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Wastewater-Based Epidemiology In Estimating Community Consumption Of Psychotropic Substances As Auxiliary Monitoring In Health System Management

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Abstract:
The illicit use of psychotropic substances due to the systematization of their consumption has become a global problem and, therefore, we seek to obtain accurate and timely information about their use by the population. However, traditional survey-based monitoring methods and reporting systems have some limitations, including data accuracy, relevance to the population, and long reporting delays in a rapidly changing drug landscape. These limitations are problematic when using the data collected to develop health and policing policies. Community wastewater analysis has been suggested as an objective and rapid method for providing supplemental data. Quantitative determination of urinary biomarkers in raw wastewater has emerged in recent years as a promising tool for estimating illicit drug consumption. Wastewater-based epidemiology seeks to obtain chemical residues and can provide suitable material for consumption by the population through the analysis of wastewater samples collected in the influent of a sewage treatment plant. Drug detection research conducted in Brazil still uses traditional monitoring methods and survey-based reporting systems, which have some limitations, including the accuracy of the data collected, its relevance to the population, and prolonged delays in reporting and reporting. action. It therefore provides data that is always out of date, especially in a drug landscape that changes routinely. Reproducible and characteristic profiles of illicit drug use were obtained in the thirteen regions analyzed in the State of Rio de Janeiro, revealing local consumption. Local drug use profiles based on wastewater measurements are in line with annual national prevalence estimates.

Keywords*: Illicit drugs; Wastewater-based epidemiology; Psychotropic substances; Drug consumption; Human rights; Violence; Public health.*

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I. Introduction

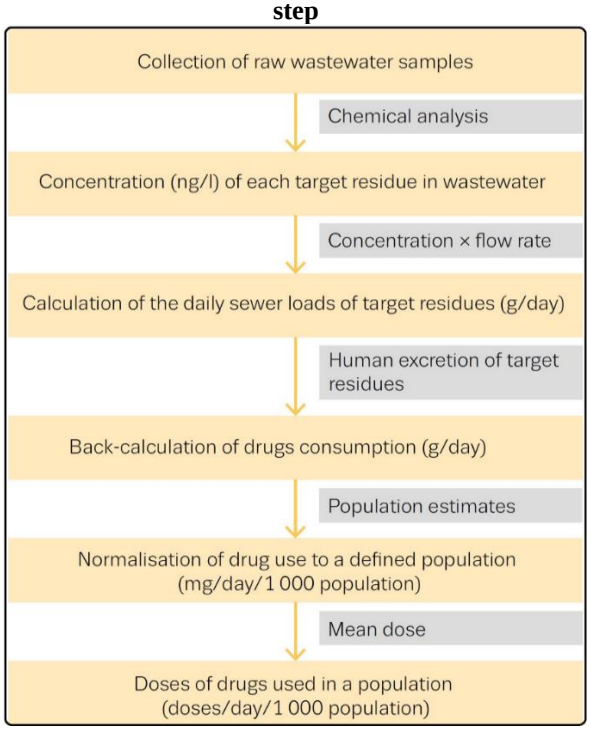
Wastewater-based epidemiology (WBE) consists in acquiring relevant information about the lifestyle and health status of the population through the analysis of wastewater samples collected at the influent of a wastewater treatment plant (Zuccato *et al.*, 2005a).

Analysis of wastewater for indicators of illicit drug use was one of the first proposed applications of WBE (Daughton, 2001). It has been successfully applied to estimate illicit drug consumption and is based on the chemical analysis of specific drug metabolites in raw wastewater as indicators of the ingestion of the parent drug (van Nuijs *et al.*, 2011a). This approach has great potential to complement current epidemiological methods in view of the objective, up-to-date results, and it can, therefore, be extended to a wider range of substances (Zuccato *et al.*, 2005a).

Thus, measuring target drug metabolic residues in raw wastewater allows the identification of the use of specific substances by a population. In WBE, the residual level of drug biomarkers in raw wastewater is used to retroactively calculate community drug consumption, which can reveal trends and subsidize public health policies (Been, Esseiva, and Delemont, 2016; Ferreira, Wermelinger, and Cruz-Hernández, 2023). According to European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (EMCDDA, 2016), the method consists of several consecutive steps that allow researchers to identify and quantify target metabolic residues of illicit drugs in raw wastewater and back-calculate the amount of the corresponding illicit drugs that would have been consumed by the population served by the wastewater treatment plant (WWTP). After entering the sewer network, these excreted agents arrive at a WWTP where wastewater samples can be collected over a defined sampling period. The general scheme for this approach is outlined in Figure 1.

The prevalence of drug use and dependence is growing as a global threat to social and economic well-being (UNODC, 2023a). Traditionally, the incidence of drug use has been valued based on a combination of toxicology reports, self-reported survey questionnaires, and crime statistics. Conservative approaches suffer from high cost, limited coverage, generating delays for the immediate need for intervention, including non-response bias in the selection of the sample from the population with the highest drug use; therefore, potentially underestimating actual drug use (Palamar, Salomone, and Keyes, 2021). In Brazil, the illicit drug epidemic continues to prevail, with the key factor being the increase in deaths related to drug use, which is associated with thousands of tragedies and family miseries. The cost of social capital, directly or indirectly related to the production, trafficking, and consumption of illicit drugs, is also enormous (Bastos *et al.*, 2017). Indeed, timely, cost-effective, and comprehensive measurement of substance use prevalence has never been more imperative before.

Figure 1. The main consecutive steps of the wastewater analysis approach and the data required for each step



Source: EMCDDA (2016)

Illicit drugs can be categorized into cannabis, opioids, stimulants, hallucinogens, inhalants, steroids, non-medical use of prescription medications, etc. (Klanjšek, Vazsonyi and Javakhishvili, 2023). Among all these drugs, after the primordial use of cannabis, stimulants constitute a special group for two reasons. Firstly, its use is frequent in the population, due to the fact of many detections in various areas by investigative police, indicating a higher global prevalence of amphetamine-type stimulants, including methamphetamine and 3,4-methylenedioxy-N-methylamphetamine (MDMA) (UNODC, 2023). Second, the pattern of stimulant use can change rapidly over time (UNODC, 2023), making it difficult to obtain timely and accurate information on its prevalence through traditional monitoring methods.

Illegal drugs enter the sewerage system either through human consumption or their illegal dumping. The parent drug may get excreted as it is or in the form of metabolites after drug administration. Benzoylecgonine is the major urinary metabolite of cocaine. Other minor human metabolites of cocaine consumption include norbenzoylecgonine and norcocaine. However, when alcohol and cocaine are co-consumed, another metabolite, cocaethylene, is formed due to the transesterification of cocaine (Wang *et al.*, 2021).

Amphetamine itself is a drug, as well as the metabolite of methamphetamine consumption. MDA (3,4-methylenedioxyamphetamine) is the common metabolite of two drugs, MDMA (3,4-methylenedioxy-N-methylamphetamine or Ecstasy) and MDEA (3,4-methylenedioxy-N-ethylamphetamine) (González-Mariño *et al.*, 2017).

Cannabinoids are a group of organic compounds found in cannabis. Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), the two major constituents of cannabinoids, and their metabolites are contaminants of emerging concern due to the limited information on their environmental impacts. As well, their releases to the water systems and environment are expected to increase due to recent legalization. Solid-phase extraction is the

most common 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. Liquid chromatography coupled with mass spectrometry is the most common technique used for the analysis of cannabinoids. THC and its metabolites, in particular, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) has been detected at concentrations up to 2590 and 169 ng/L in untreated and treated wastewater, respectively (How and Gamal El-Din, 2021).

The primary source of occurrence of illicit drugs and metabolites in the environment is the wastewater effluent containing these residues due to inefficient removal at WWTP (Yadav *et al.*, 2017). Table 1 shows the range of concentrations of selected biomarkers detected worldwide in different geographical regions (Australia, Europe, USA, South America, and Asia) in the wastewater influent and effluent.

Table 1. Variability of concentration of selected biomarkers in influent and effluent of wastewater treatment plants worldwide

Drugs and metabolites	Influent	Effluent
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
Cocaine	0.7-4700	0.2-530
Benzoylecgonine	5-7500	0.8-1500
Cannabis (THC-COOH)	<LOQ-2590	<LOD-169

LOD - Limit of Detection; LOQ - Limit of Quantification; nd - not detected

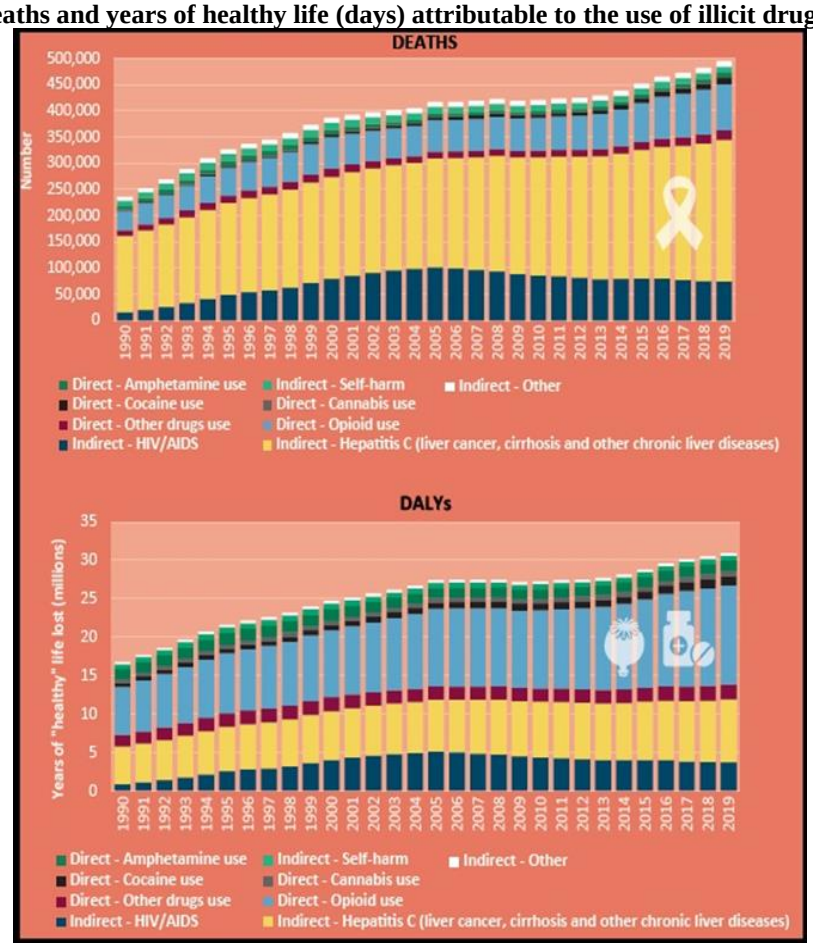
The increasing use of illicit drug and the non-medical use of prescription drugs are a growing concern for public health authorities (NIH, 2018). The impact of this use on society can be classed into two types: tangible and intangible costs. Tangible costs are the quantifiable costs to society, which can be categorized as those resulting from (1) workplace labour, (2) labour in the household, (3) healthcare and (4) crime. Examples of the quantifiable costs because of illicit drug use from each category are briefly elaborated; (1) reduction in the workforce and absenteeism, (2) premature death and illnesses, (3) pharmaceuticals, hospital, and medical costs, and (4) policing, courts, and prison (Cartwright, 2008).

Data from UNODC (2023b) deaths related to the use of illicit drugs were estimated at 500,000 in 2019, 17.5 per cent more than in 2009. Liver diseases attributed to hepatitis C are a major cause of drug-related deaths, accounting for more than half of the total number of deaths attributed to the use of drugs. Drug overdoses account for a quarter of drug-related deaths. Opioids continue to account for the most severe drug-related harm, including fatal overdoses, when used non-medically. At the global level, two-thirds of direct drug-related deaths are due to opioids, and in some subregions, the proportion can be as high as three-quarters of such deaths. More men than women die of drug overdose but excess mortality risk in women who use drugs is typically higher than in men (owing to lower mortality rates among women of corresponding age in the general population) (Figure 2).

According to Murray and Kiernan (2024), drug use becomes abusive at the appearance of dependence, which affects the set of physiological, behavioural, and cognitive manifestations in which the person prioritizes the use of a drug. This term is usually linked to the need to consume more substances to achieve the effects of previous use (Barrio *et al.*, 2017). Likewise, when a dependent person does not consume, withdrawal syndrome begins to appear. It is a cluster of symptoms that affect an individual who is suddenly deprived of any toxin or drug on which he/she is physically dependent, and which previously had been consumed regularly. The number of symptoms, as well as their intensity and duration, will depend on the type of drug, the length of time the person has consumed the substance and his/her physical and psychological state at the time of withdrawal (Devlin and Henry 2008).

Drug abuse or drug addiction is a situation when a person is addicted, he cannot control the situation of his drug use his nature of using drugs gets control over him. In other terms, when a drug abuser loses the capability to make a rational choice about whether to use drugs or alcohol, he or she is addicted. Drug addiction is a condition whereby a person experiences an overpowering thirst to look for and use drugs or alcohol regardless of the negative physical and mental consequences. Typically, drug abuse is accompanied by physical and psychological dependence on the drug and the person suffers withdrawal symptoms when the frequency or the content of the use of the drug is rapidly decreased or stopped (Schindler, 2019).

Figure 2. Deaths and years of healthy life (days) attributable to the use of illicit drugs, 1990-2019



Source: UNODC (2023b)

The main objective pursued within the study was to evaluate for the occurrence of drugs of abuse and metabolites residues in the Hydrographic Regions of the State of Rio de Janeiro (HRRJ, Brazil), as [amphetamine (AMP), methamphetamine (METH), 3,4-methylenedioxymethamphetamine (MDMA), cannabis, and cocaine] in samples of the researched metabolites obtained from tributaries due to on-site consumption by residents aged 15 to 64 years.

II. Material And Methods

Study area

The State of Rio de Janeiro is one of the 27 federative units of Brazil. It occupies an area of 43,780,172 km². It is composed of 92 municipalities, which are distributed in 8 government regions of the State of Rio de Janeiro (Latitude 23°0'1.3392" S, Longitude: 43°21'57.2184" W). It is in the southeast of the country's Southeast region, bordering the State of Minas Gerais (north and northwest), State of Espírito Santo (northeast) and State of São Paulo (southwest), in addition to the Atlantic Ocean (east and south) (IBGE, 2017).

Chemicals and materials

Standard solutions: cocaine (COC), benzoylecgonine (BZE), norcocaine (NOR), ecgonine methyl ester (EME), cocaethylene (CET), 3,4 -methylene – dioxy - N-methylamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4 – methylenedioxy - Nethylamphetamine (MDEA), methamphetamine (MET), amphetamine (AMP), 11-nor-delta-9-hydroxytetrahydrocannabinol (THC-COOH), cocaine-d_3, BZE-d_3, EME-d_3, CET-d_8, MDMA-d_5, MDA-d_5, MDEA-d_5, MET-d_5, AMP-d_6, THC-COOHd_3, in methanol (MeOH) or acetonitrile (ACN).

Chemicals: MeOH and ACN, HPLC grade (Hipersolv Chromanorm), formic acid (FA) (Normapur) and ammonium formate (AF) (Normapur). Ultra-pure water was produced using successive Milli-RO reverse-osmosis filtration and the Milli-Q Plus water purification system.

The instrumental analysis was performed with a Thermo Scientific® LC system equipped with a pump (Accela 600 pump) and an autosampler coupled with a triple quadrupole mass spectrometer, operated in the

electrospray negative ionization mode. Solid Phase Extraction (SPE) cartridges Oasis HLB (500mg/6mL) and Xbridge Phenyl 3.5mm, 3 mm×150mm HPLC column.

Sampling location and sample collection

For this research were selected the cities from Rio de Janeiro State: Resende (Latitude: 22°27'45.55"S and Longitude: 44°27'19.99"W), Petropolis (Latitude: 22°30'16.70"S and Longitude: 43°10'56.38"W), Niteroi (Latitude: 22°52'50.75"S and Longitude: 43°6'15.61"W), Rio de Janeiro Municipality (Latitude: 22°54'29.9988" S and Longitude: 43°11'46.9968" W), Cabo Frio (Latitude: 22°52'43.26"S and Longitude: 42°1'11.55"W), Nova Friburgo (Latitude: 22°17'13.69"S and Longitude: 42°32'1.31"W), and Campos dos Goytacazes (Latitude: 21°45'16.08"S and Longitude: 41°19'27.87"W). Aspects of the selected WWTPs are summarized in Table 2.

The samples were collected from 13 WWTPs varying in catchment size (38,461-394,037 inhabitants) and type of treatment technology expressed in Table 3. They were selected to have representative capacities, Rio de Janeiro locations and types of treatment. We selected the WWTPs in three capacity groups of *Equivalent Inhabitants* (EI): big with EI>200,000 EI, medium with EI ranging from 50,000 to 150,000 EI and small with EI ranging from 10,000 to 40,000 EI.

Table 2. Characteristics of the investigated wastewater treatment plants

Municipality	Wastewater treatment plant	Population assisted
Cabo Frio	Cabo Frio	222,528
Niterói	Icaraí	75,700
Resende	Alegria	50,000
Petrópolis	Quitandinha	70,000
	Palatinato	65,000
Rio de Janeiro City	Ilha do Governador	250,000
	Pavuna	120,000
	Barra da Tijuca	394,037
Nova Friburgo	Olaria	55,433
	Centro	38,461
	Conselheiro Paulino	48,655
Campos dos Goytacazes	Paraíba	247,500
	Esplanada	202,000

Every WWTP provided aliquots of composite samples from the influent, representing raw wastewater over a 24-hour period. Typically, samples were obtained for 15 consecutive days (from October 4, 2023 to October 16, 2023). At the end of sampling, 5L samples were collected in polypropylene bottles and sent to laboratory in a cool box intended to be used for 24h shipments. Upon receipt, samples were filtered and extracted according to the following protocol and the extracts were stored at 4°C before analysis (Wang *et al.*, 2021).

Compounds of interest

Human metabolic residues were targeted in wastewater (influents) as shown in Table 3.

Table 3. Compounds of interest (human metabolic residues)

Psychoactive drug (Illicit drug)	Compound of interest	Abbreviation
THC	11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol	THC-COOH
Cocaine	Cocaine	COC
	Benzoylecgonine	BE
	Cocaethylene	COE
	Ecgonine methyl ester	EME
	Norcocaine	NCOC

Amphetamine	Amphetamine	AMP
Methamphetamine	Methamphetamine	MAMP
Ecstasy	3,4-Methylenedioxymethamphetamine	MDMA
	3,4-methylene-dioxy-N-methylamphetamine	MDHMA
	3,4-methylenedioxyamphetamine	MDA

Drug consumption

The workflow in wastewater-based epidemiology model is an important tool for monitoring patterns and trends in illicit drug consumption in communities, by detecting drugs, making it possible to monitor habits and lifestyle, as well as associated outcomes in health, education, and crime (Castaldelli-Maia *et al.*, 2015).

Representative composite samples of raw wastewater are collected and analyzed for the selected substances. The back-calculation of drug consumption is performed by (a) calculating the daily sewer loads of target residues (g/day) by multiplying the concentrations of the measured target residues (ng/l) by the daily flow rates of sewage (m^3/day); (b) estimating the total consumption by applying a specific correction factor, which takes into account the average excretion rate of a given drug residue and the molecular mass ratio of the parent drug to its metabolite (Zuccato *et al.*, 2008; van Nuijs *et al.*, 2011a); (c) normalizing consumption by dividing daily values by the number of people in order to facilitate comparison among cities (mg/day/1000 population); and (d) assuming a mean dose to obtain a value in doses/day/1000 population.

The estimated illicit drug use was normalised to the number of inhabitants served by the WWTPs. The excretion rates of the selected drugs were used to calculate the drug concentrations normalised per 1000 inhabitants as well as 1000 inhabitants in the age group of 15-64 years (69.1% of the total population). The estimates of the fraction of the drugs excreted in urine provided by Zuccato, Castiglioni, and Fanelli (2005b), van Nuijs *et al.* (2011b) and van Wel *et al.* (2016) were used to obtain a consumption value for each drug within the community.

Total amount of each drug (mg/day *per* 1000 inhabitants) was back-calculated using the concentration of the parent drug and/or metabolite in influent wastewater and the daily flow rate of the parent drug and/or metabolite in the WWTPs. For these back-calculations, knowledge of several parameters is essential: (I) consumption indicators, (II) excretion rates, (III) correction factors and, (IV) average drug dose (Table 4).

For the back calculations, concentrations (ng/L) of the drugs or metabolites in WWTP influents, daily flow rate (L/day) of the WWTP influent and the correction factors are used. The back-calculation equations used for cocaine (equation 1), amphetamine (equation 2), methamphetamine (equation 3), MDMA (equation 4), and THC (equation 5) as follows:

Cocaine (g/day) = concentration of benzoylecgonine (ng/L) * flow rate (L/day) * 2.33	***equation*** (1)
Amphetamine (g/day) = concentration of amphetamine (ng/L) * flow rate (L/day) * 3.3	***equation*** (2)
Methamphetamine (g/day) = concentration of methamphetamine (ng/L) * flow rate (L/day) * 2.3	***equation*** (3)
MDMA (ecstasy) (g/day) = concentration of MDMA (ng/L) * flow rate (L/day) * 1.5	***equation*** (4)
THC (marijuana) (g/day) = concentration of THC-COOH (ng/L) * flow rate (L/day) * 10	***equation*** (5)

Table 4. Parameters used in the back-calculation process of each illicit drug

Illicit drug	Illicit drug target residue	Illicit drug excretion dose as target residue (%)	Correction factor	Dose (mg)
Amphetamine	Amphetamine	30[e]	3.3[e]	30[e]
Cocaine	Benzoylecgonine/Coca ethylene	45[e]	2.33[c]	100[d]
MDMA (Ecstasy)	MDMA	65[e]	1.5[e]	100[d]
Methamphetamine	Methamphetamine	43[e]	2.3[e]	50[e]
THC (Marijuana)	THC-COOH	0.6[e]	100[b]	125[a]

[a]Postigo, López de Alda, and Barceló (2010), [b]van Wel *et al.* (2016), [c]Zuccato, Castiglioni, and Fanelli (2005b), [d]Terzic, Senta, and Ahel (2010), [e]van Nuijs *et al.* (2011b)

Analytical methods: Solid Phase Extraction

All chemical analyses were performed by *Laboratorio de Técnicas Espectroscópicas* (LABTE), Rey Juan Carlos University, Madrid, Spain.

Analytical methodologies were based according to Hernández *et al.* (2018), which generally consisted of: *(i)* spiking samples with stable isotope-labelled internal standards (SILIS) for each analyte, in order to correct for matrix interferences and/or losses during sample treatment; *(ii)* filtration or centrifugation of samples to remove solid particles; *(iii)* off-line solid-phase extraction (SPE) for pre-concentration and clean-up; and *(iv)* analysis by liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS). Samples were filtered on

glass fiber filters (1mm, GF/B Whatman) before SPE extraction. Isotopically labelled compounds were added to 250mL of WWTP influent (250µL of a 200µg/L methanolic solution of each deuterated compound). Cartridges were conditioned by following elution of 2×5mL MeOH and 2×5mL ultra-pure water. Samples were percolated at a flow rate of 2mL/min. The SPE cartridges were then washed using 2×5mL ultra-pure water and dried for 30 min. Analytes were eluted with 2×5mL of MeOH and eluates were evaporated to dryness under a gentle stream of nitrogen. Extracts were reconstituted in 500mL of MeOH and kept frozen until analysis. A 5µL volume was injected for LC-MS-MS analysis.

Liquid chromatography tandem mass spectrometry (LC-MS-MS) measurement

LC-tandem MS (LC-MS-MS) coupled with electrospray ionization was used for sample analysis. LC separation was performed with a Waters 2695 high-performance LC separation module (Milford, MA, USA), and MS-MS analyses were performed using a Micromass Quattro triple-quadrupole mass spectrometer (Micromass, Manchester, UK). MS data acquisition and analysis was performed using MassLynx Version 4.0 software (Micromass, Manchester, UK).

GC–MS was performed on a Varian CP-3800 GC equipped with Varian Chrompak Saturn 2000 GC–MS and Varian CP-8400 autosampler (Palo Alto, CA). The column was Rxi-XLB (30mL× 0.25mm ID × 0.25-µm film thickness) from Restek (Bellefonte, PA). The oven temperature program was held at 60°C for 5 min, programmed at 30°C/min to 190°C and held for 9, followed by 20°C/min to 250°C and held for 3.67 min (Gago-Martínez *et al.*, 2004). The injector temperature was 260°C. The MS was operated in the full scan mode, 150-500 m/z mass range, under positive-ion electron impact conditions.

Statistical Analysis

Data analysis (counts, percentages, and means) were performed with Excel software (Microsoft 365®). All statistical analyses were performed using Origin 7.5. (OriginLab Corporation). Statistically significant differences of the median were judged by one-way analysis of variance (ANOVA) and least significant differences calculations at a 5% significant level.

III. Result

Occurrence of illicit drugs and metabolites in influent WWTP

The average occurrence of AMP – amphetamine; MAMP – methamphetamine; MDMA – 3,4-methylenedioxymethamphetamine; COC – cocaine; COE - cocaethylene; BE – benzoylecgonine; THC-COOH – 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol in the wastewater (in ng/L) of each city are presented in Table 5.

Seven out of the 11 targeted drug residues (as shown in Table 3) had 54.95% detection frequency (percentage of the samples containing drug residue >limit of quantification or not detected) in the wastewater influents. Quantities of drug residues in wastewater influents are, among others (e.g., excretion rate), closely related to the level of drug use (Deng *et al.*, 2020). The sample from Cabo Frio showed the highest mean concentration in influent for cocaine (4521 ng/L), cocaethylene (256 ng/L), and methamphetamine (255 ng/L). Barra da Tijuca showed the highest mean concentration in influent for amphetamine (381 ng/L), Ecstasy (436 ng/L), and benzoylecgonine (3783 ng/L); and Pavuna showed the highest mean concentration in influent for *cannabis* metabolite (THC-COOH) (641 ng/L).

Drug consumption values

The estimation was performed only for 5 illicit drugs, including cocaine, amphetamine, MDMA (ecstasy), and THC (marijuana) are presented in Table 6. The WWTPs researched were those that had the highest concentration of illicit drugs, such as: Icaraí, Ilha do Governador, Pavuna, Barra da Tijuca, and Cabo Frio. The applied methodology considers the known metabolic pathways of selected drugs, the molar ratio of metabolite to parent drug as well as the percentage of a selected drug target residues, excreted after consumption of the parent drug. They were performed in accordance with the guidelines proposed by Zuccato *et al.*, 2005b, Postigo, López de Alda, and Barceló (2010), Terzic, Senta, and Ahel (2010), van Nuijs *et al.* (2011b), and van Wel *et al.* (2016).

Table 5. Concentration (average ± standard deviation) of biomarkers (ng/L) in sewage samples collected in thirteen WWTPs of the Hydrographic Region, Rio de Janeiro State

WWTP	HRRJ	Biomarker						
		AMP	MAMP	MDMA	COC	BE	COE	THC-COOH
		Average ± standard deviation (ng/L)						
Alegria	III Resende	nd	nd	nd	nd	nd	nd	nd

Quitandinha	IV Petrópolis	<LOQ	124 ± 5	86 ± 5	1342 ± 74	1645 ± 72	<LOQ	104 ± 3
Palatinato		nd	<LOQ	<LOQ	<LOQ	<LOQ	nd	167 ± 6
Icaraí	V Niterói	111 ± 6	67 ± 4	122 ± 8	2732 ± 66	2265 ± 59	94 ± 13	303 ± 9
Ilha do Governador	V Rio de Janeiro Municipality	143 ± 8	119 ± 6	155 ± 8	2469 ± 96	3298 ± 77	137 ± 15	461 ± 12
Pavuna		74 ± 7	<LOQ	221 ± 7	2766 ± 72	2365 ± 96	166 ± 11	641 ± 14
Barra da Tijuca		381± 5	242 ± 6	436 ± 6	2231 ± 101	3783 ± 63	182 ± 22	512 ± 11
Cabo Frio	VI Cabo Frio	286 ± 6	255 ± 5	361 ± 7	4521 ± 52	2944 ± 84	256 ± 28	432 ± 11
Olaria	VII Nova Friburgo	<LOQ	<LOQ	<LOQ	1766 ± 37	2155 ± 88	nd	399 ± 13
Centro		<LOQ	<LOQ	<LOQ	1622 ± 73	1388 ± 65	<LOQ	207 ± 7
Conselheiro Paulino		nd	nd	<LOQ	nd	<LOQ	nd	238 ± 6
Paraíba	IX Campos dos Goytacazes	nd	nd	<LOQ	nd	<LOQ	nd	284 ± 7
Esplanada		nd	nd	<LOQ	nd	<LOQ	nd	277 ± 11

Abbreviations: HRRJ - Hydrographic Region - State of Rio de Janeiro; WWTP - Wastewater Treatment Plant; AMP – amphetamine; MAMP – methamphetamine; MDMA – 3,4-methylenedioxymethamphetamine; COC – cocaine; COE - cocaethylene; BE – benzoylecgonine; THC-COOH – 11-nor-9-carboxy-Δ9-tetrahydrocannabinol; LOQ – limit of quantification; (<) below LOQ; nd – not detected.

The representative excretion rates of the selected drug target residues were applied to calculate the drug abuse normalised on 1000 inhabitants as well as on 1000 inhabitants in the age group of 15-64 years. The correction factors to convert excreted amounts of individual drugs into consumed amounts were taken from the literature and are briefly summarized in Table 4. The estimated total consumption was normalised to the number of citizens served by investigated WWTPs (mg/day/1000 inhabitants).

The highest estimated illicit drug consumption rate was obtained for marijuana (4625 mg/1000 p/day (15-64 years)), followed by cocaine (867 mg/1000 p/day (15-64 years)), amphetamine (555 mg/1000 p/ day (15-64 years)), and ecstasy (139 mg/1000 p/day (15-64 years)).

The data found for marijuana are significantly close to other studies, such as those reported in some European cities, for example Turkey (3577 mg/day/1000 inhabitants) (Daglioglu, Guzel, and Kilercioglu, 2019), and Zagreb/Croatia (5214 mg/day/1,000 inhabitants) Terzic, Senta, and Ahel (2010). For cocaine consumption rate is like EMCDDA (2017) at Barcelona/Spain (965 mg/1000 p/day (15-64 years)). For amphetamine consumption rate the result are slightly smaller than the found by Zuccato *et al.* (2008) in London (690 mg/day/1,000 inhabitants). For ecstasy consumption rate the result is slightly bigger than the found by Daglioglu, Guzel, and Kilercioglu (2019) in Turkey (130 mg/day/1,000 inhabitants).

Table 6. Mean illicit drug consumption rates in 5 WWTPs of the Hydrographic Region, Rio de Janeiro State

WWTP	**HRRJ**	**Illicit drug**			
		AMP	**MDMA**	**COC**	**THC-COOH**
		mg/day/1000 inhabitants			
Icaraí	V Niterói	48	27	248	3587
Ilha do Governador	V Rio de Janeiro Municipality	<LOQ	42	389	4122
Pavuna		<LOQ	15	277	4625
Barra da Tijuca		555	139	435	4581

Cabo Frio	VI Cabo Frio	441	111	967	3988

Abbreviations: HRRJ - Hydrographic Region - State of Rio de Janeiro; WWTP - Wastewater Treatment Plant; AMP – amphetamine; MDMA – 3,4-methylenedioxymethamphetamine; COC – cocaine; THC-COOH – 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol; (<) below LOQ.

IV. Discussion

WBE has been increasingly used as an additional source of information on the consumption of illicit drugs (e.g. Zuccato *et al.*, 2008, Postigo *et al.*, 2010, Terzic *et al.*, 2010, Thomas *et al.*, 2012). The main advantage of this innovative approach is its objectivity, which is based on highly accurate chemical measurements of selected drug biomarkers in wastewater, and its suitability for near-real-time tracking of the changes in drug consumption patterns within the selected communities. Wastewater analysis can provide information about spatial and temporal variations of illicit drugs use as well as on the impact of special events such as national holidays or music festivals on drug consumption patterns (Zuccato *et al.*, 2008). The first multi-approach studies recommend that wastewater analysis can predict results from population surveys. Closer relationship between epidemiologists and legal authorities will expand the perception of the right drug situation and permit for a better evaluation of interventions (Choi *et al.*, 2018).

In recent years, there has been a rapid increase in several studies focused on monitoring illicit drug use through WBE analysis around the many countries at the regional, national, and international levels. Due to its widespread use and high prevalence rates, cannabis, cocaine, and amphetamines have been among the target substances since the first application of the approach. Better understanding the extent of its use is important for policymakers seeking to hamper criminal activities and their revenues as well as introducing new drug policies. This is particularly important concerning discussions about legalizing its use and regulating the markets (Ferreira, Wermelinger, and Cruz-Hernández, 2023).

Wastewater analyses can also be used to track temporal variability in illicit drug consumption. Zuccato *et al.* (2011) demonstrated a significant drop in cocaine use at the beginning of the economic crisis in Italy. Moreover, some studies demonstrated a pronounced increase in the consumption of stimulants during the weekend (Zuccato *et al.*, 2008, Terzic, Senta, and Ahel, 2010, Thomas *et al.*, 2012) as well as during music festivals (Guzel, 2022), and sports events (Centazzo *et al.*, 2019). Lai *et al.* (2013) demonstrated that the consumption of several illicit drugs, including MDMA, cocaine and methamphetamine on the Australian vacation island significantly increased during the national peak holiday season, however, the number of studies on seasonal variability of drug consumption is still low.

In this research consumption patterns were investigated at different scales: as detailed studies in one selected local, the Rio de Janeiro State. The results found were worryingly like those found in other national, and international studies (Zuccato *et al.*, 2005a; Zuccato, Castiglioni, and Fanelli, 2005b; Zuccato *et al.*, 2008; Terzic, Senta, and Ahel, 2010; Postigo, López de Alda, and Barceló, 2010; van Nuijs *et al.*, 2011b; Thomas *et al.*, 2012; Lai *et al.*, 2013; van Wel *et al.*, 2016; Bastos *et al.*, 2017; Yadav *et al.*, 2017; Centazzo *et al.*, 2019; Deng *et al.*, 2020; Guzel, 2022; Ferreira, Wermelinger, and Cruz-Hernández, 2023).

It is worth highlighting that the recently performed international studies indicated pronounced geographical differences in illicit drug consumption patterns across Europe. The highest cocaine use was determined in western and central Europe, while the highest consumption of methamphetamine was determined in northern and eastern Europe (Thomas *et al.*, 2012). Furthermore, significant differences in drug consumption patterns were also demonstrated within the same country (Thomas *et al.*, 2012), indicating typically higher illicit drug abuse in highly urbanized metropolitan areas.

V. Conclusion

The present study continues (seeking to improve, increase scientific partnerships and interlaboratory collaborations) in the investigation of the illicit use of cocaine, marijuana, amphetamine, methamphetamine and MDMA in the State of Rio de Janeiro using WBE.

Spatial differences in drug consumption were found between the municipalities surveyed. All drugs researched differed between municipalities in terms of purchasing power but drawing attention to similarities in consumption. Greater drug consumption was observed in vacation towns, contrasting with a region with low purchasing power that demonstrated high consumption (Pavuna).

It appears that more attention is needed to drug intervention strategies, especially in large cities. WBE data on geographic variations in illicit drug use should inform future epidemiological and sociological investigations and the development and evaluation of interventions to reduce illicit drug use and drug-related harms.

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Conflicts Of Interest

The authors declare no conflicts of interest.

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