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Occurrence and fate of illicit drugs and pharmaceuticals in wastewater from two wastewater treatment plants in Costa Rica



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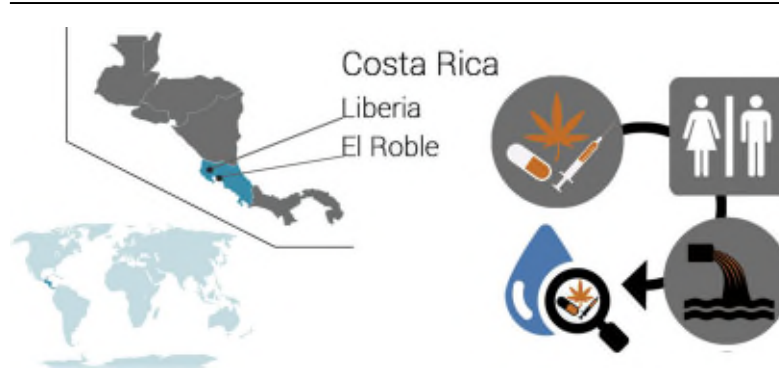
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HIGHLIGHTS

- Wastewater-based epidemiology first application in Central America
- Key parameters assessment to apply best practice protocol
- LC-HRMS analysis allowed target and suspect screening.
- Cocaine and cannabis were found to be the most consumed drugs in Costa Rica.
- Residues at low concentration were detected in surface water samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Chemical analysis of raw wastewater in order to assess the presence of biological markers entering a wastewater treatment plant can provide objective information about the health and lifestyle of the population connected to the sewer system. This work was performed in a tropical country of Central America, Costa Rica, with the aim of extending this knowledge to new world regions. This work is the first to report wastewater-based epidemiological data on the use of illicit drugs in this region of the world. Composite wastewater samples from the influents of two different wastewater treatment facilities and surface water samples from surrounding areas were collected applying the best practice protocol and analysed to investigate the occurrence and fate of selected illicit drugs of abuse and pharmaceuticals. Results showed the presence of chemical indicators of the classic drugs cocaine and cannabis at high concentration levels, besides the moderate presence of the opiates codeine and morphine. Neither the worldwide commonly used psychoactive substances of abuse such as synthetic phenethylamines, nor pharmaceuticals from the family of benzodiazepines were detected, demonstrating the spatial differences in drug use among different world regions. In addition, effluent wastewater samples were analysed and compared to influent concentrations in order to evaluate the decrease in concentration of the targeted analytes through two treatment technologies. As a final step, a wide-scope qualitative screening, including hundreds of suspect compounds, was applied in order to have a better knowledge on the presence of pharmaceuticals in waters and to assess the potential impact of the treated wastewater into the receiving aquatic ecosystems.

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1. Introduction

Chemical analysis of wastewater influents entering wastewater treatment plants (WWTPs) can provide valuable information on the health and lifestyle of the community connected to the sewer system (Daughton, 2001). In recent years several studies have been published showing the variety of licit and illicit substances which can be found in wastewater (Causanilles et al., 2016; Ort et al., 2014; Thomas et al., 2012). Loads of illicit drugs and pharmaceuticals are transported to municipal WWTPs where their concentrations can be determined by sophisticated analytical methodologies, and the results used to estimate drug use by the population. This approach, known as wastewater-based epidemiology, has allowed comparative studies between different urban centres around the world. Since the first European collaborative study in 2012 (Thomas et al., 2012), many other researchers have published their results, and the interest has been expanded to other world regions including America and Asia (Bijlsma et al., 2016; Devault et al., 2016; Du et al., 2015; Gatidou et al., 2016; Kim et al., 2015; Klupczynska et al., 2016; Lai et al., 2016). This type of studies that focus on the determination of drug residues in the raw sewage is now recognised as a complimentary tool in the assessment of drug prevalence (Amundsen and Reid, 2014; van Wel et al., 2016).

Besides this epidemiology point of view, there are also environmental implications, because the composition of raw sewage gives information that helps to decide what is the most appropriate treatment to remove or minimise those pollutants and prevent their discharge to the environment (Mara, 2003). Earlier studies in Europe (Bijlsma et al., 2012; Camacho-Muñoz et al., 2009) have shown this potential environmental threat that results from the fact that WWTPs are not specifically designed for removing illicit drug chemical indicators. Actually, some of these, such as 3,4-methylenedioxymethamphetamine (MDMA), are not removed at all by the treatment thus resulting in actual discharges into surface waters. In developing countries it is important that the treatment of municipal wastewater is efficient as much as sustainable, simple in its maintenance, and low in energy and chemicals usage (Mara, 2003). These considerations should be taken into account in order to implement the most appropriate treatment for the specific circumstances of the wastewater to be treated (which are not necessarily the same as in industrialized areas). Treatment processes can vary from wastewater stabilization ponds (WSP), where the wastewater flows at very low flow rate to enable removal by natural processes facilitated by sunlight and high temperatures during a determined residence time, to more technologically advanced systems, more appropriate when the land area required in natural systems is not available (Mara, 2003).

The study presented in this paper was performed at two locations of Costa Rica, in Central America. The goals were to investigate the presence of 15 selected chemical residues of illicit substances and pharmaceuticals in raw wastewater entering two WWTPs with different treatment technologies. The analytical information obtained was used in an attempt to apply the wastewater-based epidemiology approach for the first time to Costa Rican communities as well as to relate the loads observed to consumption by the population connected to the sewer system. Given the different treatment processes employed in the two WWTPs included in the study, their potential to reduce loads of illicit drugs and pharmaceuticals into the aquatic environment was assessed as well. Finally, since very few data are available for the occurrence of pharmaceuticals in the aquatic environment of Central America and in order to evaluate the potential impact of the wastewaters, a wide-scope qualitative screening was applied to influent and effluent wastewater as well as to surface water down and upstream from the effluent discharge point.

2. Materials and methods

2.1. Sampling sites

Samples were collected in two areas of Costa Rica, at two different WWTPs (see map in Fig. 1). The first WWTP sampled is located in the northwest of the country and serves the city of Liberia (capital of Guanacaste province). The treatment process consists of 4 WSP, connected in two series. Each pond measures 265 m long, 60 m wide and 2 m deep, containing up to 30,000 m³ of water. The average influent flow rate is 2,680 m³ per day and the hydraulic retention time varies from 24.3 days for the primary ponds to 31.5 days for the secondary ponds, being the total retention time of 55.8 days (Abarca Garbanzo, 2000). See a diagram of the system in Fig. S.1. The second WWTP sampled is located in the central west part of the country in the neighbourhood of El Roble and serves the city of Puntarenas. The process at this WWTP consists of primary settling and secondary treatment with Integrated Fixed-film Activated Sludge (IFAS). The influent wastewater is pumped to the entrance where it is separated into two parallel streams. The average influent flow rate is 6,650 m³ per day. The two WWTPs were selected for the study based on the difference in treatment technologies and to further investigate the presence of pharmaceuticals and illicit drug residues found in a preliminary collection of grab samples.

In addition, surface waters were collected at two rivers nearby: the Liberia and Tárcoles rivers. The Liberia river flows through the city of Liberia, receiving the effluent discharged from the WSPs. It is a small tributary that discharges into the Tempisque river on its way to the Gulf of Nicoya. The river Tempisque is 144 km long. The Tárcoles river originates on the southern slopes of the *Cordillera Central* volcanic range and flows in a south-westerly direction to the Gulf of Nicoya. The river is 111 km long and its watershed encompasses around 50% of the country's population based at the central valley. See Fig. 1 for information on the sampling locations.

2.2. Sewer system characterization

The two sewer systems were characterized by means of a standardized questionnaire developed by Ort and colleagues (Castiglioni et al., 2012). The information describes relevant catchment properties, such as number of inhabitants connected to the sewer system and its basis of the estimation, the type of sewer drains, whether exfiltration is expected or not, influent flow control and flow profile or variations. Wastewater chemical properties were also measured: pH, biochemical and chemical oxygen demand (BOD₅ and COD) and organic nitrogen content (Nitrogen-Kjeldahl) (Andreottola et al., 1994). A summary is presented in Table S.1. The collection of this meta-data is essential for the correct interpretation of data obtained and their normalization for further comparison.

2.3. Sample collection

Different types of samples were collected in order to address the different goals (see Table S.2 for details):

- 24-h composite influent wastewater samples at both WWTPs to relate the presence of residues of illegal drugs and pharmaceuticals to their consumption by the community included in the catchment area.
- Grab effluent wastewater samples to describe the efficiency of both treatment systems in removing those residues.
- Grab river surface water samples to investigate whether those residues can be observed in the aquatic environment.
- Pooled surface water samples at the different WSP of the system at Liberia to gain more knowledge on the fate of chemicals along this natural treatment process.



Fig. 1. Map of Costa Rica with detailed information on the location of the WWTPs and rivers included in the study (flow direction indicated by arrows <<).

For the collection of the influent 24-h composite wastewater samples (A) an ISCO 6712 portable sampler (Teledyne Isco, USA) equipped with a 12-bottle (1 L) rack and a solar panel as energy source was used. The selected operation mode was time-proportional, with a 15-minute sampling time interval of 50 mL aliquots. The 12 bottle rack was surrounded by ice cubes which assured that the sampled aliquots were directly cooled down during the 24-h cycle in order to minimise degradation. Grab effluent samples (B) were collected from the two parallel treatment lines at both WWTPs and pooled and mixed directly. To sample the river Liberia (C), two grab samples were collected 200 m upstream and downstream, respectively, from the WWTP effluent discharge point. A grab sample was taken at the river Tárcoles (C), the sampling point was located at the bridge by the coastal road 34 and 33 km on the way to the city of Jacó. To better represent each WSP (D), three samples were collected with the help of a 5-metre extension stick at the short sides of each pond (represented by a star in Fig. S.1) and then mixed into a pooled sample (represented by a blue circle, with sample number indicated inside, Fig. S.1). In total 8 pooled samples were thus collected, one representing each short side of the 4 ponds (see diagram in Fig. S.1).

2.4. Analytical methodology for quantitative analysis

Sample treatment and specific information on instrument operating conditions, both chromatographic and spectrometric, and on method validation have been described in detail elsewhere (Bijlsma et al., 2013). Details on dates of sampling, extraction, and analysis are provided in Table S.2. Briefly, 100 mL of sample were spiked with a mix of isotope labelled internal standards (IIS), vacuum filtered through 1 μm type A/E glass fibre filters, and then SPE extracted with Oasis HLB cartridges (150 mg, 6 cm^3). Two additional samples spiked at 360 ng L^{-1} served as quality control (estimating the recovery). Cartridges were stored

frozen at $-20\text{ }^\circ\text{C}$ and shipped to the laboratory at KWR Watercycle Research Institute, the Netherlands, for further sample treatment and analysis. Cartridges were eluted with methanol and extracts automatically evaporated under a gentle nitrogen stream with a Barkey Optocontrol (Germany). The final extract was reconstituted to 500 μL of 10% methanol aqueous solution.

A 20 μL sample extract aliquot was injected into a liquid chromatography coupled to a linear ion trap (LTQ) FT Orbitrap system (Thermo Electron, Bremen, Germany). Chromatographic separation of the compounds was achieved using a XBridge C18 (2.1 \times 150 mm; 3.5 μm particle size) column and an optimized gradient water:methanol, both with 0.05% formic acid. The mass spectrometer was equipped with a Heated Ion Max Electrospray Ionization (HESI) and operated in positive mode. Full-scan accurate mass spectra from m/z 100 to 600 Da were obtained at a resolution of 30,000 at full width half maximum (FWHM) (m/z 400), besides when an ion exceeded a pre-set threshold and corresponded to the target mass inclusion list specified by the user, the instrument switched to product-ion scan mode (MS^n) in the ion trap part with nominal mass measurements. In this way, relevant information for identification and confirmation, e.g., retention time, molecular weight and fragmentation, was obtained in a single analysis. All data were acquired and processed using the software Xcalibur version 2.1 (Thermo).

Mass calibration was performed prior to every batch run using a flow injection of Polytyrosine-1,3,6 solution ($[\text{M} + \text{H}]^+$ 182.01170/508.20783 and 997.39781) at a flow rate of 10 $\mu\text{L min}^{-1}$. Identification and quantification of the 15 target compounds was performed using the accurate mass of the protonated molecule within a mass window of 5 ppm. For confirmation of the identity of the compounds, in addition to the accurate mass of the precursor ion, at least one nominal mass product ion was used together with its retention time and its relative abundance, and was compared with that of the reference standards. See Supplementary material for further details (Tables S.3 and S.4).

2.5. Normalization of mass loads and estimation of drug use

Daily mass loads in mg day^{-1} 1000 inhabitants $^{-1}$ were calculated by multiplying the concentration in each sample (ng L^{-1}) by the corresponding wastewater flows (L day^{-1}) and normalizing the obtained values to 1000 inhabitants.

The normalized mass loads for benzoylecgonine (BE) and 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH) were then converted into an estimated pure drug level of consumption: in the case of cocaine, a correction factor of 3.59 was used in order to account for the BE/cocaine molar mass ratio (0.954) and the average molar fraction (29%) of a cocaine dose that is excreted as BE; in the case of cannabis, a correction factor of 182 was used taking into account 0.5% urinary excretion after smoking and the molecular weight ratio tetrahydrocannabinol (THC)/THC-COOH (Gracia-Lor et al., 2016).

2.6. Qualitative screening by UHPLC-QTOF-MS

The extracts from the pooled surface water samples collected at the WSP system in Liberia were re-analysed separately with a Waters Acquity UPLC system (Waters, Milford, MA, USA) interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters Micromass, Manchester, UK), using a Z-Spray ESI interface operating in both positive and negative ion mode. The chromatographic separation was performed using an Acquity UPLC BEH C18 1.7 μm particle size column 100×2.1 mm (Waters) at a flow rate of $300 \mu\text{L min}^{-1}$. The mobile phase used was water:methanol, both with 0.01% formic acid. Nitrogen was used as drying gas and nebulizing gas. The desolvation gas flow was set at $1,000 \text{ L h}^{-1}$ and the cone gas at 80 L h^{-1} . TOF-MS resolution was approximately 20,000 at FWHM at m/z 556.

MS data were acquired in centroid mode over an m/z range of 50–1,000 Da. Data were acquired in both positive and negative ionization modes in two separate runs. Capillary voltages of 0.7 kV and 2.5 kV were used in positive and negative ionizations modes, respectively. A cone voltage of 20 V was used. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The desolvation temperature was set to $600 \text{ }^\circ\text{C}$, and the source temperature to $130 \text{ }^\circ\text{C}$. The column temperature was set to $40 \text{ }^\circ\text{C}$.

For MS^E experiments, two sequential acquisition functions with different collision energies were created. The low energy (LE) function, selecting a collision energy of 4 eV, and the high energy (HE) function, with a collision energy ramp ranging from 15 to 40 eV in order to obtain a greater range of fragment ions. The scan time for both LE and HE function was 0.4 s.

Processing of MS data was done using ChromaLynx XS application manager (within MassLynx v 4.1; Waters Corporation). The following parameters were used for screening: mass window 150 ppm (for positive ID ≤ 5 ppm), isotope fit as well as retention time (maximum deviation of $\leq 2.5\%$) and fragmentation, when available. Software specific settings were: peak width at 5% height: 6 s, peak-to-peak baseline noise: 1,000 and threshold absolute area: 200.

A large screening of pesticides and transformation products, pharmaceuticals belonging to different therapeutic groups, veterinary drugs, X-ray agents, personal care products (PCPs) preservatives and UV filters, sweeteners, illicit drugs and a notable number of metabolites was performed. For this purpose, a homemade database with $> 1,500$ emerging contaminants was used.

The criteria used for communicating the identification level of the results were based on the following points:

- Detection, based on the presence of 1 accurate-mass ion (mass error ≤ 5 ppm) and retention time agreement (maximum deviation $\leq 2.5\%$).
- Confirmation of the identity, with at least 2 accurate-mass ions (≤ 5 ppm) and retention time ($\leq 2.5\%$).

- Tentative identification, with at least 2 accurate-mass ions justified by literature data and/or compatibility with candidate chemical structure.

The full details of the methodology applied can be found elsewhere (Hernández et al., 2015; Pitarch et al., 2016).

3. Results and discussion

3.1. Concentrations in influent wastewater

In this work, 15 target compounds were initially selected as indicators of the use of illicit and licit drugs of abuse. Compounds selected included cocaine, cannabis, opioids, some NPS and several benzodiazepines (see Table S.3). Results from the quantitative analysis of influent composite wastewater samples showed a relatively high concentration of metabolites from the classical illicit drugs cocaine and cannabis, and a moderate concentration of the chemical indicators from the opioids codeine and morphine. The same compounds were present in the influents of both studied WWTPs. None of the chemical indicators from other popular psychoactive substances such as the synthetic phenethylamines (namely amphetamine, MDMA, methamphetamine) or the pharmaceuticals from the family of benzodiazepines (namely oxazepam, temazepam, diazepam), or illicit drugs, such as heroine, were observed.

These results were in agreement with the Report on Drug Situation in Costa Rica that highlights cannabis as the most consumed drug in the country, closely followed by cocaine as the drug that has experienced a major increase in use (UNODC, 2013). A possible explanation is that the country is in the centre of the drug trafficking routes, with easy access to high purity drug at a reasonable price. The latest available prices reported by consumers in Costa Rica are 7 \$ per gram of marijuana and 17 \$ per gram of cocaine (Havocscope, 2016). Regarding the synthetic drugs prevalence, our results also agreed with data available, revealing that their use remains low and consumption is still a novel phenomenon. The use of amphetamine-type stimulants has been thus far mostly associated with music festivals and tourists coming from North America and Europe (UNODC, 2013).

Concentration data, expressed in ng L^{-1} , for each sample is shown in Table 1. Cocaine and its main human metabolite benzoylecgonine were found in the range of 525–1,050 and 2,280–3,520 ng L^{-1} in the influents of El Roble, and 763–2,710 and 2,100–4,500 ng L^{-1} in the influents of Liberia, respectively. According to human metabolism, the expected cocaine to benzoylecgonine ratio in raw wastewater should be 0.1 or lower (Castiglioni et al., 2012), in our study the ratios were 0.3 ± 0.1 and 0.5 ± 0.1 respectively, which is in line with other studies (Castiglioni et al., 2011; van Nuijs et al., 2012). The excretion ranges observed in the urine of single individuals in pharmacokinetic studies are variable (e.g. cocaine, range 1–14%), and since in wastewater the urine excreted by an entire community is collected, only a general comparison is possible (Castiglioni et al., 2011). Besides, in both sewer systems the longest travel distance from toilet to WWTP is no > 4 km, which decreases the possibility of cocaine degradation to BE in the sewer.

Cannabis use was reported based on the detection of two metabolites: THC-COOH and OH-THC. The human metabolism of cannabis varies as a function of its route of administration, and so the presence in wastewater of the two metabolites will vary depending on whether the THC has been consumed by inhalation (smoked) or by oral ingestion. OH-THC is present in much higher concentration after oral administration compared with inhalation, where it is excreted as a minor metabolite. THC-COOH has been selected as the main metabolite in many studies, because of its higher concentration after inhalation and because it has a longer half-life after both routes of administration (Grotenhermen, 2003; Schwilke et al., 2009). Other wastewater-based epidemiology studies which included both cannabis metabolites

Table 1
Concentrations in ng L⁻¹ of the illicit drug and pharmaceutical residues detected in the samples collected from two Costa Rican WWTPs.

Sample name	Sample type	Cocaine (ng L ⁻¹)	Benzoylcegonine (ng L ⁻¹)	THC-COOH (ng L ⁻¹)	OH-THC (ng L ⁻¹)	Codeine (ng L ⁻¹)	Morphine (ng L ⁻¹)
El Roble - Friday	WW influent 24-h	950	2280	124	69	462	71
El Roble - Saturday	WW influent 24-h	651	3520	187	192	460	72
El Roble - Sunday	WW influent 24-h	1050	3200	206	78	458	77
El Roble - Monday	WW influent 24-h	525	2700	158	47	538	75
El Roble - Tuesday	WW influent 24-h	709	2780	185	87	448	67
El Roble - Friday	WW effluent grab	29	340	10	20	503	15
El Roble - Tuesday	WW effluent grab	62	792	36	<LOD	665	61
Liberia - Wednesday	WW influent 24-h	1390	3410	259	101	278	77
Liberia - Thursday	WW influent 24-h	1210	3240	228	62	284	65
Liberia - Friday	WW influent 24-h	1730	2770	169	26	325	53
Liberia - Saturday	WW influent 24-h	1880	4500	502	65	363	62
Liberia - Sunday	WW influent 5-h	2710	4470	295	<LOD	143	16
Liberia - Monday	WW influent 5-h	763	2100	231	65	193	41
Liberia WSP	WW effluent grab	<LOD	1320	<LOD	44	11	<LOQ
Liberia WSP	WW effluent grab	<LOD	1260	35	102	29	<LOQ
Liberia WSP	WW effluent grab	<LOD	1450	37	200	20	<LOQ
Liberia WSP - 1.1	Pooled SW	<LOD	3440	127	75	230	33
Liberia WSP - 1.2	Pooled SW	<LOD	2900	119	71	203	30
Liberia WSP - 2.1	Pooled SW	<LOD	3280	114	458	252	29
Liberia WSP - 2.2	Pooled SW	<LOD	3030	128	<LOD	218	36
Liberia WSP - 3.1	Pooled SW	<LOD	1930	23	127	<LOQ	<LOD
Liberia WSP - 3.2	Pooled SW	<LOD	1740	<LOD	170	39	<LOQ
Liberia WSP - 4.1	Pooled SW	<LOD	763	29	133	19	<LOQ
Liberia WSP - 4.2	Pooled SW	<LOD	832	39	203	29	<LOD
River Liberia - upstream	SW grab	10	4	4	18	<LOD	<LOD
River Liberia - downstream	SW grab	<LOQ	88	<LOD	37	<LOQ	<LOD
River Tárcoles	SW grab	<LOQ	72	<LOD	86	10	<LOQ

WSP: wastewater stabilization ponds (see Fig. S.1).

WW: wastewater.

SW: surface water.

<LOD: below limit of detection.

<LOQ: below limit of quantification.

reported an insignificant amount of OH-THC (Postigo et al., 2011). It is important to highlight that in our study both cannabis metabolites were detected: in El Roble both metabolites were detected at a similar concentration range, whereas in Liberia the acid metabolite presented a higher concentration range than the hydroxyl. The cannabis biomarker more commonly used to estimate consumption, THC-COOH, was present in the range of 124–206 ng L⁻¹ in the influents of El Roble, and 169–502 ng L⁻¹ at Liberia; OH-THC was present in the range of 47–192 ng L⁻¹ in the influents of El Roble, and 26–101 ng L⁻¹ at Liberia. These results may indicate that the use of cannabis in the studied locations is not only smoked but also ingested (and the oral administration more prevalent in El Roble than in Liberia).

Finally, the opioids codeine and morphine, were found in the range of 448–538 and 67–77 ng L⁻¹ in the influents of El Roble, and 143–36 and 16–77 ng L⁻¹ at Liberia, respectively.

3.2. Normalization of mass loads and estimation of drug use

Several parameters are crucial in order to calculate the consumption of illicit drugs and pharmaceuticals using the wastewater-based epidemiology approach (Castiglioni et al., 2012). These include: (i) how representative is the average concentration in the composite sample collected (depending on the selected sampling mode), (ii) how accurate are the influent flow data and (iii) the population size estimation used for normalization, (iv) how much is known for each compound about its stability in the specific conditions of the studied sewage, and (v) what is its biotransformation rate, etc. In this preliminary study, which is the first attempt to apply wastewater-based epidemiology in Costa Rica, our efforts were concentrated in gathering the required information and applying the best possible practice protocol suggested by Castiglioni et al. (2012) in order to provide an estimate of drug use.

The biggest challenge and, at the same time, key step to ensure the reliability of the results was the sampling. According to Ort et al. (2010),

the most suitable sampling mode to account for the fluctuations in wastewater quantity or quality with time is flow-proportional. However, it requires a flow meter able to trigger the auto sampler, and such equipment was not available to us at the time of the study. As our best alternative option (Ort et al., 2010), a time-proportional mode with a high-frequency sampling interval of 15 min was selected at both locations to collect the 24-h composite samples. Cooling of the sampling equipment was done to minimise in-bottle degradation in the best possible practical way but because of the high local temperatures some degradation of relatively unstable compounds (e.g. cocaine) may have occurred at the end of every 24-h sampling cycle due to melting of the ice cubes used for cooling.

In the case of the WWTP at El Roble, the sewer system is pressurized and composed of several pumping stations that pool and direct the wastewater to the entrance of the WWTP depending on the water level. With this type of sewer, it is not so important to keep a high frequency sampling because each pulse will represent the sub-catchment rather than each individual toilet flush. However, it was noticeable that during early morning, midday and evening, the pump cycle was shorter than during the rest of the day. Therefore, high frequency sampling was applied to better represent those peak moments, concurring with mealtimes.

In the case of the WWTP at Liberia, the sewer system is gravity-drained, therefore a high frequency sampling is crucial to account for the expected wastewater pulses in a representative manner. The majority of the 24 h composite samples collected at the Liberia plant were indeed obtained by time-proportional sampling every 15 min. Unfortunately, the last two composite samples collected at Liberia only account for 5 h, since the auto sampler stopped due to heavy rainfall events. These two samples only represent the midday period (from 13 h to 18 h), and therefore were not used for back-calculation purposes.

Another challenge was the accurate measurement of the influent flow rate, which is key to back-calculate concentration in the composite

sample to daily loads. The WWTP at Roble had a flow meter installed at the entrance, so accurate and reliable data was easily acquired for each day (see Table S.5). The influent flow rate ranged from 7,351 to 8,669 m³ day⁻¹ during the 5 days of sampling. In the case of Liberia, the influent flow data was not available at the time of sampling. A flow meter was later installed at the entrance of the WSP, and we could access the continuous data recorded during 15 days in a similar dry period. The influent flow rate ranged from 2,140 to 2,900 m³ day⁻¹. This estimated range was selected and used for our normalization purposes (see Table S.6). To the best of our knowledge the range was stable during dry period, and although we are aware of the limitation, it was our best alternative option.

Another issue was the population size estimation used for normalization of daily loads. The information provided by WWTP operators was obtained from the census of house connections to the sewer. In order to check the reliability of that figure, the inhabitant equivalents (I.E.) from BOD₅ data were calculated. Two factors are important in the measurement of the wastewater quality when judged by its BOD₅: the water consumption and the amount of organic waste produced per person per day. In industrialized countries, water consumption is generally high (350–400 L person⁻¹ day⁻¹), which results in wastewater with BOD₅ levels of 200–250 mg L⁻¹ (Mara, 2003) and it is considered that 1 person represents 60 g O₂ per day (Ort et al., 2014). In developing countries BOD₅ levels in wastewater are higher, 300–700 mg L⁻¹, as the water consumption is typically much lower (40–100 L person⁻¹ day⁻¹), and it is considered that 1 person represents 40 g O₂ per day (Mara, 2003).

According to the information available at El Roble, the number of inhabitants whose households are connected to the sewer system was estimated at 35,758 (census year 2011). The I.E. calculation from BOD₅ resulted in a range of 41,550 ± 2,530, for an estimation of 40 g O₂ person⁻¹ day⁻¹, to 27,700 ± 1,690, for 60 g O₂ person⁻¹ day⁻¹. Considering that the census data fell in between the calculated I.E. range, it was selected as the most reliable figure for the normalization.

In the case of Liberia, the number of inhabitants whose households are connected to the sewer system was estimated at 14,215 (census year 2014). The I.E. calculation from BOD₅ resulted in a range of 21,170, for 40 g O₂ person⁻¹ day⁻¹ and 2,900 m³ flow, to 10,415 for 60 g O₂ person⁻¹ day⁻¹ and 2,140 m³ flow. Again the census data fell in between the calculated I.E. range, so it was selected as the most reliable figure for the normalization.

After the above considerations, daily mass loads in mg day⁻¹ 1000 inh⁻¹ were calculated as indicated in the materials and methods section. In the case of Liberia the results are presented as a range, corresponding to the normalization taking into account the minimum and the maximum daily flow respectively. Normalized mass loads are showed in Table 2. The highest mass loads corresponded to benzoylecgonine: El Roble presented an average of 650 ± 145 mg day⁻¹ 1000 inh⁻¹, and at Liberia it ranged from 524 ± 110 to 710 ± 149 mg day⁻¹ 1000 inh⁻¹. The second most abundant compound was cocaine, El Roble presented an average of 172 ± 45 mg day⁻¹ 1000 inh⁻¹, and at Liberia it ranged from 234 ± 46 to 317 ± 63 mg day⁻¹ 1000 inh⁻¹. Cannabis acid presented an average mass load in mg day⁻¹ 1000 inh⁻¹ of 38 ± 8 at El Roble and a range from 44 ± 22 to 59 ± 30 at Liberia, and OH-THC presented an average mass load in mg day⁻¹ 1000 inh⁻¹ of 22 ± 14 at El Roble and a range from 10 ± 5 to 13 ± 6 at Liberia. The opioid codeine presented an average mass load in mg day⁻¹ 1000 inh⁻¹ of 105 ± 11 at El Roble and a range from 47 ± 6 to 64 ± 8 at Liberia, whereas morphine presented an average mass load in mg day⁻¹ 1000 inh⁻¹ of 16 ± 1 at El Roble and a range from 10 ± 2 to 13 ± 2 at Liberia.

Normalized daily mass loads can be used as an indirect estimation of drugs use and for comparative purposes between different cities; in the particular case of cocaine and cannabis it was possible to estimate their consumption by back-calculation from the data

for their main human metabolites benzoylecgonine and THC-COOH, as indicated in materials and methods section.

The estimate of pure cocaine consumption at El Roble revealed an average of 2,390 ± 520 mg day⁻¹ 1000 inh⁻¹ (n = 5 days) and at Liberia, the estimation ranged between 1,880 ± 395 to 2,550 ± 536 mg day⁻¹ 1000 inh⁻¹ (n = 4 days). These results were remarkably high, but in agreement with recently reported measurements in South America that revealed an average of 3,022 mg day⁻¹ 1000 inh⁻¹ in Medellin, Colombia (Bijlsma et al., 2016). Notwithstanding the number of samples analysed was low, and the other limitations of this exploratory study discussed above, it seems that one can conclude that the level of consumption in two Costa Rican towns was above that found elsewhere in the world (Gatidou et al., 2016; Lai et al., 2016; Ort et al., 2014).

The estimate of pure cannabis consumption at El Roble revealed an average of 7,160 ± 1,460 mg day⁻¹ 1000 inh⁻¹ (n = 5 days) and at Liberia, the estimation ranged between 7,930 ± 4,020 to 10,700 ± 5,440 mg day⁻¹ 1000 inh⁻¹ (n = 4 days). These results were again remarkably high compared to other studies that we have found in literature that reported cannabis consumption figures, even though we used an updated correction factor (Bijlsma et al., 2016; Postigo et al., 2011). In addition, this result should be carefully considered since the chemical analysis of cannabis acid metabolite has several limitations due to its hydrophobic nature and therefore an incorrect load could lead to under-estimations.

These preliminary conclusions on the estimated consumption of cocaine and cannabis should be supported with additional and more detailed studies in the near future. Unfortunately, the statistical study of the distribution of the data could not be performed because the number of observations was not sufficient for the proper significance, however when plotting the data a weak increase in consumption during the weekend could be observed, representing the recreational use known as “weekend effect” (Figs. 2 and 3).

3.3. Reduction of drug concentrations from wastewater and discharge into the environment

It is known that WWTPs are an important source of organic pollutants in the environment because the removal rates of most technologies applied nowadays to treat wastewater are not completely efficient. The main concern is the potential impact that this inefficiency might have on the aquatic ecosystem after the discharge of the polluted effluents that usually contain emerging contaminants, such as pharmaceuticals and illicit drugs (Bijlsma et al., 2014; Gracia-Lor et al., 2012).

The removal rates of the WWTP can be evaluated by comparing the analyte concentrations in influent and their corresponding effluents. In the present study, a true comparison could not be made, since the hydraulic retention times at both WWTPs could not be taken into account when collecting the samples. Therefore, the comparison between average concentrations in influent and effluent was used as a rough estimation of the range of reduction rates, giving characteristic information on how efficient the treatment processes applied in the WWTPs seemed to be towards the detected illicit drugs and pharmaceuticals. The reduction potential was calculated as indicated below:

$$[1 - ((\text{mean concentration in effluent}) / (\text{mean concentration in influent}))] \cdot 100 (\%)$$

Concentrations in ng L⁻¹ of the individual samples are shown in Table 1, and the graphical summary of the potential decrease in the concentrations is presented in Fig. 4.

Differences per location and compound could be expected because of the different nature of the two treatment technologies applied: while the treatment at the WSPs of Liberia is for the most part anaerobic, and also includes photolytic degradation, the treatment

Table 2
Normalized mass loads of detected drugs expressed in $\text{mg day}^{-1} 1000 \text{ inh}^{-1}$.

		Load ($\text{mg day}^{-1} 1000 \text{ inh}^{-1}$)					
		Cocaine	Benzoylcegonine	THC-COOH	OH-THC	Codeine	Morphine
El Roble	Friday	195	469	26	14	95	15
	Saturday	158	853	45	46	111	17
	Sunday	236	721	46	18	103	17
	Monday	117	606	35	11	120	17
	Tuesday	153	600	40	19	97	14
Liberia	Wednesday	209–284	513–696	39–53	15–21	42–57	12–16
	Thursday	182–247	488–661	34–46	9–13	43–58	10–13
	Friday	260–353	417–565	25–34	4–5	49–66	8–11
	Saturday	283–384	677–918	76–102	10–13	55–74	9–13

at El Roble with IFAS is for the most part aerobic. As can be seen from Fig. 4, data from both WWTPs were in general agreement for cocaine and benzoylcegonine with reduction efficiencies ranging between 60 and 100%. In the case of cocaine such a complete removal agrees with results of previous studies that categorize this compound as very unstable in wastewater (McCall et al., 2016). In the WSP of Liberia, cocaine was detected in high concentration in the influent but not detected in any of the pooled surface water nor the effluent samples. This could be explained by its rapid transformation into BE, but also by the higher matrix effects affecting this type of sample (with higher LOQ, see Table S.4). In the samples collected at the Liberia river (where a lower LOQ was obtained due to reduced matrix effects) a dilution can be observed comparing upstream to downstream. In samples from both rivers (Liberia and Tarcoles) the residual concentration of cocaine was detectable but below LOQ. At the WWTP of El Roble, cocaine was detected in the effluent, although at lower concentration compared to the influent. The incomplete removal could be explained by the shorter retention time in this system and also because the transformation of cocaine to its metabolites has been identified to occur at higher rate under anaerobic conditions (Ramin et al., 2016) (while treatment at El Roble is mostly aerobic). In the case of benzoylcegonine, the relatively high concentration of cocaine in the influents at both locations may have caused its higher residual concentration. Besides, BE has previously been categorized as highly stable (McCall et al., 2016). The higher reduction at El Roble can be explained because BE has shown higher transformation in an aerobic system (as opposite to cocaine) (Ramin et al., 2016). Liberia river concentration of BE was higher downstream than upstream, and concentration downstream was similar in both rivers sampled. This highlights how persistent this compound is in the aquatic environment, not getting fully eliminated by the studied wastewater treatments, and therefore discharged into the aquatic environment. This finding is in contrast with some other reports (Bijlsma et al., 2012) where elimination is more complete probably due to more sophisticated treatment.

Cannabinoids and opioids are excreted as glucuronide metabolites and hydrolyzed/deconjugated in wastewater to the parent metabolite. For THC-COOH relatively high reduction rates were observed in both WWTPs, approx. to 90%, and non-detected in river samples. This was in good agreement with other studies in which it was also removed at high rates independently of the WWTP under study (Bijlsma et al., 2012). THC-OH was moderately reduced at El Roble (80%) whereas in the WSPs of Liberia the effluent contained higher levels than the influent. Less pharmacokinetic information about levels in urine is available for this biomarker; THC-COOH and its glucuronide version are considered the main metabolites in urine. Glucuronide bonds in the THC-OH conjugate are believed to be more stable than glucuronide bonds in the THC-COOH conjugate (Schwilke et al., 2009). This might result in the different cleavage rates of the conjugated molecules present in the influents. A possible explanation is that at El Roble there is not enough time for the complete deconjugation to happen due to the relatively short residence time of the water and therefore this is translated as a good removal of non-conjugated THC-OH, while the glucuronide is present and stable and therefore we are not measuring it. In contrast in Liberia, with a long residence time of the water, there might be enough time for the THC-OH-glucuronide to deconjugate stimulated by the natural process and high temperatures.

For codeine we observed an almost complete reduction in the WSP of Liberia, whereas in the WWTP of El Roble no reduction was observed and the compound appeared to be persistent throughout with an even somewhat higher concentration in the effluent than in the influent. McCall et al. (2016) reported the stability of morphine to be variable, whereas the morphine conjugates rapidly deconjugate. And codeine was reported to be stable in wastewater. This could explain our observations in the current study: in the Liberia system there is sufficient time for both MOR and COD to become deconjugated and subsequently removed (either by biodegradation or sorption). For the El Roble plant, with an estimated residence time of the wastewater of less than 1d,

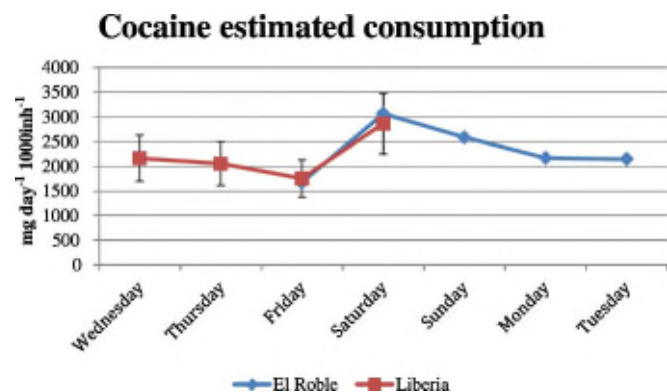


Fig. 2. Cocaine consumption in $\text{mg day}^{-1} 1000 \text{ inhabitants}^{-1}$, calculated from benzoylcegonine normalized loads in the influents of both studied locations using a correction factor of 3.59 (see text). Error bars for the results obtained from Liberia WSP correspond to the range in flow data.

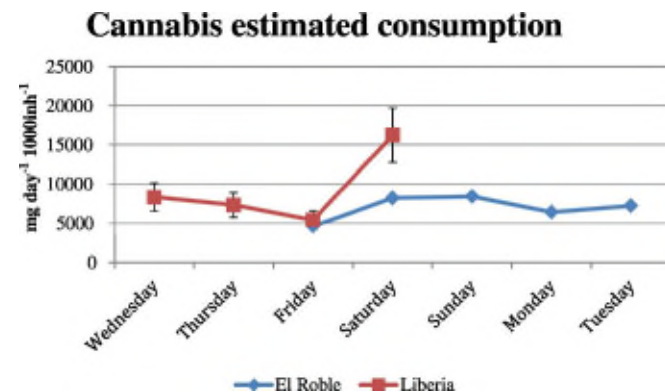


Fig. 3. Cannabis consumption in $\text{mg day}^{-1} 1000 \text{ inhabitants}^{-1}$, calculated from THC-COOH normalized loads in the influents of both studied locations using a correction factor of 182. Error bars for the results obtained from Liberia WSP correspond to the range in flow data.

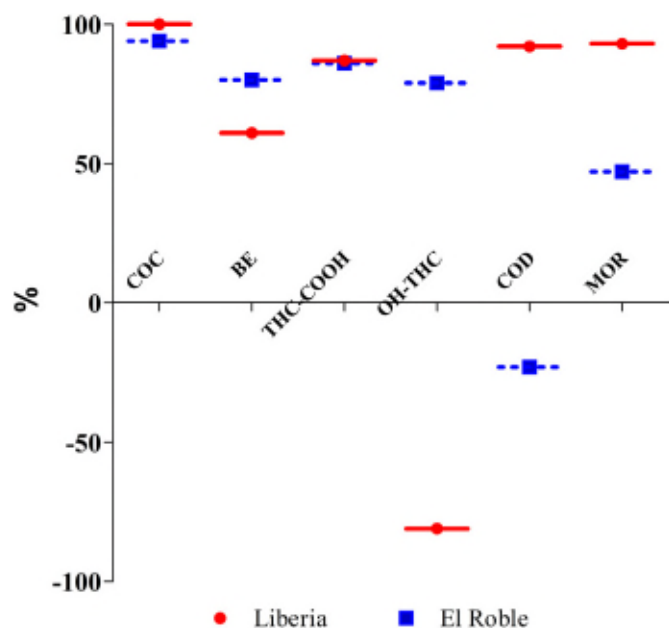


Fig. 4. Reduction potential (expressed in %) calculated for each compound from its mean concentrations in influent and effluent at both WWTPs. Positive values represent higher mean concentration in the influent than in the effluent, whereas negative values represent lower mean concentration in the influent than in the effluent. COC: cocaine; BE: benzoylecgonine; THC-COOH: carboxyl-THC; OH-THC: hydroxyl-THC; COD: codeine; MOR: morphine.

we observed a much reduced removal rate that could be explained by concomitant deconjugation of the glucuronides. The short residence time would not allow the formed deconjugated species to be transformed because of slow subsequent transformation or because the time is too short for the sorption equilibrium for COD and MOR to be reached.

To sum up, the residual low ng L^{-1} concentrations in river samples of benzoylecgonine, OH-THC, and codeine highlighted their stable and persistent behaviour in the aquatic environment, and additional research is required to investigate their possible effect on the ecosystem (Mastroianni et al., 2016).

3.4. Qualitative screening with UPLC-QTOF-MS

A subsequent step of this work consisted on the application of a wide-scope screening in order to have a comprehensive overview on the presence of pharmaceuticals in the aquatic environment. The objective of these analyses was to widen the scope of the target analytical methodology applied, and to detect and identify other emerging contaminants in the samples, including mainly pharmaceuticals and personal care products. Thus, the potential impact of effluent wastewaters on the surrounding areas could be evaluated. Results from the qualitative screening performed on the pooled samples collected at the different ponds of the WSP system at Liberia are shown in Table 3. From the 1,500 compounds included in the suspect list a relatively small amount of compounds could be identified. It should be noted that compounds not included in the suspect list might well be present in the ponds, although not identified because

Table 3

Compounds identified by UHPLC-QTOF-MS in different types of water samples: 1 influent and 1 effluent wastewater, 8 pooled surface waters from WSP Liberia (see Fig. S.1.), and 2 surface waters from the river Liberia: approx. 200 m upstream and downstream from the effluent discharge point.

Substance category	Compound ^a	River upstream	Influent	1.1	1.2	2.1	2.2	3.1	3.2	4.1	4.2	Effluent	River downstream	
Pharmaceuticals and metabolites	Acetaminophen	d	√	d	d	√	d						d	
	Atenolol	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	
	Cefotaxime		√	√	√	√	√							
	Furosemide (–)		√			(*)	(*)							
	Gemfibrozil (–)		√	√	√	(*)	(*)	√	√	√	√	√	√	
	Irbesartan	√	√	√	√	√	√	√	√	√	√	√	√	
	Naproxen	d	√	√	√	√	√	√	√	√	√	√	√	
	Sulfapyridine		√	√	√	√	√	d	d	d	d	√	d	
	Sulfamethoxazole	√	√	√	√	√	√	√	√	√	√	√	√	√
	Telmisartan	d	√	√	√	√	√	√	√	√	√	√	√	
	Salicylic acid (–)		√			(*)	(*)					d		
	Valsartan		√	√		√	√	√	√	√	√	√	√	
	OM10 ⁽¹⁾		⊕									⊕		
	ISW1b ⁽²⁾		⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	
	IbB4 (–) ⁽³⁾	⊕	⊕	⊕	⊕	(*)	(*)	⊕	⊕	⊕	⊕	⊕	⊕	
	IB5 ⁽⁴⁾		⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	
	4-Acetyl aminoantipyrine ⁽⁵⁾		√		√	√	√	√	√	√	√	√	√	√
	4-Formyl aminoantipyrine ⁽⁶⁾		√		√	√	√	√	√	√	√	√	√	√
	Clopidogrel carboxylic acid		√	√	√	√	√	√	√	√	√	√	√	√
Stimulant substance	Caffeine	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	
Sweeteners	Acesulfame (–)		d	√	√	(*)	(*)	√	√	√	√	√	√	
	Saccharin (–)	⊕	⊕	⊕	⊕	(*)	(*)	⊕	⊕	⊕	⊕	⊕	⊕	
	Sucralose (–)	√	√	√	√	(*)	(*)	√	√	√	√	√	√	
UV-filter	Benzophenone-3		√								√			
Pesticides	Imidacloprid		√		d					d	d			
	N,N-Diethyl-3-methylbenzamide (DEET)	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	

Sample codes: primary pond N°1: samples 1.1 and 1.2; primary pond N°2: samples 2.1 and 2.2; secondary pond N°3: samples 3.1 and 3.2; secondary pond N°4: samples 4.1 and 4.2. See Fig. S.1.

(d) detected, not confirmed (1 accurate-mass ion <5 ppm + retention time <2.5%).

(√) confirmed with at least two accurate-mass ions (<5 ppm) and retention time (<2.5%) with reference standard.

(⊕) tentatively identified (at least two accurate-mass ions justified by literature data and/or compatible with the candidate chemical structure).

^a A (–) sign denotes analysed in negative mode.

* Samples 2.1 and 2.2 were only injected in ESI+ mode as not enough extract was available.

¹ Elucidated metabolite of omeprazole (Boix et al., 2014).

² Elucidated metabolite of irbesartan (Boix et al., 2016).

³ Elucidated metabolite of ibuprofen (Boix et al., 2016).

⁴ Elucidated metabolite of irbesartan (Boix et al., 2016).

⁵ 4-AAA, metabolite of metamizole or dipyrone.

⁶ 4-FAA, metabolite of metamizole or dipyrone.

they were not searched for. Similarly, compounds included in the suspect list but present at very low concentrations could be missed.

From the identified compounds, three groups could be differentiated:

- (1) Persistent compounds, detected in all samples from the river upstream, also in the influent and in the ponds, to the effluent and the river downstream.
- (2) Compounds present in the influent, in the ponds, the effluent and the river downstream, but that were not present in the river upstream; therefore introduced into the aquatic environment by the inefficient treatment.
- (3) Compounds present in the influent but not present in the river downstream; therefore reduced or even removed by the treatment system.

Pharmaceuticals and their metabolites were most frequently detected. Some have been already identified as persistent in the environment in other studies. For example acetaminophen, atenolol and ibuprofen were found to be relatively resistant to photodegradation (Yamamoto et al., 2009) and in the current study they were found to be present in all samples. In general, studies that focused on the biodegradability and transformation of human pharmaceutical active ingredients in the aquatic environment have resulted in the finding of transformation products with a considerably longer half-life DT50 compared to the parent compound (Berkner and Thierbach, 2014). This highlighted the need of including transformation products in the present assessment.

The stimulant substance caffeine was also identified in all samples, which corresponds to previously reported findings in which caffeine has been identified as a distinct indicator of anthropogenic influence (Chen et al., 2002) due to its persistence. The environmental impact of artificial sweeteners has been recently evaluated, since they are newly recognised as persistent and ubiquitous in various aquatic ecosystems (Sang et al., 2014). In the WSPs of Liberia, saccharin and sucralose were identified in the different ponds as well as in river samples and therefore categorized as persistent (group 1). Acesulfame was categorized in group 3 as it was not detected in the river samples. However, photodegradation studies showed that under prolonged exposure to intensive solar irradiation (which would be the case in the WSPs of Liberia) photodegradation products of acesulfame at least six times more persistent than the parent compound were formed (Sang et al., 2014). The UV-filter benzophenone-3 was identified in the influent and effluent. Concern about its environmental persistence may still exist since earlier studies have identified photolysis products despite its categorization as photostable (Liu et al., 2011). The pesticide imidacloprid was identified in the influent and in three ponds only. The intermittent presence could be explained by its concentration level being close to the limit of detection. Finally, the insect repellent *N,N*-Diethyl-3-methylbenzamide (DEET) was tentatively identified in all samples. This is in agreement with its reported incomplete removal from wastewater (Aronson et al., 2012).

4. Conclusions

Chemical analysis of wastewater performed in the present study has revealed the presence of residues of illicit drugs and pharmaceuticals in the influents of two WWTPs in Costa Rica. Results showed high concentration levels of chemical indicators of the classic drugs cocaine and cannabis, besides the moderate presence of the opiates codeine and morphine. These findings support the established drug use pattern described by classical epidemiological tools. It is noteworthy the absence of synthetic phenethylamines such as amphetamine, methamphetamine and MDMA (ecstasy), which are commonly found in wastewaters collected in other regions of the world; as well as the absence of benzodiazepines and other drugs such as heroine. In order to obtain reliable loads and estimated consumption, an assessment to apply the best practice protocol was performed.

The analysis of effluents revealed the incomplete removal of some of the compounds in the two different treatment processes applied in the WWTPs investigated in the study. Additional qualitative screening of a large number of pharmaceuticals allowed to conclude that several compounds were present in the river samples of the surrounding areas, thus showing that WWTP discharges lead to appreciable levels of emerging contaminants in fluvial waters in Costa Rica, the possible impacts of which needs further elucidation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2017.04.202>.

References

- Abarca Garbanzo, J.A., 2000. Evaluación fisicoquímica y microbiológica del sistema de lagunas facultivas para el tratamiento de las aguas residuales de la ciudad de Liberia, Guanacaste. Informe de Proyecto para optar por el grado de Bachiller en Ingeniería en Biotecnología, Cartago.
- Amundsen, E.J., Reid, M.J., 2014. Self-reports of consumption of amphetamines, cocaine and heroin in a survey among marginalized drug users. *Sci. Total Environ.* 487, 740–745.
- Andreottola, G.B.L., Poggiali, L., Zaffaroni, C., 1994. A methodology for the estimation of unit nutrient and organic loads from domestic and non-domestic sources. *Eur. Water Pollut. Contr.* 4, 13–19.
- Aronson, D., Weeks, J., Meylan, B., Guiney, P.D., Howard, P.H., 2012. Environmental release, environmental concentrations, and ecological risk of *N,N*-diethyl-*m*-toluamide (DEET). *Integr. Environ. Assess. Manag.* 8 (1), 135–166.
- Berkner, S., Thierbach, C., 2014. Biodegradability and transformation of human pharmaceutical active ingredients in environmentally relevant test systems. *Environ. Sci. Pollut. Res. Int.* 21 (16), 9461–9467.
- Bijlsma, L., Emke, E., Hernández, F., de Voogt, P., 2012. Investigation of drugs of abuse and relevant metabolites in Dutch sewage water by liquid chromatography coupled to high resolution mass spectrometry. *Chemosphere* 89 (11), 1399–1406.
- Bijlsma, L., Emke, E., Hernández, F., de Voogt, P., 2013. Performance of the linear ion trap Orbitrap mass analyzer for qualitative and quantitative analysis of drugs of abuse and relevant metabolites in sewage water. *Anal. Chim. Acta* 768 (0), 102–110.
- Bijlsma, L., Serrano, R., Ferrer, C., Tormos, I., Hernández, F., 2014. Occurrence and behavior of illicit drugs and metabolites in sewage water from the Spanish Mediterranean coast (Valencia region). *Sci. Total Environ.* 487, 703–709.
- Bijlsma, L., Botero-Coy, A.M., Rincón, R.J., Peñuela, G.A., Hernández, F., 2016. Estimation of illicit drug use in the main cities of Colombia by means of urban wastewater analysis. *Sci. Total Environ.* 565, 984–993.
- Boix, C., Ibáñez, M., Zamora, T., Sancho, J.V., Niessen, W.M.A., Hernández, F., 2014. Identification of new omeprazole metabolites in wastewaters and surface waters. *Sci. Total Environ.* 468–469, 706–714.
- Boix, C., Ibáñez, M., Sancho, J.V., Parsons, J.R., Voogt, P.d., Hernández, F., 2016. Biotransformation of pharmaceuticals in surface water and during waste water treatment: identification and occurrence of transformation products. *J. Hazard. Mater.* 302, 175–187.

- Camacho-Muñoz, D., Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2009. An affordable method for the simultaneous determination of the most studied pharmaceutical compounds as wastewater and surface water pollutants. *J. Sep. Sci.* 32 (18), 3064–3073.
- Castiglioni, S., Bagnati, R., Melis, M., Panawennage, D., Chiarelli, P., Fanelli, R., Zuccato, E., 2011. Identification of cocaine and its metabolites in urban wastewater and comparison with the human excretion profile in urine. *Water Res.* 45 (16), 5141–5150.
- Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernández, F., Reid, M., Ort, C., Thomas, K.V., van Nuijs, A.L.N., de Voogt, P., Zuccato, E., 2012. Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. *Environ. Sci. Technol.* 47 (3), 1452–1460.
- Causanilles, A., Emke, E., de Voogt, P., 2016. Determination of phosphodiesterase type V inhibitors in wastewater by direct injection followed by liquid chromatography coupled to tandem mass spectrometry. *Sci. Total Environ.* 565, 140–147.
- Chen, Z., Pavelic, P., Dillon, P., Naidu, R., 2002. Determination of caffeine as a tracer of sewage effluent in natural waters by on-line solid-phase extraction and liquid chromatography with diode-array detection. *Water Res.* 36 (19), 4830–4838.
- Daughton, C.G., 2001. In: Daughton, C.G., Jones-Lepp, T. (Eds.), *Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues*. ACS Symposium Series 791. American Chemical Society, Washington, D.C., pp. 348–364.
- Devault, D.A., Néfau, T., Levi, Y., Karolak, S., 2016. The removal of illicit drugs and morphine in two waste water treatment plants (WWTPs) under tropical conditions. *Environ. Sci. Pollut. Res.* 1–11.
- Du, P., Li, K., Li, J., Xu, Z., Fu, X., Yang, J., Zhang, H., Li, X., 2015. Methamphetamine and ketamine use in major Chinese cities, a nationwide reconnaissance through sewage-based epidemiology. *Water Res.* 84, 76–84.
- Gatidou, G., Kinyua, J., van Nuijs, A.L.N., Gracia-Lor, E., Castiglioni, S., Covaci, A., Stasinakis, A.S., 2016. Drugs of abuse and alcohol consumption among different groups of population on the Greek Island of Lesbos through sewage-based epidemiology. *Sci. Total Environ.* 563–564, 633–640.
- Gracia-Lor, E., Sancho, J.V., Serrano, R., Hernández, F., 2012. Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. *Chemosphere* 87 (5), 453–462.
- Gracia-Lor, E., Zuccato, E., Castiglioni, S., 2016. Refining correction factors for back-calculation of illicit drug use. *Sci. Total Environ.* 573, 1648–1659.
- Grotenhermen, F., 2003. Pharmacokinetics and pharmacodynamics of cannabinoids. *Clin. Pharmacokinet.* 42 (4), 327–360.
- Havocscope, 2016. Global Black Market Information. Accessed on 21/12/2016 from <http://www.havocscope.com/tag/costa-rica/>.
- Hernández, F., Ibáñez, M., Botero-Coy, A.-M., Bade, R., Bustos-López, M.C., Rincón, J., Moncayo, A., Bijlsma, L., 2015. LC-QTOF MS screening of more than 1,000 licit and illicit drugs and their metabolites in wastewater and surface waters from the area of Bogotá, Colombia. *Anal. Bioanal. Chem.* 407 (21), 6405–6416.
- Kim, K.Y., Lai, F.Y., Kim, H.-Y., Thai, P.K., Mueller, J.F., Oh, J.-E., 2015. The first application of wastewater-based drug epidemiology in five South Korean cities. *Sci. Total Environ.* 524–525, 440–446.
- Klupczynska, A., Dereziński, P., Krysztofiak, J., Kokot, Z.J., 2016. Estimation of drug abuse in 9 Polish cities by wastewater analysis. *Forensic Sci. Int.* 260, 14–21.
- Lai, F.Y., O'Brien, J., Bruno, R., Hall, W., Prichard, J., Kirkbride, P., Gartner, C., Thai, P., Carter, S., Lloyd, B., Burns, L., Mueller, J., 2016. Spatial variations in the consumption of illicit stimulant drugs across Australia: a nationwide application of wastewater-based epidemiology. *Sci. Total Environ.* 568, 810–818.
- Liu, Y.-S., Ying, G.-G., Shareef, A., Kookana, R.S., 2011. Photostability of the UV filter benzophenone-3 and its effect on the photodegradation of benzotriazole in water. *Environ. Chem.* 8 (6), 581–588.
- Mara, D., 2003. *Domestic Wastewater Treatment in Developing Countries*. Earthscan, London · Sterling, VA.
- Mastroianni, N., Bleda, M.J., López de Alda, M., Barceló, D., 2016. Occurrence of drugs of abuse in surface water from four Spanish river basins: spatial and temporal variations and environmental risk assessment. *J. Hazard. Mater.* 316, 134–142.
- McCall, A.-K., Bade, R., Kinyua, J., Lai, F.Y., Thai, P.K., Covaci, A., Bijlsma, L., van Nuijs, A.L.N., Ort, C., 2016. Critical review on the stability of illicit drugs in sewers and wastewater samples. *Water Res.* 88, 933–947.
- van Nuijs, A.L.N., Abdellati, K., Bervoets, L., Blust, R., Jorens, P.G., Neels, H., Covaci, A., 2012. The stability of illicit drugs and metabolites in wastewater, an important issue for sewage epidemiology? *J. Hazard. Mater.* 239–240 (0), 19–23.
- Ort, C., Lawrence, M.G., Rieckermann, J., Joss, A., 2010. Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review. *Environ. Sci. Technol.* 44 (16), 6024–6035.
- Ort, C., van Nuijs, A.L.N., Berset, J.-D., Bijlsma, L., Castiglioni, S., Covaci, A., de Voogt, P., Emke, E., Fatta-Kassinos, D., Griffiths, P., Hernández, F., González-Mariño, I., Grabic, R., Kasprzyk-Hordern, B., Mastroianni, N., Meierjohann, A., Nefau, T., Östman, M., Pico, Y., Racamonde, I., Reid, M., Slobodnik, J., Terzic, S., Thomaidis, N., Thomas, K.V., 2014. Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis. *Addiction* 109 (8), 1338–1352.
- Pitarch, E., Cervera, M.I., Portolés, T., Ibáñez, M., Barreda, M., Renau-Pruñonosa, A., Morell, I., López, F., Albarrán, F., Hernández, F., 2016. Comprehensive monitoring of organic micro-pollutants in surface and groundwater in the surrounding of a solid-waste treatment plant of Castellón, Spain. *Sci. Total Environ.* 548–549, 211–220.
- Postigo, C., de Alda, M.L., Barceló, D., 2011. Evaluation of drugs of abuse use and trends in a prison through wastewater analysis. *Environ. Int.* 37 (1), 49–55.
- Ramin, P., Libonati Brock, A., Polesel, F., Causanilles, A., Emke, E., de Voogt, P., Plosz, B.G., 2016. Transformation and sorption of illicit drug biomarkers in sewer systems: understanding the role of suspended solids in raw wastewater. *Environ. Sci. Technol.* 50 (24), 13397–13408.
- Sang, Z., Jiang, Y., Tsoi, Y.-K., Leung, K.S.-Y., 2014. Evaluating the environmental impact of artificial sweeteners: a study of their distributions, photodegradation and toxicities. *Water Res.* 52, 260–274.
- Schwilke, E.W., Schwoppe, D.M., Karschner, E.L., Lowe, R.H., Darwin, W.D., Kelly, D.L., Goodwin, R.S., Gorelick, D.A., Huestis, M.A., 2009. Δ^9 -tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC plasma pharmacokinetics during and after continuous high-dose oral THC. *Clin. Chem.* 55 (12), 2180–2189.
- Thomas, K.V., Bijlsma, L., Castiglioni, S., Covaci, A., Emke, E., Grabic, R., Hernández, F., Karolak, S., Kasprzyk-Hordern, B., Lindberg, R.H., Lopez de Alda, M., Meierjohann, A., Ort, C., Pico, Y., Quintana, J.B., Reid, M., Rieckermann, J., Terzic, S., van Nuijs, A.L.N., de Voogt, P., 2012. Comparing illicit drug use in 19 European cities through sewage analysis. *Sci. Total Environ.* 432 (0), 432–439.
- UNODC, 2013. Reporte de situación: Tráfico de Drogas y Amenazas del Crimen Organizado en Costa Rica.
- van Wel, J.H.P., Kinyua, J., van Nuijs, A.L.N., Salvatore, S., Bramness, J.G., Covaci, A., Van Hal, G., 2016. A comparison between wastewater-based drug data and an illicit drug use survey in a selected community. *Int. J. Drug Policy* 34, 20–26.
- Yamamoto, H., Nakamura, Y., Moriguchi, S., Nakamura, Y., Honda, Y., Tamura, I., Hirata, Y., Hayashi, A., Sekizawa, J., 2009. Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: laboratory photolysis, biodegradation, and sorption experiments. *Water Res.* 43 (2), 351–362.