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Illicit Drugs in Wastewater Treatment Plants A case study: Rio de Janeiro, Brazil

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KEYWORDS

Wastewater-Based Epidemiology;
Drug consumption;
Occurrence;
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ABSTRACT: Intake of illicit drugs should be expressed not merely for the reason of the public health aspects but also in an environmental context concerning the contamination of surface waters. Wastewater-based epidemiology consists in acquiring relevant information about the standard of living and in population health status athwart the investigation of wastewater samples collected at the influent of a wastewater treatment plant (WWTP). This method has been applied to the examination on samples from 4 WWTPs situated in Rio de Janeiro Municipality, Brazil, to investigate the presence of illicit drugs and their metabolites. These included cocaine (COC), benzoylecgonine (BE, cocaine metabolite), amphetamine (AMP), methamphetamine (METH), and 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH, THC metabolite). Concentrations of COC and its main metabolite BE ranged from 201.3 to 2751.5 ng/L and from 630.7 to 5849.2 ng/L, respectively. Amphetamine-like stimulants ranged from 21.7 to 110.0 ng/L for AMP, and from 55.3 ng/L to 477.4 ng/L for METH. THC-COOH ranged from 188.8 to 940.2ng/L. The concentrations found, besides being noteworthy to public health, may likely have important repercussion influence at the functioning of the environment. It is important to detach that COC and amphetamines (including metabolites as well) have potent pharmacological activities and their incidence as multifarious assortments in the ecosystem must hurt aquatic organisms and, consequently, in human health. However, unfortunately, there is no current regulation demanding the determination of the occurrence of these pollutants at the environment. In conclusion, investigates on the spreading configuration of these illicit drugs and their potentially harmful impact on our environment needs immediate attention and regulatory limits.

INTRODUCTION

The illicit drug is a worldwide problem with significant direct or indirect adverse effects on human health and social welfare [1]. They fall into the categories of opioids, cocaine, cannabis, amphetamine-type substances (ATSs) [2]. ATSs currently demand the most attention by law enforcement agencies [3, 4]. Globally, ATSs and ecstasy are the second most commonly consumed after cannabis, and they are good-looking to clandestine laboratory operators because of the easy availability of the precursor chemicals and their ease of manufacturing [5]. However, the ATSs consume regularly normally are superior the heroin and cocaine intake combined [5], and millions of individuals are reported to be current users of ATS drugs. Human use of these psychoactive substances is virtually universal [4]. In according with the latest estimates of consumption of illicit drugs supported by the United Nations Office on Drugs and Crime (UNODC), approximately 275 million of the global population, who is approximately 5.6 per cent of the worldwide population aged 15-64 years, used drugs at least once during 2016 [6]. Some 31 million people who use drugs suffer from drug use illnesses, connoting that their drug consume is detrimental to the point where they may need treatment. However, most importantly, one out of ten, in other words almost 27 million people around the world are taking into account drug consumers [6].

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Notwithstanding, because of its numerous facets, it is an extremely complex phenomenon to understand and measure. The flaws in the methods used to measure them limit present knowledge about how drug use provokes improvements and directly or indirectly affects society as a whole. It is in this context that the analysis using "Wastewater-Based Epidemiology" (WBE) made possible the real knowledge of the number of illicit drugs consumed by the population, besides the possibility of the quantitative identification of them. Large amounts of illicit drugs are consumed, metabolized and eliminated from the human body in urinary and faecal excretions and the eliminated parental composites and metabolites are measurable in wastewater [7]. Thus, have been released into the environment during manufacturing, trafficking, and post-consumption, resulting in the wide detection of this kind of forthcoming drugs universally [8] by the fact of their deficient or elimination/degradation in the wastewater treatment plants (WWTP), due to the standard sewage treatment processes. On the other hand, these compounds may have potent pharmacological and biological activities and their presence generally at low concentrations in surface water, often mixed with the residues of many therapeutic pharmaceuticals and other organic compounds, may lead to unexpected pharmacological interactions causing toxic effects to aquatic organisms. Besides, they can cause a wide range of health and environmental problems [9].

It is a matter of concern that in Brazil, around 59% of the sewage produced is not treated and discarded *in nature*. Consequently, the pollution of water sources occurs, compromising the quality of drinking water concerning the presence of illegal substances and its major metabolites, which are only partially removed in a conventional drinking-water-treatment plant. This impurity may represent a risk to humans, especially considering that 17% of Brazilian cities are not fully served by the water distribution network [10].

Detection of illicit drugs in surface waters and effluents was developed and improved by Daughton [11] using the mass spectrometry technique, which provided an estimate of consumption in a given region of interest. Subsequently, Zuccato et al. [12] published a new calculation method (WBE) for obtaining local and real-time estimates of drug consumption. These authors pronounced the occurrence of cocaine (COC) and its metabolite benzoylecgonine (BE) in wastewaters and surface waters estimating pioneering collective cocaine usage. WBE's ability to provide useful and timely information on spatial variations (within and among countries) and temporal variations (every day, weekly, monthly and yearly) in illicit drug use have been demonstrated [7, 8, 11-27]. Presented as an objective, evidence-based and non-invasive approach, it has seen countless progress. Nevertheless, as with every approach trying to model a complex phenomenon, no matter how sophisticated, it will always only provide a partial description of it. It is shown in Figure 1 a schematic diagram of WBE processes. If the average dose and abuse frequency of a particular drug is known, the number of drug abusers can also be estimated.

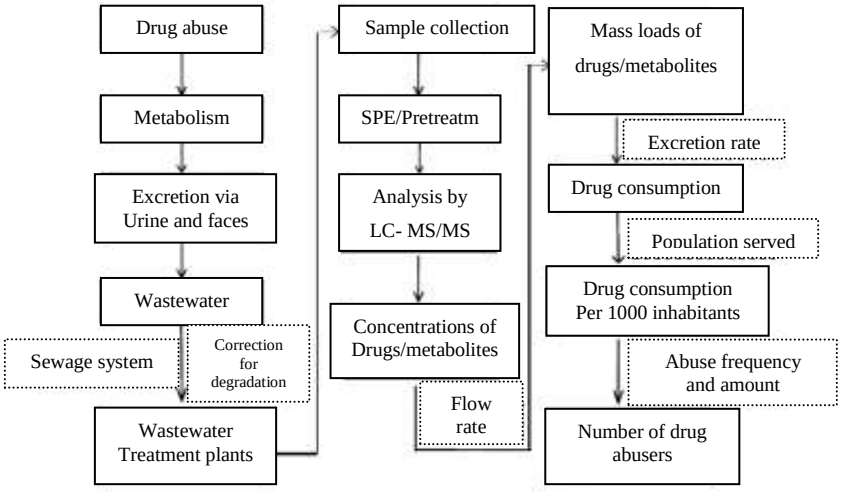

Figure 1. Schematic diagram of wastewater-based epidemiology [28].

Pharmacokinetics of illicit drugs

The founding principle of WBE is related to the mechanism by which the consumption of illegal (and prescription) drugs can be detected and eventually quantified by quantifying the incidence of the parent compound or its metabolites in biological matrices [11-27]. Each xenobiotic will undergo specific pharmacokinetic and metabolization processes, which, depending on the constituent, may last longer times and emission products will thus be found in urine up to several days after administration [29]. As stated by Zuccato et al. [9] the ideal drug marker should be an important and unique excretion of the drug under study that is stable in wastewater. Furthermore, it should additionally show limited inter and intra-individual variability and must be expelled at comparable rates indifferent of the administration route and dose.

Yet, none of the available drug residues fulfils all criteria. Firstly, pharmacokinetics and excretion rates depend on the individual, meanwhile, body mass, and other factors associated with the state of the consumer will influence the excretion rate for a given illicit drug [30]. Secondly, elimination degrees depend on the management route, as chemical compounds will have different bioavailability by the fact of how they enter the organism. This is an important aspect concerning pharmacokinetic studies available in the literature and frequently referenced in WBE methods. Countless of this evidence has been carried out using administration routes, which do not reflect current user practices.

Cannabis

Cannabis (*Cannabis sativa* L), family *Cannabidaceae*, is universally the utmost prevalent illicit drug. The major active compound in cannabis is Δ^9-tetrahydrocannabinol (THC) and over 20 metabolites have been identified in human urine and faeces, which THC is eliminated from the human body in a proportion of 65% is eliminated in the faeces and approximately 20% in urine [31]. It is worthy to detach that THC readily oxidizes to form THC-COOH (11-nor-9-carboxy-Δ^9-tetrahydrocannabinol), which is more unalterable, and in a small extent it's possible also be detected the THC-COOH, OH-THC (11-Hydroxy-Δ^9-tetrahydrocannabinol). The most important metabolites encountered after the administration of cannabis and their respective excretion rates are reported in Table 1. In WBE studies, THC-COOH has usually remained the goal substance to monitor the ingesting of cannabis.

Table 1. Summary of the metabolites and their analogous excretion rates measured in urine after administration of cannabis

Administered compound	Major metabolites of interest	Urinary excretion Rate (%)	Administration Route	Reference
THC	THC-COOH	0.5 – 0.6	smoke	[32]
	OH-THC	2		

Cocaine

COC has been the target of most WBE studies published in the literature, mainly because of its widespread use [11-27]. After consumption, between 85 and 92% of the preliminary cocaine dose is eliminated by urine within approximately 24h [33], as shown in Table 2. Liver carboxylases are responsible for the formation of BE. However, this reaction can also occur spontaneously at physiological pH [30]. COC, ecgonine methyl ester (EME) and BE can then be supplementarily altered to form negligible metabolites, which we can highlight: ecgonine (ECG), norcocaine (NCOC), and cocaethylene (CE), the latter being excreted exclusively when cocaine and alcohol are coadministered [30].

Table 2. Summary of the metabolites and their equivalent elimination taxes detected in urine subsequently administration of cocaine by nasal insufflation

Major metabolites of interest	Urinary excretion Rate (%)	Administration Route	References
Cocaine	1-15	Nasal insufflation	[30,34,35]
Benzoylecgonine	15-55		
Ecgonine methyl ester	32-49		

Amphetamine-type stimulants

Amphetamine-type stimulants (ATS) represent a class of compounds derived from phenethylamine to which a methyl group is linked to the α carbon. Among the various molecules, the most encountered ones are (±)-Amphetamine, (±)-Methamphetamine, (±)-3,4-Methylenedioxymethamphetamine (MDMA), (±)-3,4-Methylenedioxethylamphetamine (MDEA), (±)-3,4-Methylenedioxyamphetamine (MDA) and Ephedrine. However, with the ever-growing number of new psychoactive substances appearing on the market every year [36], this group of compounds has seen an important increase in recent years. Synthesis of ATS in illegal laboratories tends to produce racemic mixtures since criminals do not possess the knowledge or the instrumentation to obtain enantiopure drugs. This is not the case for industrial of pharmaceutical productions where products generally contain only one enantiomer or determined proportion of each of them.

The metabolic process involved in the excretion of MDA, MDMA, MDEA and the other derivatives has not yet been studied extensively. In the particular case of MDMA, an important variability in excretion rates of the parent compound has been attributed to its nonlinear elimination, which increases as a function of the administered dose [37]. In some WBE studies, an excretion rate of 12-47% of the initial dose of MDMA after consumption [38]. Specific metabolites of MDMA (i.e., 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA)) have been reported in the literature and can be used as markers of MDMA consumption in WBE studies [39]. Table 3 summarizes the metabolites and expected excretion rates after administration of the major ATS.

Table 3. Summary of the metabolites and their equivalent elimination taxes detected in urine subsequently administration of the major ATS.

Administered compound	Major metabolites of interest	Urinary excretion Rate (%)	Administration Route	References
Amphetamine	AM	1-70	oral	[30]
Methamphetamine	METH	2-76	oral	[30,35]
	AM	7		
MDMA	MDMA	12-47	oral	[40-42]
	HMMA	11-49		

The purpose of this research was thence to examine the intake patterns COC, BE, amphetamine (AMP), methamphetamine (METH), and 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH, THC metabolite)) in Rio de Janeiro Municipality, Brazil. The focus of the research encompassed a comparison of wastewater-based intake estimates with the existing epidemiological data.

MATERIALS AND METHODS

Reagents and materials

Standards of BE, METH, AMP, THC, and THC-COOH were obtained at concentrations of 1 mg/mL in 1 mL methanol. Cocaine was acquired at 1 mg/mL in 1 mL acetonitrile. These standards were purchased from Sigma-Aldrich Brazil Ltda. (São Paulo, Brazil). Deionized water was obtained from Milli-Q purification system (Merck Millipore, Goiânia, Brazil). Deuterated

internal standards (cocaine-d_3, BE-d_3, cocaethylene-d_3, amphetamine-d_5, methamphetamine-d_5, THC-d_3 and THC-COOH-d_3) were purchased from Sigma-Aldrich Brazil Ltda. (São Paulo, Brazil) at 100 mg/mL in 1 mL methanol, and Cocaine-d_3 at 100 mg/mL in acetonitrile.

Liquid chromatography-mass spectrometry (LC-MS) grade methanol, isopropanol, acetonitrile, and formic acid were obtained from Thermo Fisher Scientific (São Paulo, Brazil). Hydrochloric acid (HCl) 36.5%–38% was acquired from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA). Reagent grade ammonium hydroxide and dichloromethane and were obtained from Pharmco-Aaper (São Paulo, Brazil). Nalgene^{TM} certified wide-mouth amber high-density polyethylene (HDPE) 250 mL bottles, Whatman^{TM} glass microfiber filters (outside diameter 4.7 cm, particle retention 1.6 mm, and thickness 0.26 mm), EMD Millipore (Merck Millipore, Goiânia, Brazil) all-glass filter holder assembly (1000 mL), Sarstedt Inc 10 mL sc tubes 16 mmx100 mm, and 350 mL fused insert vials were obtained from Thermo Fisher Scientific (São Paulo, Brazil). Oasis MCX cartridges (150 mg/6 mL) were produced by Waters (Waters Technologies do Brasil Ltda, Rio de Janeiro, Brazil) while Strata NH_2 (200 mg/3 mL) cartridges, including HPLC columns utilised for the chromatographic separation (Synergi Polar; 4 μm, 150 mm×3 mm and Kinetex PFP; 2.6 μm, 100 mm×2.1 mm), were factory-made by Phenomenex (São Paulo, Brazil). Glass-fibre filters (GF/C) were obtained by Whatman (Biochem, Belo Horizonte, Minas Gerais, Brazil).

Standards were diluted with LC-MS grade methanol at a ratio of 1:10 to final concentrations of 100 mg/mL. Ten millilitres of standard stock solution mixture was prepared at 1 mg/mL and a serial dilution utilizing a 1:10 dilution factor was made until a concluding concentration of 0.001 mg/mL was obtained in methanol. Deuterated internal standards were diluted with LC-MS grade methanol at a ratio of 1:10 from the original ampoule to a concluding concentration of 10 mg/mL. Subsequently, 10 millilitre of internal standard (IS) mixture was prepared at 0.1 mg/mL by 1:10 dilution in methanol. Standard and IS working solutions were stored in amber vials at –20°C. Working solutions were prepared by serial 1:10 dilutions in MilliQ water (Merck Millipore, Goiânia, Brazil), and were stored in amber vials at 4°C.

Wastewater samples collection

For this research, wastewater samples were collected from WWTPs located at distinguished four municipal boroughs of Rio de Janeiro Municipality. Pavuna WWTP has a capacity of 1097 L/s and serves a population of 498,553 inhabitants, activated sludge system. Alegria WWTP has a capacity of 1529 L/s and serves a population of 688,000 from four main sub-basins: Centro, Mangue, Catumbi, Alegria, Faria-Timbó and São Cristóvão, long-term activated sludge system. Penha WWTP has a capacity of 765 L/s and serves a population of 344,051 inhabitants, activated sludge system. Barra Bonita WWTP has a capacity of 42 L/s and serves a population of 18,923 inhabitants, activated sludge system (Figure 2).

The 24-h composite samples of untreated wastewaters were collected during between the spring period (September/2018), and early summer (December/2018) at the inlet of the municipal WWTPs (Pavuna, Penha, Alegria, Barra Bonita). For collection, the 24-h composite samples utilised time-intervals of 30 min. Each 24h-composite samples was obtained by collecting a 200 mL aliquot of the sanitary affluent using time-proportional automatic sampler wastewater at 4-hour interval [43]. For each 200 mL aliquot was transferred to a 5-L plastic bottle and then stored in a refrigerator at 4°C. At the end of the sampling collection, the collective sample was homogenized and 1.5-L of this sample was transferred to an amber flask (acidified on-site after sub-sampling) and transported under refrigeration to the laboratory [17]. The samples obtained during the sampling campaign were frozen promptly subsequently the collection and kept in the freezer (−20°C) until laboratory tests.

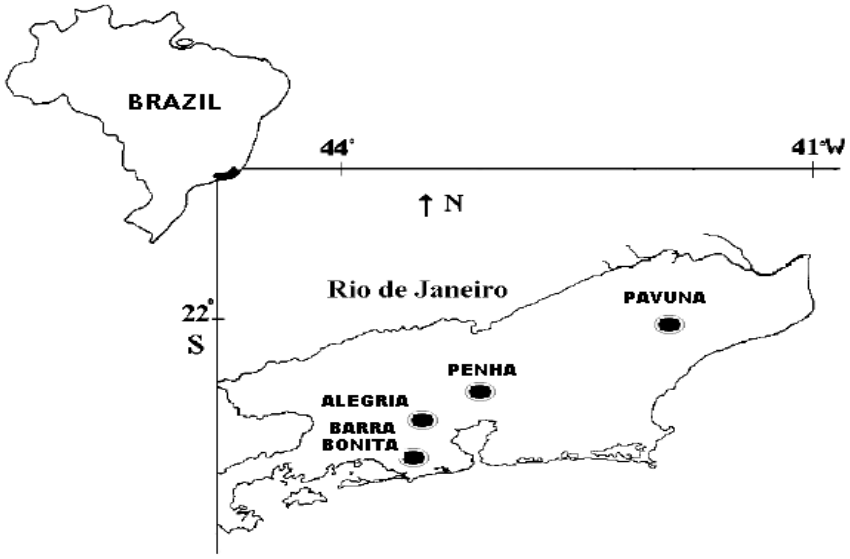

Figure 2. Map of Rio de Janeiro Municipality with indicated sampling locations.

Analytical methodology

The sample preparation and instrumental analysis were performed according to Senta et al. [44]. Briefly, the wastewater samples (125 mL) were spiked with surrogate standards (120 ng/L) and filtered using GF/C filters.

The Solid-phase extraction (SPE) method was performed with Oasis HLB Cartridges. Commercial HLB Oasis® cartridges (6mL, 500mg) were obtained from Waters Corporation (Waters Technologies do Brasil Ltda, Rio de Janeiro, Brazil).

The HLB cartridges were equilibrated with three mL of methanol (MeOH), four mL of acetonitrile (ACN) and five mL of ultrapure water. After percolation of the filtered samples (1 L), the cartridges were vacuum dried (10 mm Hg) for 10min, and eluted with six mL of an ACN:MeOH, 60:40 (v v^{-1}) solution, and evaporated to dryness under a nitrogen stream. The dry extract was dissolved in 1.0 mL of 0.1% aqueous formic acid: ACN, 90:10 (vv^{-1}) solutions and transferred to vials for LC-MS/MS analysis.

LC-MS/MS analysis

The LC-MS/MS analysis was performed using a liquid chromatograph Agilent 1200 series, with a binary pump, connected to a 6410 triple quadrupole mass spectrometer equipped with electrospray ionization source (Agilent Technologies, Palo Alto, CA, USA) [10]. Separation used a Zorbax SB-C18 (30 mm× 2.1mm, 3.5 µm) reversed-phase HPLC column (Agilent Technologies, Palo Alto, CA - USA). Formic acid solutions (0.1%, v v^{-1}) (eluent A) and ACN (Eluent B) were used as mobile phase solvents. The following gradient program was performed: initial condition at 10% of eluent B kept for 0.5min, followed by a linear gradient reaching 80% of eluent B in 5min. This condition was kept for 2 min, and then a 4 min linear gradient back to 10% of eluent B. To equilibrate the column for the next run, the initial condition was held for 5min. The flow and the column temperature rate were held at 0.3 mL min^{-1} and 35°C, and 10 µL of each sample was injected into the LC-MS/MS system.

The electrospray source was operated in the positive polarity. High purity nitrogen was used as desolvation (350°C; 10 L min^{-1}), nebulizer and collision gas. Nebulizer pressure was kept at 30 psi and the capillary voltage set at 2500 V. The fragmentor was optimized at 120 V for all analytes, which were analyzed in multiple reactions monitoring (MRM) mode, recording the transitions between the precursor ion and the three most abundant product ions for each target analyte. The most intense transition was used as a quantifier ion (MRM_1) and the others as a qualifier ion (MRM_2). The identification of the analytes was confirmed by comparing the retention time and the MRM_1/MRM_2 ratio

with the corresponding values of analytical standards measurements. The method accuracy was in the range from 83% to 116% and extraction recovery between 60% and 94%. The method quantification limits were between 0.1 and 5 ng/L.

Daily loads and drug use estimate calculations

The method offered by Zuccato et al. [9] was used for the evaluation of drug consumption. So, the ingesting of individual drugs is expressed as the number of average doses per 1000 inhabitants, and for this procedure, it was planned by multiplying the population normalized representative average mass loads of selected drug biomarkers by the equivalent correction factors, then dividing with the corresponding average dose size.

All screened analytes were considered as potential indicators of drug consumption throughout the sampling campaign. The potential loss of metabolites from wastewater during sample extraction was corrected through labelled internal standards and the isotope dilution method. Narcotic mass loadings were calculated from analyte concentrations in raw wastewater (in units of ng/L) for daily wastewater flows using Eq. (1):

Mass Load (mg/day) = Raw Concentration (ng/L) × Flow (L/d) × (1mg/1,000,000ng) (1)

The drug consumption estimates are then obtained by normalizing the mass load of illicit drugs to the estimated contributing population and subsequently subjected to a correction factor, which accounts for metabolic excretion of the compounds [45-50].

Apart from some exceptions, the correction factors used in the calculation of drug consumption were taken from the paper published by Zuccato et al. [9]. The estimation of COC consumption was made by using a later proposed correction factor of 3.6 [50], while the estimation of methadone consumption was performed by using recently proposed correction factor of 2.0 [48]. The consumption data were transformed into the number of average drug doses by applying the data on the size of a dose presented in Table 4.

Table 4. Characteristics of wastewater treatment plants (WWTPs) included in the study

Data	Wastewater treatment plants (WWTPs)			
	Pavuna	**Alegria**	**Penha**	**Barra Bonita**
Number of inhabitants	498,553	695,050	397,200	22,555
Number of treated drug consumers/1000 inhabitants[∞]		2,23		
No. of inhabitants served by WWTP	498,553	688,000	344,051	18,923
WW flow (L/s)	1097	1529	765	42

[∞]Number of treated drug consumers/1000 inhabitants — data for 2018 obtained from Municipal Health Secretary of Rio de Janeiro municipality

Statistical analysis

Data analysis (counts, percentages, means) was performed with Excel software (Microsoft Office Excel 2016). All statistical analyses were performed using Origin 7.5. (OriginLab Corporation). Normal distribution was checked by Kolmogorov-Smirnov and Shapiro-Wilk tests for all variables. Statistical significant differences of the median were judged by one-way analysis of variance (ANOVA) and least significant differences calculations at a 5% significant level.

RESULTS AND DISCUSSION

The lack of previous data regarding the presence of illicit drugs in wastewater prompted this analysis of the presence of selected types of drugs in wastewater from a number of WWTPs in Rio de Janeiro Municipality.

A total of 32 samples of untreated wastewater were analyzed during the study period. Samples were taken every month from September to December 2018, at the following intervals: September (n = 2), October (n = 2), November (n = 2) and December (n = 2). Therefore, it was decided to analyze the occurrence of illegitimate drugs in wastewater. The obtained data provide the first more detailed information on the incidence of illicit drugs in wastewaters. The samples were taken and subsequently analyzed by LC-MS/MS. The incidences of the most commonly used drugs, methamphetamine; cocaine; the main cocaine metabolite in wastewater, BE,

AMP, METH, THCCOOH were compared with the results reported in other studies.

The level of cannabis in wastewater from the monitored sites was calculated based on the concentration of the metabolite THC-COOH. The concentrations of METH, AMP, COC, and BE were calculated directly from the measured values of the parent drugs in the wastewater.

Founded on the level of drugs in the wastewater and the conforming daily flow rate of wastewater in a treatment plant, the daily quantities of the drugs in the wastewater were obtained.

These values were specifically related to 1000 inhabitants connected to the WWTP. The obtained results represent the specific loads of the drugs in wastewater related to the population on a given day (mg/day/1000 inhabitants). Thus, drug consumption values in selected cities correlate with the specific loads of the drugs in wastewater.

The consumption of illicit drugs in society is currently one of the biggest social problems and is also emerging as a possible environmental problem. The occurrence of illicit drugs in environmental matrices, mainly surface water, arises as a matter combined with the contribution of effluents from WWTPs and, in countries with a low rate of wastewater treatment such as Brazil, it is associated with the impact of crude sewage as an important pollutant. Therefore, due to the complexity of the matrices and the importance of the determination of these substances, the development of reliable analytical methods is a real necessity.

An important indicator related to the fight against illicit drugs is the amount of substance circulating or consumed in a city, state or country. At present, this data is obtained indirectly through extrapolations from socio-epidemiological investigations [17]. Such investigations use data on drug seizures, medical hospitalization records, population investigations and interviews with users. However, these methods are obtained in a very onerous, slow manner, and do not provide real-time knowledge about the consumption and/or trafficking of narcotics in a region [14].

The occurrence of illicit drugs and metabolites in wastewaters

The concentration of illicit drugs in WWTPs from the monitored sites is presented in Table 5.

Concentrations of COC and its main metabolite BE ranged from 201.3 to 2751.5 ng/L and from 630.7 to 5849.2 ng/L, respectively. The highest specific loads of COC used were found in Alegria (91.7 mg/day/1000 inhabitants), followed by Pavuna (68.4 mg/day/1000 inhabitants), Barra Bonita (34.6 mg/day/1000 inhabitants), and Penha (11 mg/day/1000 inhabitants).

Amphetamine-like stimulants (AMP and METH) ranged from 1.7 to 110.0ng/L for AMP. The highest mean specific load of AMP in wastewater during the monitored days was found also at the Alegria WWTP (19 mg/day/1000 inhabitants), and the lowest value was measured in Barra Bonita WWTP (1.8 mg/day/1000 inhabitants). The concentrations of METH in wastewater ranged from 55.3ng/L (Barra Bonita) to 477.4ng/L (Pavuna). The highest concentrations were typically found in areas with known drug problems or with inhabitants of low socio-economic backgrounds, such as some areas in Rio de Janeiro Municipality, like Pavuna. In this study, the Pavuna WWTP had the highest mean specific load of METH in the wastewater (109.6 mg/day/1000 inhabitants), followed by Alegria (92.1 mg/day/1000 inhabitants), Penha (56.3 mg/day/1000 inhabitants) and Barra Bonita (8.3 mg/day/1000 inhabitants).

The concentration of THC-COOH ranged from 188.8 to 940.2ng/L. The loads for this drug were (37.7 mg/day/1000 inhabitants) in Alegria, (32.3 mg/day/1000 inhabitants) in Pavuna, (28.5 mg/day/1000 inhabitants) in Penha, and (22.2 mg/day/1000 inhabitants) in Barra Bonita, indicating that cannabis is used in many large areas at the studied region.

The concentration of illicit drugs and metabolites (ng/L) in the WWTPs from the monitored sites (1-Alegria, 2-Penha, 3-Pavuna, and 4-Barra Bonita) and estimated mean consumption is in good agreement with published results for some other studies [3, 4, 7, 8, 15, 16, 27, 28 and 41].

Chemical analysis of wastewater performed in the present study has revealed the presence of residues of illicit drugs in the influents of the WWTPs analyzed. Samples of wastewater generated by more than 1.6 million inhabitants of four areas were analyzed for illicit drugs including METH, COC, BE, AMP, and TCHCOOH. The most commonly used hard drug was cocaine. The expected would be cannabis due to be cheap or even free and often produced by groups of end-users [51].

Residues of illicit drugs have become widespread surface water contaminants in populated areas. Like for therapeutic pharmaceuticals, this contamination appears to be common, consumers being the major source. After consumption, these substances can be excreted as the parent compound and/or metabolites in urine and faeces. Several widely used drugs are excreted unchanged or as active metabolites in high percentages after consumption and continuously discharged into domestic wastewaters. Several substances can, therefore, reach WWTPs in substantial amounts and, if they escape degradation, can be released into surface water, what is worse.

Table 5. The concentration of illicit drugs and metabolites (ng/L in WWTPs from the monitored sites (1-Alegria, 2-Penha, 3-Pavuna and 4- Barra Bonita) and the estimate of medium consumption. Municipality of Rio de Janeiro, 2018.

Drugs and Metabolites	Concentration (ng/L)				Consumption of Drugs (mg/day/1000 inhabitants between 15 and 64 years old)				Chemical Group
	WWTP-1	WWTP-2	WWTP-3	WWTP-4	WWTP-1	WWTP-2	WWTP-3	WWTP-4	
AMP	88.8	34.3	110	21.7	92.1	56.3	109.6	8.3	*Amphetamine-like stimulants*
METH	345.2	212.6	477.4	55.3					
THC-COOH	940,2	437.4	188.8	662.3	37.7	28.5	32.3	22.2	*Cannabis*
BE	5849.2	630.7	2580.9	1285.1	91.7	11	68.4	34.6	*Cocaine*
COC	2751.5	201.3	1732.2	823.8					

CONCLUSIONS

This study signifies the first detailed analysis of drug ingesting in Rio de Janeiro Municipality based on wastewater analysis. The analysis of sewage waters of the 4 main WWTPs that treat the wastewaters of Rio de Janeiro Municipality provides valuable information about illicit drugs that are used in this area.

The discoveries support some of the existing hypotheses about regional features, but also provide additional evidence about geographical particularities. Understanding illicit drug consumption remains a difficult task; however, the results of this research illustrate how the combination of different and complementary data sources allows for obtaining a more accurate picture of the situation. The information can be used to monitor changes in drug use, identify potential dangers, promote the setup of targeted surveys, prevention campaigns and/or police actions), understand the structure of drug markets and guide future drug policies. Although wastewater analysis does not provide direct information about users, its ability to provide close to real-time data and its potential integration in existing monitoring programs make it a valuable tool to help under-standing illicit drug consumption.

Accurate and timely information about the scale and dynamics of drug consumption is important for assessing the needs of law enforcement and public health services in a community. Careful analysis of sewage samples can provide valuable information on the scale of drug consumption, but with a comprehensive investigation into the kinetics of drug-flow, this technique can also provide valuable support in identifying trends in drug use patterns. Such information can add additional quantitative weight to the findings of sociological and general population surveys.

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Conflicts of interest

The author declares no conflicts of interest.

Ethical consideration

The present work had the approval of the Research Ethics Committee of the Sergio Arouca National School of Public Health (ENSP/FIOCRUZ).

REFERENCES

1. Schulte M.T., Hser Y.I., 2014. Substance Use and Associated Health Conditions throughout the Lifespan. Public health reviews. 35(2), https: //web – beta. archive. org /web/20150206061220 / http : //www . publichealthreviews. eu/upload/pdf _files /14/00 _Schulte_Hser .pdf.

2. Hall W., Degenhardt L., Sindicich N., 2008. Illicit drug use and the burden of disease. In: Heggenhougen K., Quah S., editors. International Encyclopedia of Public Health. Elsevier. pp.523–530.

3. Logan B.K., 2001. Amphetamines: an update on forensic issues. J Anal Toxicol. 25, 400–404.

4. Griffiths P., Meacham M., McKetin R., 2008. Illicit drug trends globally. In: Heggenhougen K., Quah S., editors. International encyclopedia of public health. Elsevier, 515–523.

5. UNODC. United Nations Office on Drugs and Crime. Amphetamines and Ecstasy: Global ATS Assessment. United Nations Publication. 2014. https://www. unodc. org/documents/scientific/2014_Global_Synthetic_Drugs_ Assessment_web.pdf (Accessed November 12, 2018).

6. United Nations Office on Drugs and Crime. World Drug Report 2018. https://www. unodc.org/wdr 2018/ prelaunch/WDR18_Booklet_1_EXSUM.pdf (Accessed July 5, 2018).

7. Nefau T., Karolak S., Castillo L., Boireau V., Levi Y., 2013. Presence of illicit drugs and metabolites in influents and effluents of 25 sewage water treatment plants and map of drug consumption in France. Sci Total Environ. 461-462, 712-722.

8. Hu P., Guo C., Zhang Y., Lv J., Zhang Y., Xu J., 2019. Occurrence, distribution and risk assessment of abused drugs and their metabolites in a typical urban river in north China. Front Environ Sci Eng. 13(4), 56-67.

9. Zuccato E., Castiglioni S., Bagnati R., Chiabrando C., Grassi P., Fanelli R. 2008. Illicit drugs, a novel group of environmental contaminants. Water Res. 42, 961–968.

10. Campestrini I., Jardim W.F. 2017. Occurrence of cocaine and benzoylecgonine in drinking and source water in the São Paulo State region, Brazil. Sci Total Environ. 576, 374-380.

11. Daughton C.G., 2001. Illicit Drugs in Municipal sewage: Proposed new non-intrusive tool to heighten public awareness of societal use of illicit/abused drugs and their potential for ecological consequences. In: Daughton C.G., Jones-Lepp T., editors. Pharmaceuticals and personal care products in the environment: scientific and regulatory issues. Symposium Series. Washington, D.C.: American Chemical Society. pp. 348–364.

12. Zuccato E., Chiabrando C., Castiglioni S., Calamari D., Bagnati R., Schiarea S., Fanelli R., 2005. Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. Environ Health. 7, 1–7.

13. Gheorghe A., van Nuijs A., Pecceu B., Bervoets L., Jorens P.G., Blust R., Neels H., Covaci A., 2008. Analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography-ion trap tandem mass spectrometry. Anal Bioanal Chem. 391, 1309–1319.

14. Postigo C., López de Alda M.J., Barceló D., 2009. Drugs of abuse and their metabolites in the Ebro River basin: occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. Environ Int. 36, 75-84.

15. Terzic S., Senta I., Ahel M., 2010. Illicit drugs in wastewater of the city of Zagreb (Croatia) – estimation of drug abuse in a transition country. Environ Pollut. 158, 2686-2693.

16. Harman C., Reid M., Thomas K.V. 2011. *In situ* calibration of a passive sampling device for selected illicit drugs and their metabolites in wastewater, and

subsequent year-long assessment of community drug usage. Environ Sci Technol. 45, 5676-5682.

17. van Nuijs A.L.N., Mougel J.F., Tarcomnicu I., Bervoets L., Blust R., Jorens P.G., Neels H., Covaci A. 2011. Sewage epidemiology- a real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium. Environ Int. 37, 612-621.

18. Maldaner A.O., Schmidt L.L., Locatelli M.A.F., Jardim W.F., Sodré F.F., Almeida F.V., Pereira C.E.B, Silva C.M., 2012. Estimating Cocaine Consumption in the Brazilian Federal District (FD) by Sewage Analysis. J Braz Chem Soc. 23(5), 861-867.

19. Mackuľak T., Skubák J., Grabic R., Ryba J., Birošová L., Fedorova G., Swt. 2014. National study of illicit drug use in Slovakia based on wastewater analysis. Sci Total Environ. 494-495, 158-165.

20. Ort C., Eppler J.M., Scheidegger A., Rieckermann J., Kinzig M., Sörgel F., 2014. Challenges of surveying wastewater drug loads of small populations and generalizable aspects on optimizing monitoring design. Addiction. 109, 472-481.

21. Ostman M., Fick J., Näsström E., Lindberg R.H., 2014. A snapshot of illicit drug use in Sweden acquired through sewage water analysis. Sci Total Environ. 472, 862-871.

22. Been F., Bijlsma L., Benaglia L., Berset J.D., Botero-Coy A.M., Castiglioni S., Kraus L., Zobel F., Schaub M.P., Bücheli A., Hernández F., Delémont O., Esseiva P., Ort C., 2016. Assessing geographical differences in illicit drug consumption - A comparison of results from epidemiological and wastewater data in Germany and Switzerland. Drug Alcohol Depend. 161, 189-199.

23. Kankaanpää A., Ariniemi K., Heinonen M., Kuoppasalmi K., Gunnar T., 2016. Current trends in Finnish drug abuse: Wastewater based epidemiology combined with other national indicators. Sci Total Environ. 568, 864-874.

24. Krizman I., Senta I., Ahel M., Terzic S., 2016. Wastewater-based assessment of regional and temporal consumption patterns of illicit drugs and therapeutic opioids in Croatia. Sci Total Environ. 566-567, 454-462.

25. Lai F.Y., O'Brien J.W., Thai P.K., Hall W., Chan G., Bruno R., Ort C., Prichard J., Carter S., Anuj S., Kirkbride K.P., Gartner C., Humphries M., Mueller J.F., 2016. Cocaine, MDMA and methamphetamine residues in wastewater: Consumption trends (2009-2015) in South East Queensland, Australia. Sci Total Environ. 568, 803-809.

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

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

28. Feng L.Z., Zhang W., Li X.Q., 2018. Monitoring of regional drug abuse through wastewater-based epidemiology—A critical review. Science China Earth Sciences. 61, 239–255.

29. The Merck Manual – Second home edition. 2004. Section 2. Drugs, Chapter 11, Drug administration and kinetics. http:www.merckhomeedition.com/home.html (Accessed June 15, 2018).

30. Jenkins A.J., 2007. Pharmacokinetics: drug absorption, distribution, and elimination. In: Karch S.B., editor. Drug abuse handbook. Boca Raton: CRC Press. pp. 167.

31. Pertwee R.G., 2005. Pharmacological actions of cannabinoids. Handb Exp Pharmacol. 168, 1-51.

32. Huestis M.A., 2007. Human cannabinoid pharmacokinetics. Chem Biodivers. 4(8), 1770–804.

33. Jatlow P., 1988. Cocaine: Analysis, pharmacokinetics, and metabolic disposition. Yale J Biol. Med. 61, 105–113.

34. Jufer R.A., Wstadik A., Walsh S.L., Levine B.S., Cone E.J., 2000. Elimination of cocaine and metabolites in plasma, saliva, and urine following repeated oral administration to human volunteers. J Anal Toxicol. 24, 467–477.

35. Baselt R.C., Cravey R.H., 2014. Disposition of toxic drugs and chemicals in man. 10th Edition. Foster City, California: Biomedical Publications. pp.76.

36. Evans-Brown M., Gallegos A., Francis W., Christie R., Cunningham A., Sekula J., Almeida A, Sedefov R.,

2015. New psychoactive substances in Europe an update from the EU Early Warning System. Luxembourg: Publications Office. http://dx.publications.europa.eu/ 10.2810/372415.

37. Mueller M., Peters F.T., Huestis M.A., Ricaurte G.A., Maurer H.H., 2009. Simultaneous liquid chromatographic-electrospray ionization mass spectrometric quantification of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and its metabolites 3,4-dihydroxymethamphetamine, 4-hydroxy-3-methoxymethamphetamine and 3,4-methylenedioxyamphetamine in squirrel monkey and human plasma after acidic conjugate cleavage. Forensic Sci Int. 184, 64–68.

38. Khan U., Nicell J.A. 2011. Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy. Environ Int. 37, 1236–1252.

39. Been F., Rossi L., Ort C., Rudaz S., Delémont O., Esseiva P. 2014. Population normalization with ammonium in wastewater-based epidemiology: Application to illicit drug monitoring. Environ Sci Technol. 48(14), 8162–8169.

40. De La Torre R., Farré M., Ortuño J., Mas M., Brenneisen R., Roset P.N., Segura J., Camí J. 2000. Non-linear pharmacokinetics of MDMA (ecstasy) in humans. Br J Clin Pharmacol. 49, 104–109.

41. Segura M., Ortuño J., Farré M., McLure J.A., Pujadas M., Pizarro N., Llebaria A., Joglar J., Roset P.N., Segura J., de La Torre R. 2001. 3,4-Dihydroxymethamphetamine (HHMA). A major in vivo 3,4-methylenedioxymethamphetamine (MDMA) Metabolite in humans. Chem Res Toxicol., 14(9), 1203–1208.

42. Pizarro N., Ortuño J., Farré M., Hernández-López C., Pujadas M., Llebaria A., Joglar J., Roset P.N., Mas M., Segura J., Camí J., de la Torre R., 2002. Determination of MDMA and its metabolites in blood and urine by gas chromatography-mass spectrometry and analysis of enantiomers by capillary electrophoresis. J Anal Toxicol. 26(3), 157–165.

43. Krizman I., Senta I., Ahel M., Terzic S. 2016. Wastewater-based assessment of regional and temporal consumption patterns of illicit drugs and therapeutic opioids in Croatia. Sci Total Environ. 566-567, 454-462.

44. Senta I., Krizman I., Ahel M., Terzić S., 2013. Integrated procedure for multiresidue analysis of dissolved and particulate drugs in municipal wastewater by liquid chromatography–tandem mass spectrometry. Anal Bioanal Chem. 405, 3255–3268.

45. Terzic S., Senta I., Ahel M. 2010. Illicit drugs in wastewater of the city of Zagreb (Croatia) — Estimation of drug abuse in a transition country. Environ Pollut. 158, 2686–2693.

46. Baker D. R., Kasprzyk-Hordern B. 2011. Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography-positive electrospray ionisation tandem mass spectrometry. J Chromatogr A. 1218(12), 1620−1631.

47. Postigo C., López de Alda M.J. Barceló D. 2011. Evaluation of drugs of abuse use and trends in a prison through wastewater analysis. Environ Int. 37, 49–55.

48. Thai P.K., Lai F.Y., Bruno R., van Dyken E., Hall W., O'Brien J., Prichard J., Mueller J.F., 2016. Refining the excretion factors of methadone and codeine for wastewater analysis—combining data from pharmacokinetic and wastewater studies. Environ Int. 94, 307–314.

49. Gracia-Lor E., Zuccato E., Castiglioni S., 2016. Refining correction factors for back-calculation of illicit drug use. Sci Total Environ. 573, 1648–1659.

50. Castiglioni S., Bijlsma L., Covaci A., Emke E., Hernández F., Reid M., Ort C., Thomas K.V., van Nuijs A.L., de Voogt P., Zuccato E., 2013. Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. Environ Sci Technol. 47, 1452–1460.

51. Ferreira A.P., 2019.Estimaciones del consumo de drogas ilícitas derivadas del análisis de aguas residuales: Una revisión crítica. Rev Univ Ind Santander Salud. 51(1), 69-80.