Journal of Human Environment and Health Promotion
Print ISSN: 2476-5481 Online ISSN: 2476-549X

Assessing the Presence and Variability of Illicit Drugs and Metabolites in Wastewater Treatment Plants of Southeast Brazil: A Comprehensive Wastewater-Based Epidemiology Study

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ARTICLE INFO

Article type:
Original article

Article history:
Received: 15 MARCH 2023
Revised: 6 APRIL 2023
Accepted: 3 MAY 2023

DOI: 10.52547/jhehp.9.2.63

Keywords:
Wastewater-based epidemiology
Illicit drug
Addiction
Drug consumption
Public health

ABSTRACT

Background: Wastewater analysis has emerged as a promising methodology for real-time monitoring of illicit drug consumption. The main objective of this article is to identify the occurrence and distribution of illicit drugs and their metabolites in the wastewater treatment plants (WWTP) of municipalities with adequate sewage coverage systems, in terms of water and sewage services, in the Hydrographic Regions of the State of Rio de Janeiro (HRRJ).
Methods: Diurnal composite samples of influent were collected from 13 wastewater treatment plants between December 27, 2022, and January 10, 2023, to detect the presence of illicit drugs, including Ecstasy, Amphetamine, Methamphetamine, Cocaine, Benzoylecgonine, and Cannabis. The excretion products were quantified using liquid chromatography coupled with mass spectrometry.
Results: The highest per capita consumption was observed for Cannabis, with a consumption rate of 432mg/day/1,000 inhabitants. Benzoylecgonine was the second most consumed drug, with a rate of 190mg/day/1,000 inhabitants, followed by Cocaine with a rate of 98mg/day/1,000 inhabitants. Amphetamine had a consumption rate of 104mg/day/1,000 inhabitants, while Methamphetamine had a rate of 42mg/day/1,000 inhabitants. 3,4-Methylene-dioxy-N-methylamphetamine had the lowest consumption rate, with a rate of 23.8mg/day/1,000 inhabitants.
Conclusion: The consumption patterns of illicit drugs were analyzed, and it was observed that stimulant drugs such as Cocaine, Ecstasy, and Amphetamines were consumed to a greater extent in municipalities located on the coast or with greater purchasing power. In contrast, Cannabis consumption was found to be more widespread across the surveyed locations, with higher consumption rates observed in regions with high traffic.

1. Introduction

Drug addiction is a significant contributor to mental and social disorders, which can disrupt the organization of society, resulting in addiction, heightened usage, trafficking, and public health issues. In addition, drug addiction inevitably leads to alterations in social and family behavior, coupled with adverse consequences such as reduced work commitment, decreased individual motivation, depletion of financial resources, and a need for spiritual intervention. As such, the inappropriate use and addiction of drugs pose a range of biological, psychological, social, and spiritual risks

How to cite: Pacheco Ferreira A, Wermelinger ED, Cruz-Hernández MJ. Assessing the Presence and Variability of Illicit Drugs and Metabolites in Wastewater Treatment Plants of Southeast Brazil: A Comprehensive Wastewater-Based Epidemiology Study. *J Hum Environ Health Promot.* 2023; 9(2): 63-71.

to individuals [1]. The category of emerging contaminants encompasses psychoactive substances, belonging to the set of illicit drugs and their metabolites. This group includes Cocaine (COC), Methamphetamine (MET), Amphetamine (AMP), and Opioids [2, 3]. The use of illicit drugs, and the abuse of licit drugs, has become a prevalent aspect of modern-day lifestyle across the globe, irrespective of economic status. According to the United Nations Office on Drugs and Crime (UNODC) around 284 million individuals aged 15-64 used drugs worldwide in 2020, representing a 26% increase over the previous decade. Furthermore, the current rate of drug use among young people is higher than those of previous generations in many countries. In Africa and Latin America, individuals below the age of 35 constitute the majority of those undergoing treatment for drug-related disorders [4]. Despite remaining stable in recent years, it is worth noting that more than 29 million individuals continue to suffer from drug use disorders, as per the UNODC report. The incomplete elimination of illicit drugs from sewage treatment plants following consumption and subsequent release into wastewater, particularly toilet use, has led to the emergence of these substances in treatment facilities. This can occur either at the start of the wastewater collection process, at the entrance to the sewage network (in the effluent), or the end of the process during final discharge, potentially resulting in environmental consequences [5]. Various detection surveys have revealed the presence of residual levels of drugs and/or metabolites in multiple regions of surveyed countries, ranging from <1ng/L to >10µg/L [6]. There is a growing interest in using wastewater as a tool for analyzing and detecting illicit substances, as it enables the estimation of drug consumption rates within a specific region, thereby facilitating the acquisition of authentic data. This approach not only enhances the mitigation of drugs but also enables more direct actions in the field of public health. It is important to note that this technique, known as wastewater-based epidemiology (WBE), is non-invasive and can identify the per capita pattern of drug use among communities linked to municipal wastewater treatment plants (WWTPs) without harming individuals′ privacy [7, 8] The WBE is a versatile technique that furnishes information on both temporal (daily, weekly, monthly, and yearly) and spatial (transnational) variations in drug usage, thus enabling the comprehension of drug use trends over the short term and long term. The insights obtained from WBE can serve as a complementary source of data to traditional socio-epidemiological methods such as surveys, arrest data, and crime statistics [9, 10]. It is noteworthy that the calculation of drug use within the population typically entails the measurement of drug loads normalized as stable biomarkers, accounting for the human excretion factor [11-13]. The prevalence of drug addiction in our society is substantial, prompting social scientists and professionals to take a keen interest in the phenomenon from various perspectives, including detection research, in order to inform the development of public policies aimed at addressing this significant public health challenge.

1.1 Occurrence and fate of illicit drugs in a WWTP

In general, drugs that are consumed either enter the sewage system via disposal or are illegally discarded. Following usage, the drugs consumed may be excreted in their original form or as metabolites. Benzoylecgonine (BZE) is the primary human metabolite resulting from COC usage. Other metabolites stemming from COC use, such as Nor-benzoylecgonine and Nor-cocaine (NORC), are less predominant. However, when alcohol and COC are co-consumed concomitantly, a further metabolite known as Cocaethylene emerges as a result of the transesterification process [14]. After usage, the urinary metabolites of AMP that are excreted comprise *3,4-*methylenedioxyamphetamine (MDA), which is a common metabolite of two drugs, namely, AMP and MET, as well as 3,4-methylenedioxy-N-methylamphetamine (MDMA) or Ecstasy, and 3,4-methylenedioxy-N-ethylamphetamine (MDEA) [15]. Cannabinoids are a class of organic compounds found in Cannabis. The two primary constituents of Cannabinoids, Δ^9-tetrahydrocannabinol (THC) and Cannabidiol (CBD), as well as their metabolites, are considered emerging contaminants concern due to the limited knowledge regarding their environmental effects. Moreover, with the recent legalization of Cannabinoids, the release of these compounds into water systems and the environment is anticipated to escalate. The most commonly utilized technique for the extraction and pre-concentration of Cannabinoids in water samples, as well as a clean-up step after the extraction of Cannabinoids from solid samples, is solid-phase extraction. Liquid chromatography coupled with mass spectrometry is the most frequent technique used method for Cannabinoids analysis. THC and its metabolites, specifically 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), have been detected at concentrations of up to 2590 and 169 ng/L in untreated and treated wastewater, respectively [16]. Sewage effluent is the primary source of analysis for illicit drugs and their metabolites in the environment, as it enables the detection of such substances that have been consumed by the population that the WWTP serves [17]. Table 1 shows the range of concentrations of selected biomarkers detected in influent and effluent wastewater in different geographical regions, including Australia, Europe, the United States of America, South America, and Asia.

1.2 Illicit drug ingesting

To estimate the daily drug consumption, the formula expressed in equation 1, which means:

Daily drug consumption (mg/d/1000 inhabitants) = [C*F*(R/E)]/P (eq. 1). (1)

where C is the concentration of the target biomarker, F is the total flow during the sampling, R is the ratio of the parent drug to its metabolite, E is the average excretion rate of the drug, and P is the population using the WWTP. Notably, COC consumption is prevalent in North and South America, Asia, and Europe, while amphetamine-type substances are most popular in Asia, North America, Oceania, and Europe [18, 19]. According to a study conducted by Tscharke et al. (2016) in Australia, Cannabis showed consistent consumption rates throughout the week. However, the consumption of stimulants (such as COC, and AMP) and some new psychoactive substances (NPS) (synthetic cathinone, 3,4-methylenedioxypyrovalerone, and methylone) was found to be higher during the weekends [20]. The main objective of this article is to identify the occurrence and distribution of illicit drugs and their metabolites in the WWTP of municipalities with adequate sewage coverage systems, in terms of water and sewage services, in the Hydrographic Regions of the State of Rio de Janeiro (HRRJ).

Table 1: Range of concentrations of biomarkers of wastewater treatment plant international

Selected biomarkers	Influent	Effluent
Cocaine	0.7-4700	0.2-530
Benzoylecgonine	5-7500	0.8-1500
Cannabis (THC-COOH)	<LOQ-2590	<LOD-169
Amphetamine	<LOQ-4310	<LOD-210
Methamphetamine	<LOQ-2000	0.4-370
MDMA or Ecstasy (3,4-(methylenedioxy)methylamphetamine)	<0.5-455	<LOD-376
MDA (3,4-(methylenedioxy)amphetamine)	ND-1637	ND-902
MDEA (3, 4-methylenedioxy-N-ethyl-amphetamine)	1.4-114	ND-12

* Source: Yadav et al. [17]

2. Materials and Methods

2.1 Study area

The State of Rio de Janeiro is one of the 27 federative units of Brazil and is located in the southeast of the country. It is spread over an area of 43,780,172km^2 and is comprised of 92 municipalities that are distributed across eight government regions of the State of Rio de Janeiro (Latitude 23°0'1.3392''S, Longitude: 43°21'57.2184''W). The estimated population of the state was 17,463,349 in 2021. The state shares borders with the State of Minas Gerais (north and northwest), the State of Espírito Santo (northeast), and the State of São Paulo (southwest), and is also bounded by the Atlantic Ocean to the east and south [21].

2.2 Chemicals and Materials

The standard solutions of various drugs and their metabolites, including COC, BZE, NORC, Ecgonine Methyl Ester (EME), Cocaethylene (CET), MDMA, MDA, MDEA, MET, AMP, and 11-Nor-Delta-9-Hydroxyetrahydrocannabinol (THC-COOH), as well as Cocaine-d3, BZE-d3, EME-d3, CET-d8, MDMA-d5, MDA-d5, MDEA-d5, MET-d5, AMP-d6, THC-COOHd3, in methanol (MeOH) or acetonitrile (ACN), were purchased from LGC Standards (São Paulo, Brazil). The solvents used in this study, namely, methanol (MeOH) and acetonitrile (ACN), were of high purity and obtained as HPLC grade (Hipersolv Chromanorm) from VWR (São Paulo, Brazil) (Scharlab Brazil S/A). f Formic acid (FA) (Normapur) and ammonium formate (AF) (Normapur) were also purchased from VWR for use in the liquid chromatography-mass spectrometry (LC-MS) analysis. Ultra-pure water was produced through a series of Milli-RO reverse-osmosis filtration and the Milli-Q Plus water purification system (Merck Millipore, Rio de Janeiro, Brazil). Solid Phase Extraction (SPE) was performed using Oasis HLB cartridges (500 mg/6 mL) and Xbridge Phenyl 3.5 mm, 3 mm×150 mm HPLC columns, both were purchased from Merck (Rio de Janeiro, Brazil). The instrumental analysis was conducted on a Thermo Scientific® LC system equipped with a pump (Accela 600 pump) and an autosampler, which was connected to a triple quadrupole mass spectrometer (Thermo Scientific TSQ Quantum Access Max, Thermo Scientific™, Belo Horizonte, Brazil) and operated in the negative electrospray ionization mode.

Table 2: Characteristics of the investigated wastewater treatment plants

Municipality	WWTP	Population assisted	Type of treatment	Hydrographic Region
Resende	Alegria	50,000	Anaerobic Sludge Blanket	III
Petrópolis	Quitandinha	70,000	Biofiltration	IV
	Palatinato	65,000	Biofiltration	
Niterói	Icaraí	75,700	Activated sludge	V
Rio de Janeiro	Ilha do Governador	250,000	Activated sludge	V
	Pavuna	120,000	Activated sludge	
	Barra da Tijuca	394,037	Activated sludge	
Cabo Frio	Cabo Frio	222,528	Anaerobic Sludge Blanket	VI
Nova Friburgo	Olaria	55,433	Activated sludge	VII
	Centro	38,461	Activated sludge	
	Conselheiro Paulino	48,655	Activated sludge	
Campos dos Goytacazes	Paraíba	247,500	Activated sludge	IX
	Esplanada	202,000	Activated sludge	

2.3 Sampling location and sample collection

The samples were collected from 13 WWTPs varying in catchment size (38,461-394,037 inhabitants) and utilizing various treatment technologies, as detailed in Table 2. We selected the WWTPs in three capacity groups of equivalent inhabitants (EI): big with EI >200,000, medium with EI ranging from 50,000 to 150,000, and small with EI ranging from 10,000 to 40,000. The samples were collected from

December 27, 2022, to January 10, 2023, with each WWTP providing aliquots of composite samples from the influent, representing raw wastewater over 24 h. The 24 - hour composite samples were obtained by collecting 200 mL aliquots of sanitary effluent every 4 h using a time - proportional automatic sampler wastewater. A total of 5L of each composite sample was collected and transported to the laboratory for analysis in a cool box. Upon receipt, the samples were filtered, extracted, and the extracts were stored at 4°C before analysis [22]. The selection of WWTPs for this study was based on their EI for the municipalities′ latitude and longitude, namely Resende (22°27'45.55"S - 44°27'19.99"W), Petropolis (22°30'16.70"S - 43°10'56.38"W), Niteroi (22°52'50.75"S - 43°6'15.61"W), Rio de Janeiro (22°54'29.9988"S - 43°11'46.9968"W), Cabo Frio (22°52'43.26" S - 42°1'11.55"W), Nova Friburgo (22°17'13.69"S - 42°32'1.31"W), and Campo dos Goytacazes (21°45'16.08"S - 41°19'27.87"W). Aspects of the selected WWTPs are summarized in Table 2.

2.4 Compounds of interest

Eleven substances and metabolites were identified in wastewater (influents) as exposed in Table 3.

Table 3: Compounds of interest (human metabolic residues)

Psychoactive drug (Illicit drug)	Compound of interest	Abbreviation
THC	11-Nor-9-carboxy-Δ9-tetrahydrocannabinol	THC-COOH
Cocaine	Cocaine	COC
	Benzoylecgonine	BE
	Cocaethylene	COE
	Ecgonine methyl ester	EME
	Nor-cocaine	NCOC
Amphetamine	Amphetamine	AMP
Methamphetamine	Methamphetamine	MAMP
Ecstasy	3,4-Methylenedioxymethamphetamine	MDMA
	3,4-methylene-dioxy-N-methylamphetamine	MDHMA
	3,4-methylenedioxyamphetamine	MDA

2.5 Analytical methods: Solid Phase Extraction

The analytical methodologies employed in this study were based on the approach proposed by Hernández et al. (2018), which involved several steps. Firstly, samples were spiked with stable isotope-labeled internal standards for each analyte to correct for matrix interferences and/or losses during sample treatment. Secondly, solid particles were removed from the samples via filtration or centrifugation. Thirdly, offline solid-phase extraction (SPE) was utilized for pre-concentration and clean-up of the samples. Finally, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was employed for the analysis of the samples [23]. Isotopically labeled compounds were added to 250mL of influent (250µL of a methanolic solution of 200µg/L of an individually deuterated compound). The SPE cartridges were conditioned by eluting 2mL of methanol and 2mL of ultrapure water, followed by percolation of the samples were percolated at a flow rate of 2mL/min. The cartridges were then washed with 2mL of ultrapure water and dried for 30 min. The analytes were eluted by adding 2mL of methanol, and the eluates were evaporated to dryness using nitrogen. The resulting extracts were reconstituted in 500mL of methanol and stored frozen. For the LC-MS/MS analysis, a volume of 5µL was injected into the chromatograph.

2.6 LC-MS/MS methodology

Chromatographic separation was executed employing a gradient elution with a flow rate of 0.4mL/min, consisting of solvents A(acetonitrile) and B (ammonium formate buffer, 5mM, pH 4). The gradient was programmed as follows: 0-3min, 2% solvent A; 3-22min, increase to 90% solvent A; 22-24min, 90% solvent A; 24-24.5min decrease to 2% solvent A; 24.5-30min, 2% solvent A. Methanol was used for the calibration standards, with a range of 12.5-400µg/L for all target compounds. A calibration curve was drawn for each compound using the internal standard method with the addition of labeled compounds.

2.7 Method validation

The performance of the analytical methods was evaluated based on the approach proposed by Castiglioni et al. (2013), which involved the determination of several key parameters. These parameters included linearity, limits of detection (LOD), limits of quantification (LOQ), filtration recovery (FR), extraction recovery (ER), matrix effect (ME), accuracy, and repeatability on at least two concentration levels. The evaluation also included retention times with standard deviations, optimization results indicating acceptable peak shapes, injection repeatability, and column efficiency, as shown in Figure 1 [24].

2.8 Drug consumption

The analysis of wastewater entering municipal WWTPs facilitates the estimation of illicit drug consumption through a back-calculation methodology that takes into account the populations connected to the WWTP [25]. Specifically, this involves the measurement of parent substances such as AMP, MET, and MDMA, as well as two urinary metabolites, (BZE for cocaine and THC-COOH for Cannabis in the influent wastewater [26]. The community usage of drugs is an essential parameter for WBE back-calculation [27]. To determine the consumption per capita (mg/day/1000 inhabitants, the concentrations (ng/l) were multiplied by the wastewater daily flow rates (L/day) and divided by the population served by each WWTP. This gave the population-normalized loads (mg/1000 people/day) for detecting drug-target residues (DTR) in the age group 15-64 years, using Equation 2 as proposed by Zuccato et al. (2008) [12]:

$$Q\ (\text{mg/day/1000 inh.}) = Q_{day}/U_{ex} \times M_{ratio} \times 1000/N_{inh}$$

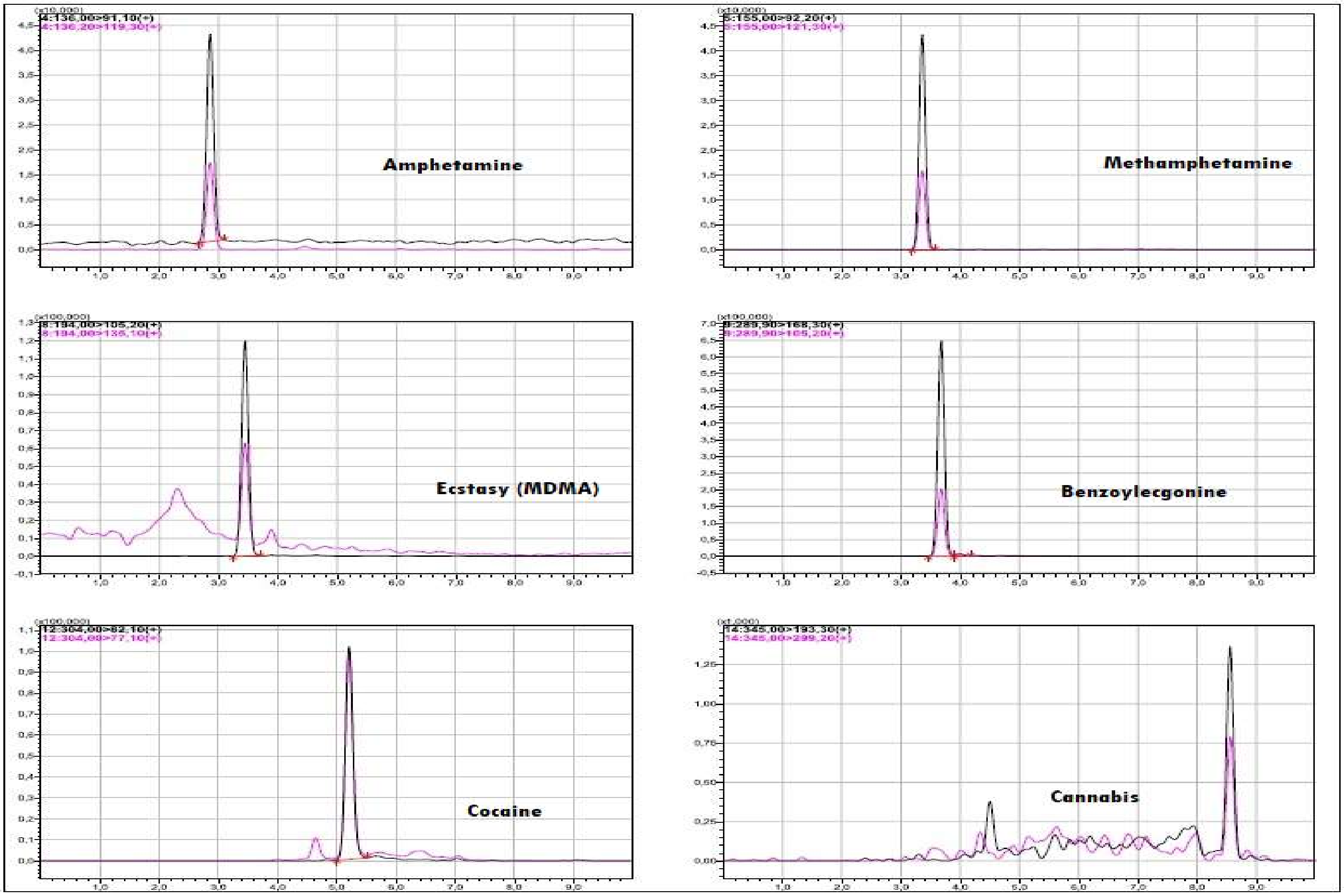

Figure 1: LC-MS/MS chromatograms of detected substances (AMP, MAMP, MDMA (Ecstasy), BE, COC, THC-COOH) in real wastewater samples representing concentrations in ng/L

Q_{day} is the load of the drug target residue, U_{ex} is the percentage of the drug target residue urinary excretion, M_{ratio} is the parent drug/drug target residue molar ratio and N_{inh} is the number of inhabitants served by the WWTP. The schematic design of the methodology outlined in this study is presented in Figure 2. This methodology has become an important tool for monitoring patterns and trends in drug consumption in municipalities and allows for the tracking of habits and lifestyles, obtaining associated results in health, education, and crime [28].

2.9 Statistical Analysis

Statistical analyses were performed using the Origin 9 software (OriginLab).

3. Results and Discussion

3.1 Method validation parameters

A linear response ($R^2 > 0.99$) was observed between LOQ-1000 ng/mL for all drug residues. LOD and LOQ were established to be within the ng/L range (Table 4). The analytical procedure relies on solid phase extraction (SPE) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the analysis of drugs in sewage, according to van Nuijs et al. [29].

3.2 Incidence of illicit drugs and metabolites

Out of the 11 drug residues targeted in the study, six displayed a high detection frequency (DF) of ≥50% in the wastewater influents. The quantities of drug residues in wastewater influents are closely linked, among other factors such as excretion rate [30]. The overall frequency of detection for each of the target compounds in the influent sewage water samples analyzed is shown in Table 5. Additionally, the mean concentrations and the concentration ranges (expressed in ng/L) of each compound per location are also displayed. Six types of illicit drugs and/or metabolites were found in at least one of the influents analyzed. The samples from Cabo Frio showed the highest mean concentrations in influents for BZE (2448ng/L), COC (534ng/L), the Cannabis metabolite (THC-COOH, 433ng/L), and MDMA (196ng/L). This finding might be attributed to the fact that Cabo Frio is a significant traveler hub, experiencing high MDMA and COC consumption, and being a crucial part of the Fluminense tourism route, serving as the main destination of the so-called Costa do Sol. However, the highest levels of AMP were found in influents from Barra da Tijuca, with concentrations ranging from 286 to 979ng/L, and an average of 781ng/L. No drug residues were detected at the Alegria WWTP. According to Zuccato et al. (2008), the measured AMP concentration can be influenced by the presence of MET [12]. The highest mean AMP concentration was measured in Barra da Tijuca WWTP (781ng/L). The AMP concentration was not detected on all WWTPs.

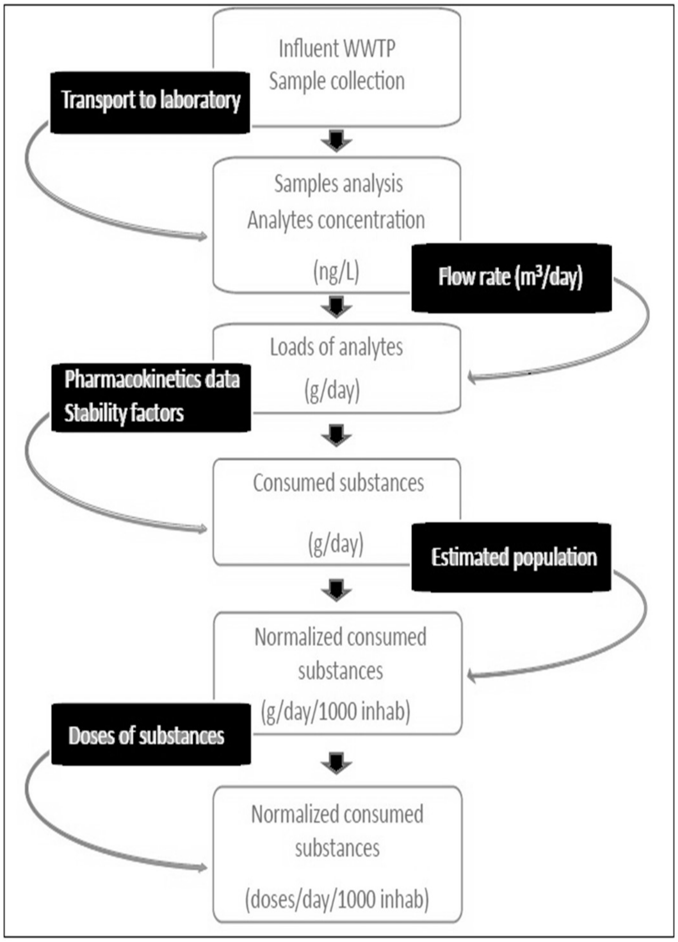

Figure 2: Sampling flow schematic diagram

3.3 Drug consumption values (mg/day/1,000 inhabitants)

Equation 2 enables the determination of the number of used drugs and metabolites [12]. This involves deriving the daily amounts of the targeted drugs and metabolites based on their concentrations in the sewage flow. The resulting values correspond to the per capita number of drugs and/or metabolites in the wastewater relative to the population on a daily basis. Ferreira (2021) underscored the highly addictive nature of AMP, and as a result, its users tend to be dependent and consume the drug daily [31, 32]. The Barra da Tijuca WWTP exhibited the highest average amphetamine level during the examination period, measuring 104mg/day, while the lowest value was observed at Ilha do Governador WWTP measuring 21mg/day. Regarding MET, the Barra da Tijuca WWTP had the highest mean specific load at 42mg/day, followed by Pavuna WWTP with31mg/day, Cabo Frio WWTP with 12mg/day, and Ilha do Governador WWTP with 8mg/day. The presence of high levels of MET in WWTPs has been linked to higher crime rates in the surrounding areas. These findings are consistent with statistics on AMP and Ecstasy use. Similar to AMP, MET is a highly addictive drug, and the measured values indicate that its consumption is consistent among users [33]. The levels of MDMA (or Ecstasy) were found to be highest in two of the WWTPs, namely Cabo Frio (21mg/day) and Pavuna (23.8mg/day), with mean values of 16.7mg/day, 11.8mg/day, 10.4mg/day, 9.6mg/day, 8.5mg/day, and 8mg/day for Ilha do Governador, Barra da Tijuca, Olaria, Centro, Icaraí, and Conselheiro Paulino. These findings were compared to those measured in a study conducted in Santiago de Compostela, Brussels, Milan, Zagreb, and Turku [34]. In a drug survey in 19 European cities, Thomas et al. (2012) reported that the highest quantities of Ecstasy were found in the Dutch cities of Amsterdam, Eindhoven (67mg/day), and Utrecht (615mg/day) [35]. However, our investigation indicates that the mean specific levels at Ilha do Governador WWTP and Pavuna WWTP were significantly lower than those observed in the aforementioned cities. The amount of COC used by consumers was found to be highest at Cabo Frio WWTP, with a mean value of 98mg/day, followed by Barra da Tijuca WWTP (65mg/day), Icaraí WWTP (22mg/day), Ilha do Governador WWTP (26mg/day), Pavuna WWTP (17mg/day), Conselheiro Paulino WWTP (8mg/day), Quitandinha WWTP (5mg/day), and Olaria WWTP (5mg/day). BZE is the main metabolite of COC and is relatively stable in sewage wastewater. Although is the most abundant metabolite of COC, the values reported for BE excretion results from the different routes of administration of COC vary from 24 up to 52% for intranasal and intravenous administrations [12, 24]. Data obtained related to BE amount were found in Cabo Frio WWTP (190mg/day), Barra da Tijuca WWTP (79mg/day), Icaraí WWTP (40 mg/day), Ilha do Governador WWTP (47.5mg/day), Pavuna WWTP (33.3 mg/day), Olaria WWTP (9.8mg/day), Quitandinha WWTP (9.5mg/day), and Conselheiro Paulino WWTP (7.6mg/day). Cannabis (marijuana) was the most frequently used soft drug in the researched areas; probably due to being cheap and, unfortunately, easily available. The mean concentration of its secondary metabolite (THC-COOH) was greater in coastal areas such as Cabo Frio and the population areas of the State with a lower income population, but which are mostly those where the highest number of drug seizures occur, thus presenting an expressive regional drug trade. Data obtained related to THC-COOH amount were found in Cabo Frio WWTP (432mg/day), followed by Barra da Tijuca WWTP (231mg/day), Pavuna WWTP (211mg/day), Conselheiro Paulino WWTP (211mg/day), Ilha do Governador WWTP (189mg/day), Icaraí WWTP (181mg/day), Esplanada WWTP (177mg/day), Paraíba WWTP (136mg/day), Centro WWTP (107mg/day), Olaria WWTP (99mg/day), Quitandinha WWTP (82mg/day), and Palatinato WWTP (67mg/day).

Table 4: Validation parameters for wastewater influent

Analyte	R^2 (ng/ml)	LOD (ng/L)	LOQ (ng/L)	Concentration level used for validation (ng/ml)	Recovery (%)	Inter-day repeatability (%RSD)	Instrumental repeatability (%RSD)
THC-COOH	LOQ-750 (0.9957)	0.797	2.32	50	108	5	4
				250	98	4	3
COC	LOQ-1,000 (0.9950)	0.294	0,980	50	105	9	1
				250	99	3	1
BE	LOQ-500 (0.9970)	0.553	1.4	50	99	8	1
				250	99	4	2
AMP	LOQ-300 (0.9950)	0.318	0.989	50	96	10	2
				250	98	4	1
MAMP	LOQ-300 (0.9901)	0.0948	0.316	50	102	10	1
				250	102	4	1
MDMA	LOQ-300 (0.9900)	0.258	0.859	50	103	9	3
				250	101	6	3

*AMP-Amphetamine, BZE-Benzoylecgonine, COC-Cocaine, R^2-Linearity range, MAMP-Methamphetamine, MDMA-3,4-methylenedioxymethamphetamine, THC-COOH-11-nor-9-carboxy-△9-tetrahydrocannabinol. Source: van Nuijs et al. [29].

Table 5: The overall detection frequency and mean concentrations (ng/L) of drug residues in influent sewage water samples from six WWTPs in Rio de Janeiro

WWTP	HRRJ	Compounds					
		AMP	MAMP	MDMA	COC	BE	THC-COOH
Alegria	III (Resende)	-	-	-	-	-	-
Quitandinha	IV (Petropólis)	-	-	-	29 (13–52)	108 (82–147)	82 (56–104)
Palatinato		-	-	-	-	-	67 (34–84)
Icaraí	V (Niterói)	155 (107-216)	61 (37-94)	81 (44–122)	205 (118-292)	231 (148–303)	181 (133–303)
Ilha do Governador	V (Rio de Janeiro)	85 (29-109)	36 (22-55)	155 (109-187)	115 (55-149)	612 (467-878)	189 (109-261)
Pavuna		104 (81-158)	56 (32-67)	221 (178-259)	98 (72-143)	444 (251-553)	211 (139-245)
Barra da Tijuca		781 (286-979)	242 (132-367)	109 (88-166)	329 (203-485)	896 (657-1433)	231 (155-322)
Cabo Frio	VI (Cabo Frio)	286 (218-367)	54 (27-85)	196 (122-258)	428 (315-628)	2448 (1950-2783)	432 (333-501)
Olaria	VII (Nova Friburgo)	-	-	97 (66–145)	22 (16–45)	125 (77–198)	99 (67–157)
Centro		-	-	89 (67–129)	-	-	107 (76–155)
Conselheiro Paulino		-	-	76 (39-88)	40 (23–78)	99 (63-167)	209 (143-238)
Paraíba	IX (Campos dos Goytacazes)	-	-	-	-	-	136 (74–184)
Esplanada		-	-	-	-	-	177 (142–229)

*AMP-Amphetamine, MAMP-Methamphetamine, MDMA-3,4-methylenedioxymethamphetamine, COC-Cocaine, BE-Benzoylecgonine, THC-COOH-11-nor-9-carboxy-△9-tetrahydrocannabinol, HRRJ-Hydrographic Region of the State of Rio de Janeiro, LOQ-limit of quantification, (-) below LOQ

Cannabis levels were found to be significantly higher in some WWTPs than in others and were generally comparable or lower than those reported in other studies, such as those conducted in some European cities, such as Amsterdam (192mg/day), and Paris (124mg/day) [35]. It is possible to speculate that obtaining more specific data by investigating different WWTPs in the city and correlating them with income per capita, employment, and level of education could be useful in identifying appropriate strategies and actions and effective health education policies. However, further studies are needed to explore these epidemiological aspects. Despite the problems and the limitations described in other studies, WBE can be considered an additional tool to the available indirect indicators of drug consumption and abuse, which can help institutions understand and monitor drug addiction and the effectiveness of health education policies for drug prevention.

4. Conclusion

The present study evaluated the presence of illicit drugs and metabolites in WWTPs located across different HRRJ and identified the patterns of drug consumption in the region. The lack of data on illicit drugs in sewage across Brazil, even with specific studies, served as the inspiration for investigating illicit drugs in wastewater from several WWTPs in Rio de Janeiro, considering the cities with larger populations. The use of illicit drugs is a sensitive issue that cannot be directly revealed through epidemiological surveys due to ethical reasons, especially since drug use and dependence are illegal. This can lead to biased responses and make questionnaire-based studies less reliable, particularly since drug users may be unaware of the actual drug or substance mixture they are consuming. In contrast, WBE offers more realistic data, revealing drug use to its true extent and allowing estimation of the amount consumed and the types of drugs present in the population. We used the data obtained from the WWTPs to compare them with data obtained in other studies, which allowed us to gain a deeper understanding of the problem and the method used. The comparison showed differences in drug consumption, mainly due to population size and other characteristics. The urgent need for wastewater-based monitoring of illicit drugs arises from the fact that policymakers need more evidence-based facts and up-to-date information on trends and amounts of consumption to combat illicit drugs more effectively.

Authors' Contributions

Aldo Pacheco Ferreira: study design; data collection; conducting statistical analysis; manuscript revision. Eduardo Dias Wermelinger: providing administrative, technical, and material support. Maria José Cruz-Hernández: manuscript revision.

Funding

The Project receives a research productivity grant from the National Council for Scientific and Technological Development (CNPQ).

Conflicts of Interest

The Authors declare that there is no conflict of interest.

Acknowledgements

The authors express their gratitude to the Universitat Jaume I for providing some of the standards used and for kindly analyzing sewage samples. They also extend special thanks to the National Council for Scientific and Technological Development (CNPq). This work was approved by the Research Ethics Committee, Sergio Arouca National School of Public Health, Protocol 07/2018.

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