nuijs et al. 2011b; Banta-Green et al. 2009), surely not only because of the higher population but also because of the lifestyle associated to big cities.

In addition to these results, our recent data from Castellón sewage waters (March 2011 and April 2012) show concentrations in influents about the same magnitude. Average weekly concentrations in 2011 and 2012 for cocaine were 400 ng/L and 450 ng/L, and for BE 1000 ng/L and 1400 ng/L, respectively. Furthermore, recent improvements in the analytical methodology for THC-COOH, especially better method sensitivity and lower limits of detection and quantification, have facilitated the

determination of THC-COOH in influent waters from Castellón, with average weekly concentrations of 300 ng/L and 600 ng/L in 2011 and 2012, respectively. For this purpose, the use of new-generation more sensitive LC-MS/MS instrumentation was essential (Bijlsma et al., 2013).

3.3 Occurrence of illicit drugs in effluent waters: removal efficiency of STPs

The efficiency of the treatment processes of STPs can be estimated from illicit drugs concentrations in influents and from their corresponding effluents, taking into account the residence time of water in the plant (Bijlsma et al. 2012; Postigo et al. 2010). In this study, we applied the same approach for estimating the removal efficiency of each STP, which requires the analysis of illicit drugs in influent and effluent samples. It is important to notice that removal was calculated considering an average residence time of 24 h. As the residence time of the STPs varied between 12 and 24 h, there will be some uncertainties associated with the calculations presented in this work. Therefore, data given in this paper must be taken as a rough, albeit useful, estimation of the removal efficiency of the STPs due to the large number of samples collected (data from 21 influent and 21 effluent samples for each STP).

Figure 3 shows the average removal efficiencies for several illicit drugs in three STPs. Nearly all values were higher than 60% with a few exceptions that mostly correspond to the STP of Benicasim during the festival event of July. The removal of amphetamine, cocaine and its main metabolite BE was ≥ 75% for all STPs, which compares well with the range of 81 - 99% reported by Baker and Kasprzyk-Hordern (2013), Huerta-Fontela et al. (2008), Kasprzyk-Hordern et al. (2008) and Repice et al. (2013). Removal of the other cocaine metabolites, cocaethylene, norbenzoylecgonine (norBE) and norcocaine was ≥ 75% for all STPs, except for norBE by the Castellón STP. Baker et al. (2013) reported removals with the use of activated sludge of 42% and 68% for norbenzoylecgonine and cocaethylene, respectively. MDMA presented high variability with values from 34% to 89%. This is in agreement with data reported by Bijlsma et al. (2012), who also found highly variable removal efficiencies for MDMA in five Dutch STPs. Since MDA and THC-COOH were not commonly detected in influent samples, the removal for these compounds could only be estimated for the STP serving Benicasim during the festival week.

In general, the removal efficiencies were not much different over the three weeks studied along the year (June, January and April). This implies that the applied treatment does not seem to be highly affected by meteorological conditions (i.e. temperature, which ranges on average from 5 °C in January to 30 °C in June at this latitude) and that bioactivity/degradation was similar in summer and winter.

The STP of Benicasim, designed to treat sewage water of a small community (approx. 15.000 inh.) showed satisfactory removal (75 - 100%) for all compounds, except MDMA. However, some difficulties were observed during the music festival (**Figure 3**). As stated in the previous section, concentrations of illicit drugs in influent waters during this festival were considerably higher (see **Figure 1**), as a consequence of the strong increase of population and consumption of illicit drugs linked to these events. It seems that the drastic increase in population during the festival week, added to the common increase of tourists in summer, creates some difficulties in the treatment processes of sewage water.

More recent data obtained from Castellón STP (2011 and 2012) have enabled a new estimation of removal efficiencies for cocaine, BE as well as THC-COOH. The removal of cocaine and BE was consistent with data from previous years, with efficiencies higher than 80%. The removal efficiency for THC-COOH could not be estimated in our analysis from 2008 and 2009 samples due to the above-mentioned difficulties in determining this cannabis biomarker in influent samples. However, with an improved analytical methodology, we were able to use our data from 2011 and 2012 to estimate the THC-COOH removal, which was found to be higher than 90% in both years.

As previously stated, the removal efficiency was estimated using concentrations of analytes in solely sewage waters. The lower levels commonly found in effluents are assumed to be a result of removal in the STP, due to microbial degradation, or other transformation processes. However, the analysis of suspended particular matter (SPM) has also been suggested to prevent under-reporting. The analysis of both sewage water (influent and effluent) and SPM provides a better estimation and more realistic knowledge on removal and environmental impact of compounds by STP systems, as removal from wastewater does not necessarily mean degradation (Baker et al. 2012; Baker and Kasprzyk-Hordern 2013). For most illicit drugs included in this study, less than 5% was estimated to be present in SPM (Baker et al. 2012), thus potential under-

reporting was assumed to be low. THC-COOH was not included in that study, but partition to SPM might be greater for this compound compared with the other selected drugs, owing to its less polar character. Furthermore, the stability of illicit drugs in sewage is an important issue. Amphetamine, methamphetamine, MDA, MDEA, MDMA and THC-COOH are generally stable up to 72 h at 4 °C (Castiglioni et al. 2006). However cocaine, norcocaine and cocaethylene are not stable under these conditions (decrease > 10%), and can be transformed into BE and norBE through chemical hydrolysis (Castiglioni et al. 2013). In this study, the bias due to cocaine transformation is expected to be relatively low as the estimated mean residence time of wastewater in the sewers investigated is 3 h and the conditions for sampling and sample storage minimize degradation of the selected compounds. The highest uncertainty within this study seems to be related to the sampling (Ort et al. 2010). In this work, sampling error was estimated to be relatively high (around 30%) (Castiglioni et al. 2013) due to the time proportional sampling interval used (every hour). This uncertainty has been reduced for the studies performed in 2011 and 2012 by taking a sample every 15 min.

As demonstrated in this work, the results obtained from the analysis of sewage water give useful information on trends of drugs usage from the population and the removal of these compounds by STPs. However, for the correct interpretation of these results it is important to take into account some of the uncertainties associated with the determination of illicit drugs in sewage (Castiglioni et al. 2013; van Nuijs et al. 2011b).

3.4 Environmental loads

Weekly loads (g) of illicit drugs and metabolites discharged via sewage effluents into the aquatic ecosystem were estimated from daily concentrations of each compound detected in effluent water (ng/L) and the daily flow of effluent water discharged along the week (**Table 1**). Loads (g/week) of illicit drugs (MDMA, cocaine, BE, cocaethylene and THC-COOH) from effluent waters of Castellón could be updated for 2011 and 2012, and are also included in **Table 1**.

The effluents of the three STPs studied are discharged into the Mediterranean Sea. Occasionally, effluents of the Castellon STP are also used for irrigation. The discharges for each individual compound were generally below 10 g per week, with only a few exceptions, mainly BE, with discharges up to 49 g/week in Castellón (2012).

The main discharges predominantly corresponded to cocaine and its metabolites, which were released by all three STPs. This might be expected as cocaine (and BE) was the most abundant drug found in sewage waters. Although the determination of THC-COOH in influent waters was troublesome, it could be determined on several occasions in effluents, which are less prone to matrix interferences when using LC-MS/MS. Consequently, the environmental loads of THC-COOH could be estimated. Weekly discharges of THC-COOH were 6.4 g and 3.2 g for Castellón (June 2008 and April 2012, respectively) and 9.9 g for Benicasim (July 2008). Although concentrations of MDMA in influent waters were not very high (sub-ppb level), this compound was frequently detected in the effluents. Similarly to THC-COOH, this fact might be due to less matrix interferences affecting LC-MS/MS analysis in this type of samples making detection of illicit drugs easier, or owing to the low removal of MDMA by the STPs (see **Figure 3**). Weekly discharges of MDMA were mostly below 10 g in the three STPs evaluated.

A notable increase in weekly discharges was observed for Benicasim in July, as a consequence of the much higher levels of illicit drugs in sewage waters. This was expected as there was a dramatic increase in population and drug consumption during this week. The highest weekly discharges towards the aquatic ecosystem corresponded to MDMA and BE, which is in agreement with the high consumption of ecstasy and cocaine during the festival. Loads for cocaine, MDA and amphetamine were also higher during this week, but in all cases below 30 g.

Overall, the amounts of illicit drugs discharged into the environment reported in this work are of the same order than those reported by van der Aa et al. (2013), who estimated daily discharges ranging from 1 to 5 g of BE and 1 to 10 g of MDMA. The only exception in their study (80 g/day of MDMA) coincided with a big dance party on the day before sampling, similarly to what we observed in the present work during the festival week of Benicassim.

The lack of aquatic ecotoxicological data for narcotic substances makes it difficult to do proper environmental risk assessment. Environmental risk characterization ratios (RCRs) were calculated by van der Aa et al. (2013), in order to evaluate the potential risk associated with the presence of illicit drugs in the aquatic ecosystem. Measured environmental concentrations (MEC) found in surface water were divided by the predicted no effect concentrations (PNECs) and for values < 1 no

potential risk to the aquatic environment was assumed. PNECs derived by the Ecological Structure Activity Relationships (ECOSAR) modeling were given for methamphetamine (2.30 µg/L), MDMA (2.70 µg/L), cocaine and BE (both 4.90 µg/L) (van der Aa et al. 2013). Although the authors of that manuscript recognized the limitations and uncertainties of this approach, it may give an indication of possible environmental effects. In the present work, we did not perform analysis of surface/sea water, but from the measured concentrations in effluents and considering the PNEC values, no short-term environmental risk might be expected under the treatment and normal circumstances applied in the STPs under study. In the future, the analysis of surface waters and biota might give useful information on the potential impact of illicit drugs in the aquatic environment.

4. Conclusions

The results obtained in this study from analysis of sewage waters gave valuable information of illicit drugs use in three locations on the Spanish Mediterranean coast. Moreover, as both influent and effluent samples were analyzed in three one-week periods, it was feasible to evaluate the efficiency of treatment process applied by the STPs. In general, the removal of illicit drugs by the three STPs was satisfactory (mostly above 75%) under normal circumstances, which limited the discharge of these emerging contaminants towards receiving environmental waters. In one case, during a large music festival, where the population and drug consumption dramatically increased, there was a notable increase in illicit drug concentrations in influents. This was also reflected in higher analyte levels in the effluents and in the loads to the aquatic environment. One interesting aspect is the usefulness of the methodology applied on the detection of short-time changes in drug use at local level and the effects on STP processes, and consequently on the potential impact on the aquatic environment.

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Table 1. Weekly loads (g) and range of daily loads (g) (in brackets) of illicit drugs discharged in the environment via effluent waters by the three STPs studied.

STP	Amph.	MDA	MDEA	MDMA	Metamph.	Cocaine	Cocaethylene	BE	NorBE	NorCoc	THC-COOH
CS0806 (a)	0.10 (0-0.10)	-	-	9.86 (1.08-2.17)	0.09 (0-0.09)	5.65 (0.4-1.58)	1.15 (0-0.46)	5.50 (0-1.72)	9.07 (0.98-1.67)	1.25 (0.10-0.31)	6.44 (0-6.44)
CS0901	- (b)	-	0.09 (0-0.09)	2.17 (0-0.74)	-	6.87 (0-2.22)	-	8.23 (0-4.12)	14.78 (1.85-3.14)	2.54 (0-0.56)	-
CS0904	-	-	-	-	-	1.64 (0.16-3.21)	-	10.87 (0.67-2.78)	8.04 (0.71-1.55)	1.94 (0.24-0.30)	-
CS1103	-	na (c)	na	11.73 (0-2.99)	-	6.02 (0.24-1.55)	2.43 (0-0.49)	12.5 (0-5.15)	na	na	-
CS1204	-	na	na	4.56 (0.35-1.35)	-	4.08 (0.14-1.36)	na	49.32 (5.12-8.18)	na	na	3.20 (0-0.81)
BU0806	0.21 (0.01-0.10)	-	-	0.88 (0-0.32)	-	0.54 (0.04-0.14)	-	9.68 (0.66-2.38)	2.46 (0.14-0.64)	0.22 (0-0.06)	-
BU0901	-	-	-	0.30 (0-0.14)	-	4.11 (0.39-0.94)	0.27 (0-0.07)	27.55 (1.73-6.52)	3.31 (0.20-0.84)	0.71 (0.08-0.12)	-
BU0904	-	-	-	-	-	1.65 (0.18-0.31)	-	12.02 (1.29-2.34)	3.35 (0.33-0.74)	0.64 (0.06-0.12)	-
BE0806	0.63 (0.07-0.11)	-	0.12 (0-0.06)	0.68 (0.05-0.22)	0.32 (0.04-0.06)	0.44 (0.02-0.20)	-	0.56 (0.054-0.132)	0.27 (0.02-0.06)	0.26 (0.04-0.04)	-
BE0807 (d)	12.97 (0.02-7.18)	28.34 (0.15-12.30)	0.07 (0-0.07)	405.96 (1.05-145.79)	0.08 (0-0.04)	16.21 (0.05-7.55)	2.61 (0.02-0.15)	121.67 (0.15-61.16)	3.62 (0.13-1.01)	0.41 (0.03-0.10)	9.91 (0-3.76)
BE0901	-	-	-	0.70 (0.04-0.21)	-	0.67 (0.08-0.11)	0.06 (0-0.02)	2.33 (0.18-0.47)	0.30 (0-0.05)	0.18 (0.02-0.03)	-

(a) CS Castellón. BU Burriana. BE Benicasim. CS0806 means: Castellón, June 2008

(b) –: no loads could be estimated as the illicit drugs was not detected in any of the influent samples analyzed
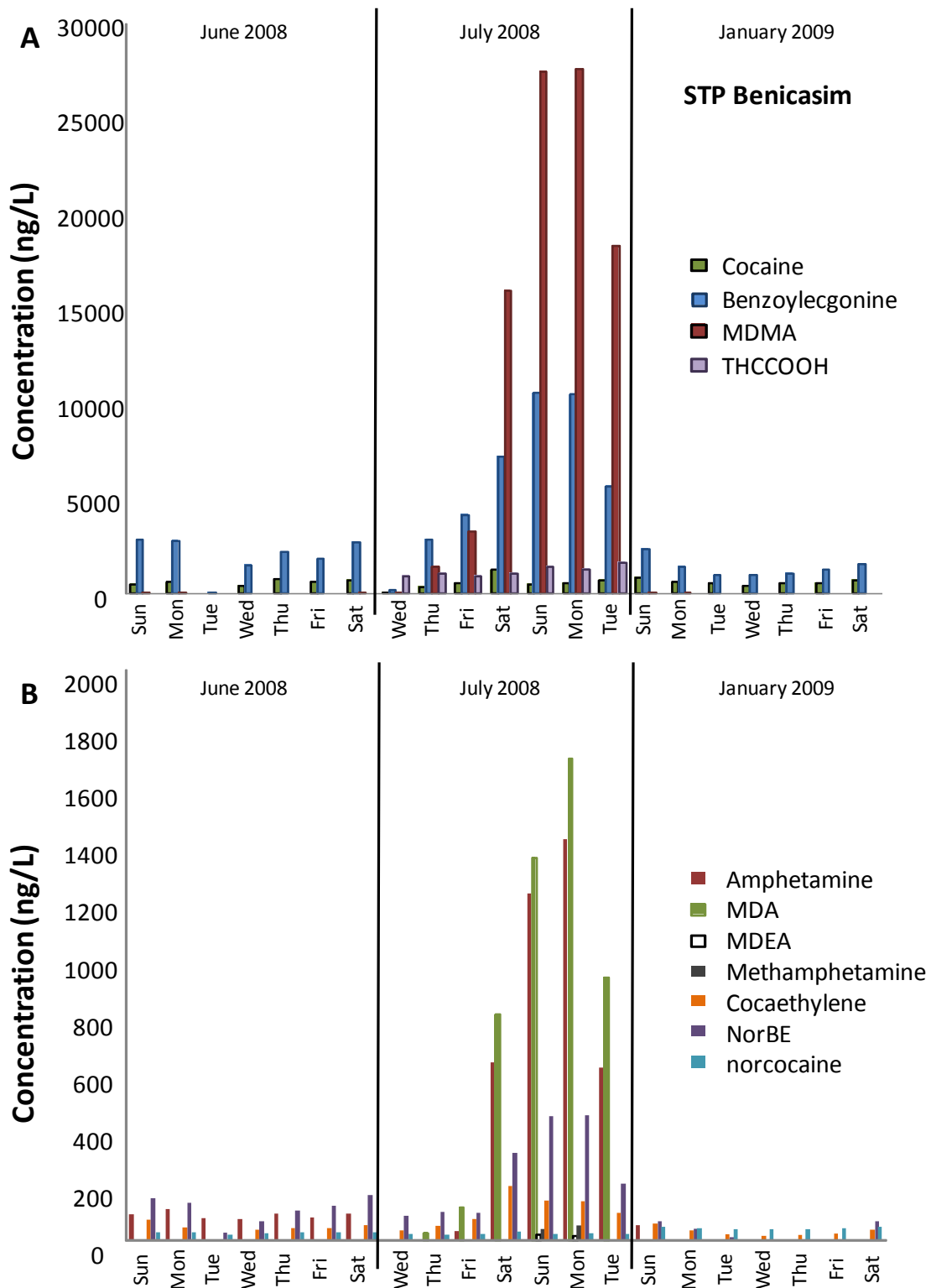
(c) na: not analysed

(d) Music event

Figure captions

Figure 1. Weekly profile of illicit drugs and metabolites concentrations (ng/L) in influent sewage waters from the Benicasim STP in June, July (music event) and January. Top: Major compounds. Bottom: Minor compounds
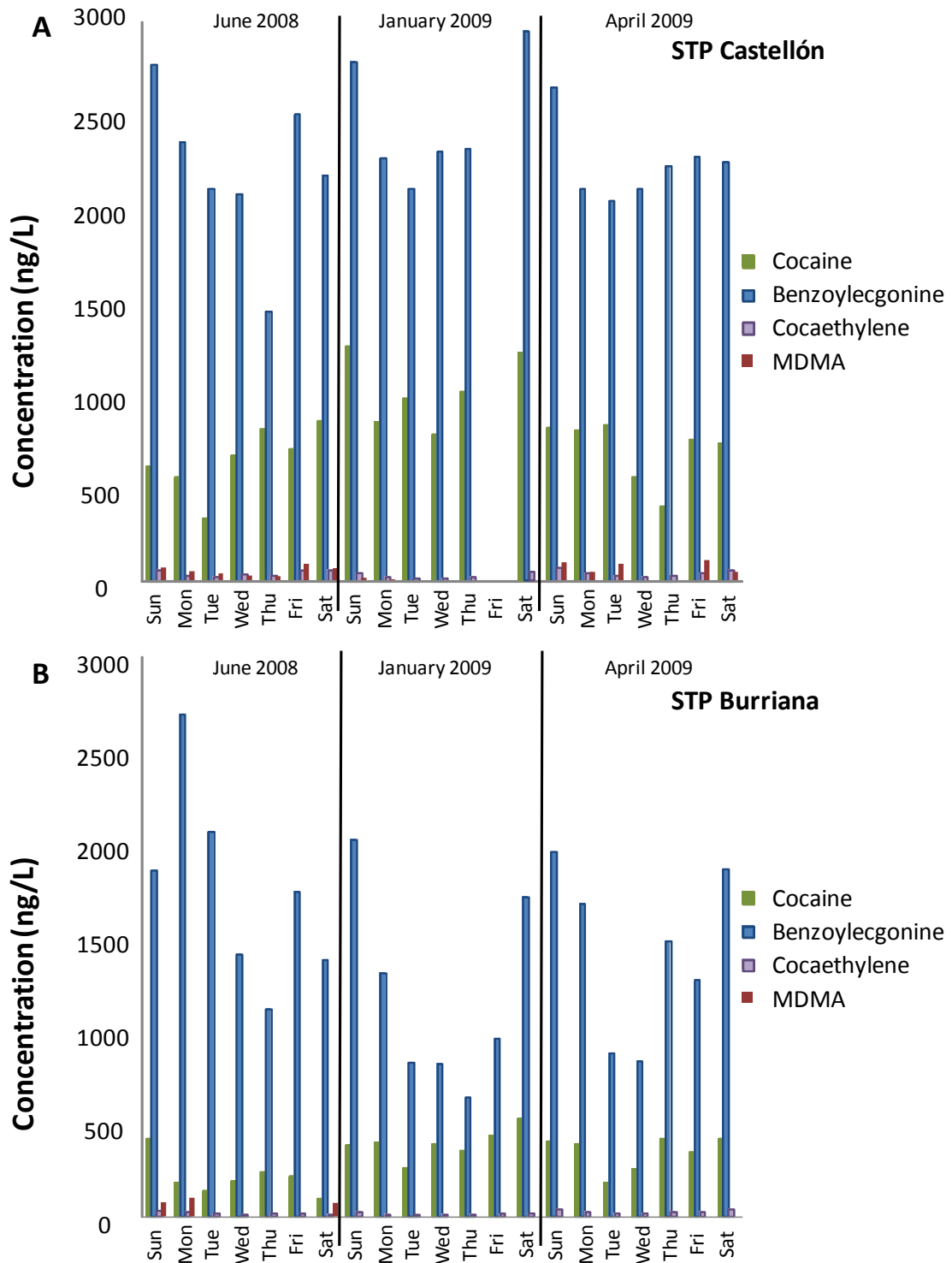
Figure 2. Weekly profile of concentrations (ng/L) of major illicit drugs and metabolites in influent sewage waters from the STP of Castellón and Burriana in June, January and April.

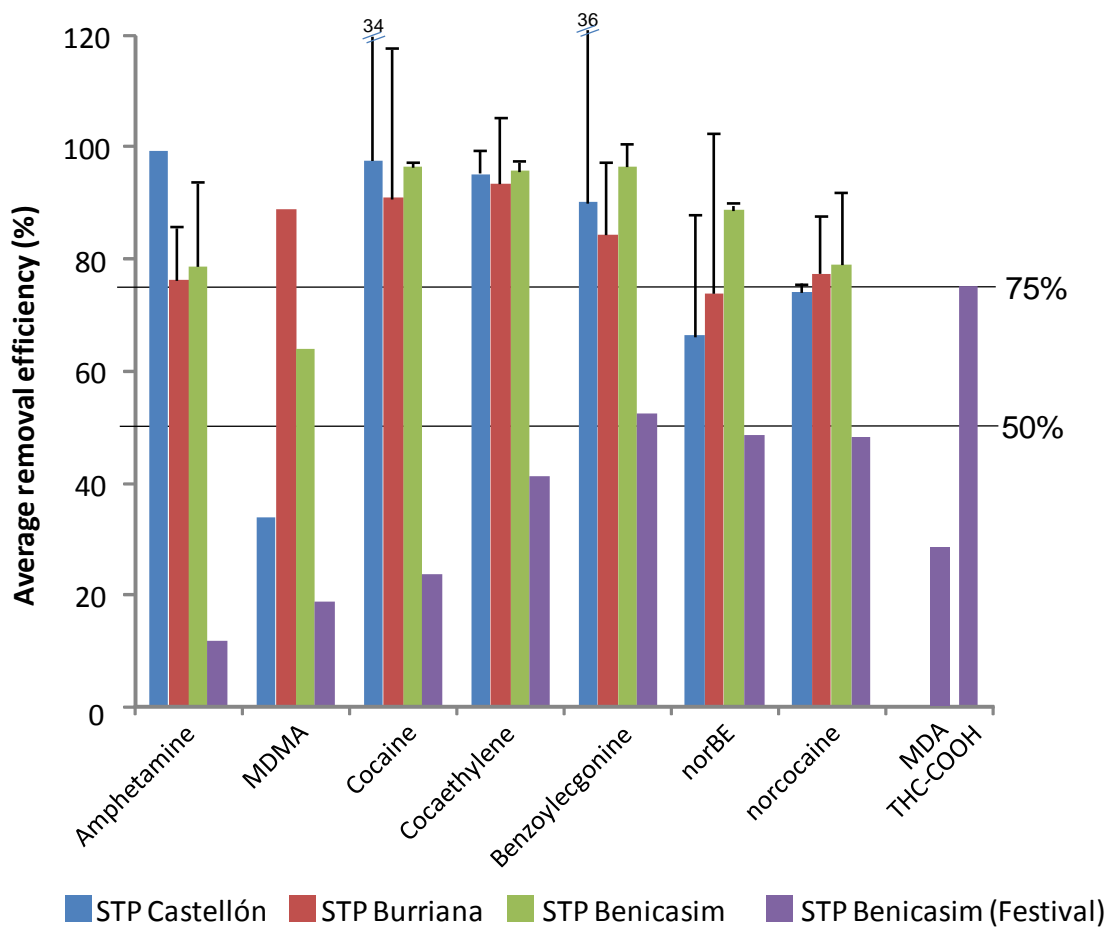**Figure 3.** Average removal efficiencies (%) for illicit drugs in different STPs.

FIGURE 1

FIGURE 2

FIGURE 3