

Drugs of Abuse and Metabolites in Urban Wastewater: A Case Study, Rio De Janeiro Municipality, Brazil

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Abstract— The use of drugs of abuse is a concern for social and public health aspects, but also for environmental contamination. However, the study of these pollutants becomes important since the magnitude, nature and consumption patterns can be evaluated through the range of compounds detected in wastewater treatment plant (WWTP). Wastewater-based epidemiology depend on the principle that traces of compounds, which a population is exposed to or consume, are excreted unchanged or as metabolites in urine and/or faeces, and at last end up in the sewer network. Quantifying target metabolic residues in raw urban wastewater permits identifying the exposure or use of substances of interest in a community. For this purpose, it was used an HPLC-MS/MS analytical method to assess the concentrations of 2 drug of abuses and metabolites (cocaine and cannabis) in influent samples from Penha WWTP and Ilha do Governador WWTP, in Rio de Janeiro Municipality. A systematic use of cocaine and cannabis was identified by detection in all samples analyzed. This study corroborates with other findings related to the methodological approach which largely can be applied to monitor the use of drug of abuses, giving important subsidies to both environmental and health control systems.

Index Terms— Drug of abuse, Wastewater-Based Epidemiology, Environmental Detection, Public Health.

I. INTRODUCTION

There are some hypotheses regarding the etiology of the word drug. One is that it may have originated from the Arabic word *drowa*, which means wheat candy, or else from the Dutch term *drooge vate*, associated with dry leaves, since the medicines were made from the vegetables. In French it is that the term known today is described as *drogue*, to designate medicine or pharmaceutical product. Currently, the term drug is associated with substances that change the user's behavioural aspects, giving him pleasure sensations, and is defined by the World Health Organization (WHO) as "any substance not produced by the body that has the property of acting on one or more of its systems, producing changes in its functioning" [1].

Drugs of abuse go into the sewage system, unchanged or as metabolites, most commonly after their consumption and excretion and after illegal discharges as result of police interventions [2]. Data on the occurrence of these compounds in influent waters from wastewater treatment plant (WWTP) provide valuable information on drug use and consumption tendencies at local level [2]-[3]-[4]-[5].

With the increase of drug consumption in recent decades, in particular cocaine and cannabis, the social impact on society has also propagated [6]. These consequences include rising healthcare costs, crime rates, and economic losses. Therefore, it is imperative that policy makers gain knowledge of the trends, usage levels, hot spots, and overall prevalence of drug of abuse consumption, in order to develop proper prevention campaigns and effective intervention strategies.

Estimation of drug use at the population level is traditionally performed mainly via socio-epidemiological methods, such as population surveys and seizure data. These data are subject to significant uncertainties in measurement and selection, for example, self-reporting bias from false reports, unaware or misinformed consumers, and limited population coverage of the study [7]-[8].

Drug of abuse consumption is actually an important public health concern that needs to be well defined to be managed. A new concept to assess collective drug consumption, based on measuring concentrations of drug of abuses and their excreted human metabolites in untreated sewage, was proposed by Daughton [9] and developed by Zuccato et al. [10]. The technique is based on the principle that excreted drug residuals in the sewage can be collected by the downstream end of urban drainage systems and subsequently analysed. Later, this approach, termed wastewater-based epidemiology (WBE). Levels of drug of abuses (parent compounds or metabolites) in wastewater can be used to back-calculate the total consumption of drug of abuse by the population served by a particular WWTP.

Sampling campaigns for the purpose of detection of excreted drug residuals (drug biomarkers) are usually performed at the inlet to WWTPs. There have been many studies to monitor temporal and spatial patterns of drug use in selected urban sewer catchments [11]-[12]-[13] allowing, more recently, for the undertaking of international comparative studies [2]-[14]. These findings demonstrated that wastewater analysis techniques have the potential to complement conventional surveillance data. Estimation of drug use via wastewater analysis has been further expanded to include monitoring during special events (e.g. music festivals) [15]-[16], as well as within smaller communities (e.g. prisons) [17]-[18]. Despite the claim for protection of anonymity and non-intrusive approaches, various ethical concerns and debates have been raised with regards to the privacy of individuals and how far upstream one can perform monitoring in this way [19].

Drug of abuse are recognised as environmental pollutants of

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emerging concern to both human and ecological health, whose impact on ecosystems is still poorly understood [20]. There has been a growing interest in the study of these substances due to their high consumption and continuous discharge in environmental compartments, after their insufficient elimination in WWTPs. The nature and quantity of these pollutants are frequently related to the characteristics, state of health and habits of the populations that discharge these WWTPs. Therefore, the study of these pollutants has a double aspect: epidemiologically the magnitude, nature and consumption patterns can be evaluated through the range of compounds detected in the WWTPs, environmentally trace, and manage the danger that these substances can be traced represent for human and environmental health [4].

Regarding the relationship of drugs to health, the World Drug Report 2017 [21] aims to present data and strategies to analyze social issues of marginalization, gender, incarceration, confrontation and combat the world drug problem with prevention and treatment policies, punishments and alternative measures, among others, embracing the goals of the 2030 Sustainable Development Agenda " [22].

It is detached by Campos [23] that in Brazil, in particular in Rio de Janeiro, people living in a slums conjuncture live in a scenario where crime, impunity and social injustice are prevalent, fuelled by expressions of uprooting and vulnerability facing the violence of the State, militias or trafficking.

The increasing use of drugs of abuse and the non-medical use of prescription drugs are a growing concern for public health authorities [24]. According to the data provided by population surveys, around 255 million people worldwide, which is approximately 5.6 percent of the world population aged 15 to 64 years, used drugs at least once during 2016 [21]. Around 31 million people who use drugs of abuse suffer from their disorders, which mean that their use of drugs is harmful to the point where they may need treatment. According to the same report, cannabis users (*Cannabis sativa L.*) comprise the largest number of users. Figure 1 presents the global trends in estimated number of drug users and disorders derived from their use in the period of 2006-2015 [21].

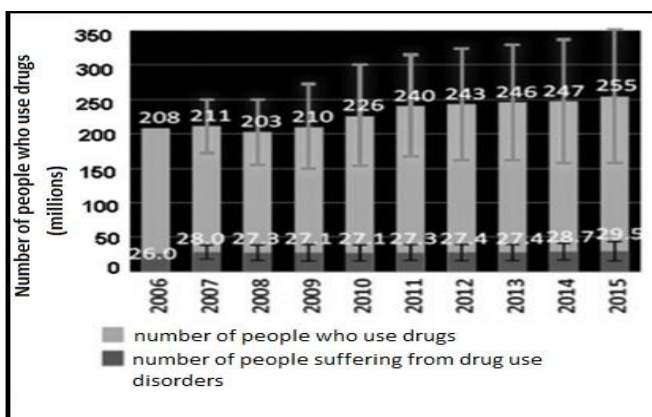


Fig 1. Global trends in estimated number of drug users and disorders arising from their use in the period 2006-2015 [21]

The aim of the present work was to study the occurrence and distribution of drugs of abuse (cocaine and cannabis), in the influent from WWTP Penha and WWTP Ilha do Governador,

Rio de Janeiro Municipality, Rio de Janeiro State, Brazil (Figure 2). These areas are affected by different anthropogenic pressures, including wastewater release, industrial effluents, and municipal activities. For these purposes, a highly sensitive analytical method was developed and validated for the determination of the main drugs of abuse and their metabolites in WWTPs.

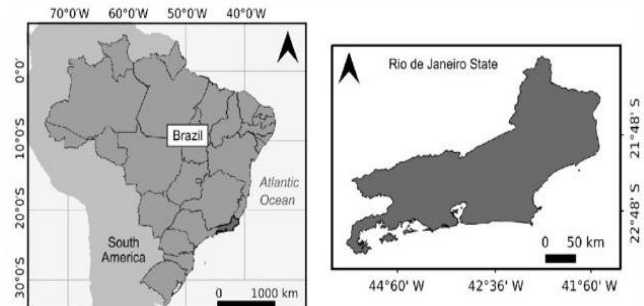


Fig 2. Location of Rio de Janeiro municipality, Rio de Janeiro state, Brazil

II. MATERIALS AND METHODS

A. Characterizing the WWTP

It is noteworthy that the wastewater samples collected comes from catchment areas where several schools, universities, hospitals, business offices, shops, entertainment venues and clubs are located, and in a minor extent some industries.

B. WWTP Penha

The WWTP Penha (22°49'57"S 43°16'0"W) operates with biofilters and activated sludge, treating a flow of 1600 L/s. The pre-treatment starts by medium and fine screening, with removal of solids, followed by removal of sands, oils, and grease. Greases are incinerated. The primary treatment consists of an accelerated lamellar settling in four tanks, with optional physical and chemical treatment, and of three tanks of equalization/homogenization. The secondary biological treatment is performed by means of a continuous-flow activated sludge system with conventional aeration in six aerated tanks by surface aeration, followed by a secondary lamellar settling, in 12 settlers of rectangular plant, with biological sludge recirculation.

C. WWTP Ilha do Governador

The WWTP Ilha do Governador (22°47'43"S 43°11'14"W) operates with activated sludge, treating a flow of 525 L/s. The treatment process works with the steps: the elevated influent pass through a screening formed by grids – a coarse and a medium, responsible for removing coarse solids, followed by the removal of oils, greases and sands, in two grit chambers/degreasers. The resulting by-products of this pre-treatment are later deposited in controlled landfill. The influent is then fed to a homogenization tank e with 4000 m3 working volume, whose function is to regularize peaks of pollutant load. The subsequent primary treatment consists of a preliminary step of physical and chemical treatment, with application of aluminium sulphate, hydrated lime and a polyelectrolyte on two lines of coagulation/flocculation tanks, with primary settling in two tanks of circular plant with

bottom and surface scraper. The primary effluent is then fed to the secondary treatment, which is performed by means of activated sludge system, in six aeration tanks provided of two surface aerators each and sludge recirculation tank. The next step is the secondary settling in two settlers of circular plant, with bottom and surface scraper.

D. Chemicals and materials

Standard solutions of cocaine (COC), benzoylecgonine (BZE), norcocaine (NOR), ecgonine methyl ester (EME), cocaethylene (CET), 11-nor-delta-9-hydroxytetrahydrocannabinol (THC-COOH), cocaine-d₃, BZE-d₃, EME-d₃, CET-d₈, THC-COOH-d₃, in methanol (MeOH) or acetonitrile (ACN), were purchased from LGC Standards (S.J. dos Campos, SP, Brazil).

MeOH and ACN, HPLC grade (Hipersolv Chromanorm), formic acid (FA) (Normapur) and ammonium formate (AF) (Normapur) were purchased from VWR (Bela Aliança, São Paulo- SP, Brazil). Ultra-pure water was produced using successive Milli-RO reverse-osmosis filtration and the Milli-Q Plus water purification system (Merck Millipore, Rio de Janeiro, Brazil).

Solid Phase Extraction (SPE) cartridges Oasis HLB (500 mg/6 mL) and Xbridge Phenyl 3.5 mm, 3 mm × 150 mm HPLC column were purchased from Waters (Guyancourt, France).

Analysis was carried out with a Thermo Accela pump and Accela sampler coupled to a triple quadrupole mass spectrometer Quantum Access Max equipped with Xcalibur software (ThermoFisher Scientific, Jundiaí, São Paulo, Brazil).

E. Sample collection

The 24-h composite influent wastewater samples were collected daily over five consecutive days in 2018, starting on Monday September 10th and ending on Friday September 14th. Sampling was carried out using cooling auto samplers to obtain 24-h flow-weighted composite influent samples with a sampling frequency of at least 6 times per hour according to the local WWTP procedures. At the end of sampling, 2 L samples were collected in polypropylene bottles and sent to laboratory in a cool box intended to be used for 24-h shipments. Upon receipt, samples were filtered and extracted according to the following protocol and the extracts were stored at 4°C before analysis.

F. Target analytes

The following compounds were analyzed: The cocaine group that contains COC and its metabolites, BZE, EME, CET and norcocaine (NOR), and the cannabinoid THC-COOH.

G. Analytical Methods

Solid-phase extraction (SPE)

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

The complete run time of 26 min, with analytes eluting between 0.8 and 19 min, provided effective analysis time. Average mass accuracy for all target drugs of abuse was below 5 ppm and the standard deviation of these errors was from 0.1 to 0.9, which point to a very low deviation of the instrument response. Purity score values higher than 82.4% were at all times obtained in HPLC-MS/MS identification.

In sewage, recoveries ranged from 61% for THC-COOH to 119% for BZE. Because of the loss during SPE extraction and the matrix effect, the efficiency values ranged from 24% to 57% in influents. Linearity was confirmed for all of the compounds in their relative concentration range using the IS (Internal Standard) addition method with correlation coefficient *r* values above 0.99 (Table 1).

Data analysis (counts, percentages, and means) were performed with Excel software (Microsoft Office Excel 2016 for Apple™). The level of significance was calculated by two sample *t* test with unequal variance performed with the STATA MP statistical software package v14.1 for Apple™ (Stata Corp).

Table 1. Analytical validation data

Molecules	Recovery (%) (400 ng/L)	Efficiency (%) (400 ng/L)	Linearity (<i>r</i> ²)	Intra-run precision (%)	LOQ (ng/L)
COC	107	36	0.999	2.42	20
BZE	119	54	1.000	0.52	10
EME	110	24	0.997	3.58	40
NOR	105	34	0.994	2.35	20
CET	109	41	0.999	4.02	5
THC-COOH	61	57	0.998	14.41	5

Intra-run precision: three time analyses of a 400 ng/L spiked wastewater sample; Linearity range: 12.5, 25, 50, 100, and 200 µg/L; LOQ - limits of quantification.

III. RESULTS AND DISCUSSION

Concentrations measured in influent wastewater samples from WWTP Penha and WWTP Ilha do Governador are summarized in Table 2 and Table 3.

Table 2. Concentrations (ng/L) of drug of abuses detected in replicate samples from the WWTP – Penha, Rio de Janeiro, 2018

Compound analysed	Sample collection	Compound concentration (ng/L)					
		Mean	SD	Lower 95% CI of Mean	Upper 95% CI of Mean	LI	LM
COC	1 th	102.2	14.83709	99.19	105.2	67	198
	2 nd	111.8	8.99663	109.98	113.62	90	156
	3 rd	120.4	11.60171	118.05	122.75	54	167
	4 th	101.5	14.31378	98.6	104.4	53	162
BZE	1 th	242.7	18.83732	238.88	246.51	167	293
	2 nd	247.9	40.87297	239.61	256.18	133	369
	3 rd	288.8	21.39042	284.47	293.14	189	344
	4 th	211.6	22.62252	207.02	216.19	112	282
THC-COOH	1 th	39.6	6.64669	38.26	40.95	26	89
	2 nd	42.8	5.89669	41.61	44	16	86
	3 rd	38.2	4.53204	37.28	39.12	15	67
	4 th	44.9	4.8286	43.92	45.87	19	74

SD - Standard Deviation; CI - confidence interval; LI - limit inferior; LS - limit superior

It was found in all influent samples. COC and its metabolite BZE showed concentration values in both WWTP. The mean values of COC exceeded 100 ng/L in all analysis, BZE exceeded 200 ng/L in all samples from WWTP Penha and 300 ng/L in all samples from WWTP Ilha do Governador. THC-COOH was found in all samples at average concentration of 38.2 and 44.9 ng/L for WWTP Penha and 39.7 and 65.4 for WWTP Ilha do Governador, respectively. These concentrations are very similar to the range of compounds detected in untreated wastewater from WWTPs in Colombia and in Europe. On the other hand, the values found for COC and BZE are significantly lower than those found in another Brazilian study. COC levels ranged from 519 ± 29 to 1260 ± 79 ng/L whereas for BZE, its major metabolite concentration varied between 1228 ± 98 and 4297 ± 298 ng/L [25].

Table 3. Concentrations (ng/L) of drug of abuses detected in replicate samples from the WWTP - Ilha do Governador, Rio de Janeiro, 2018

Compound analysed	Sample collection	Compound concentration (ng/L)					
		Mean	SD	Lower 95% CI of Mean	Upper 95% CI of Mean	LI	LM
COC	1 th	143.4	8.52177	141.67	145.12	112	198
	2 nd	141.6	13.61692	138.85	144.36	98	199
	3 rd	192.6	34.08487	185.70	199.51	102	288
	4 th	155.4	20.39633	151.26	159.53	83	211
BZE	1 th	396.3	55.37131	385.08	407.52	299	543
	2 nd	399.5	50.16016	389.34	409.66	302	555
	3 rd	381.1	63.60296	368.22	393.99	278	555
	4 th	403.7	41.35421	395.32	412.08	299	516
THC-COOH	1 th	57.3	10.13916	55.25	59.36	31	86
	2 nd	65.4	7.94253	63.79	67.01	39	92
	3 rd	45.1	5.99382	43.89	46.32	33	73
	4 th	39.7	4.65916	38.75	40.64	26	51

SD - Standard Deviation; CI - confidence interval; LI - limit inferior; LS - limit superior

A number of drug of abuses have so far been detected in raw municipal wastewater as well as treated wastewater effluent

and receiving surface waters due to incomplete removal of these drugs by conventional WWTP processes [4]. Even after the prevalence of illicit substances/metabolites in different

water sources was established and in light of growing concern about their unknown effects, very few studies have reported on their degradation behaviour and eco-toxicological impacts, with most studies based on detection/analysis, or usage patterns/trends. There are largely unknown natures of the ecological effects from these substances, stating that while the half-life of some drug of abuses is short, they become more prevalent with continued population usage and environmental release and as such, they cannot be ignored. Therefore, these substances are considered ubiquitous contaminants that are continuously discharged from WWTPs into the receiving environment, but with essentially unknown effects on human and environmental health from chronic low-level exposure.

Due to the complexity involved in the issue, it is important to analyze the use of drugs, as well as their effects and derived political practices, based on the articulation and mediation of factors of an individual, sociocultural and political-economic nature. Therefore, by virtue of the consequences of use in the lives of individuals and communities, as well as prevailing prejudices and stigmas, the inclusion of this topic in the public policy agenda is accompanied, with the health sector having a prominent role [26].

Consumption of drug of abuses is a new concern for water management that must be considered not only because of the social and public health aspects but also in an environmental context in relation with the contamination of surface waters. Indeed, WWTP effluents contain drug residues that have not been eliminated since WWTP treatments are not completely efficient in their removal.

It should be noted that the violence resulting from the illicit drug market has a direct impact on the quality of life of people, affecting health, even though this does not result directly from the actions related to prevention or health care. The daily stress of people who live or need to go through locations dominated by violent criminal factions strongly affects public health and can result in diseases of various shades, some of which are only detectable in a relevant time course. Peace of mind itself is a dimension of the quality of life that public health needs to be aware of. It is not correct, therefore, to simplify the ills to public health that result to the figure of the user.

IV. CONCLUSION

The increasing use of drug of abuses and the nonmedical use of prescription medications are a growing concern for public health authorities. In consequence, environmental existence of chemical pollutants through negligenced interconnectedness of human actions and activities influence the environment in countless ways. These impacts ascending from specially deposition of drug of abuses harbours underestimated risks to ecosystem and the lives, which live there. The individual use of these pharmacologically active substances generates great but underappreciated levels of other toxicologically potent and associated bioactive metabolites through purposeful and inadvertent discharge to the environment via excreta and by illegal disposal. These facts have important in many environmental health aspects as well as on population health.

The length of time that drug of abuses remain in the health system varies widely depending on the extent of the local sewage collection network. The biological nature, temperature and composition of the sanitary tributary present high variation among different regions, due to rainfall precipitation, consumption culture, climate, among other factors. Due to such variations, the degradation of drug of abuses should be considered in the estimation of consumption. For the estimation of cocaine use through the epidemiology of the sewage, 10% uncertainty is assumed in the determination of BE concentration, due to the stability of COC and BZE.

The results, although preliminary are innovative in the study region, being extremely significant, and of concern from an environmental and public health point of view. It proves the relevance of the sewage epidemiology method that should be considered as an efficient tool to support harm-reduction activities and prevention policy. This study could be the reason for the launching of a large-scale study with repeated campaigns, intended for risk reduction management and health purpose.

For wastewater-based epidemiology to produce reliable estimates of drug of abuse use and to inform the development of novel applications, the most urgent future research needs are to improve the methodology by checking and reducing uncertainty factors for each single step; to improve the comparability of results produced by different researchers or studies by adopting a common protocol of action, which will include ethical standards; and to develop methods to integrate wastewater analysis with established methods of drug epidemiology.

In conclusion, research on the presence of drug of abuses, particularly as active compounds in the environment, are vital to improve knowledge of the types consumed, its occurrence, being able to unfold to analyse the exposure and the impact of these compounds in the environment, giving, also, important subsidies for the health management.

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