DRUGS OF ABUSE AND METABOLITES IN URBAN WASTEWATER: A CASE STUDY, MUNICIPALITY OF RIO DE JANEIRO, 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 ultimately end up in the sewer network. For this purpose, it was used a high-performance liquid chromatography - tandem mass spectrometry (HPLC-MS/MS) analytical method to assess the concentrations of two drug of abuses and metabolites (cocaine and cannabis) in influent samples from Penha and Ilha do Governador WWTPs, in the Municipality of Rio de Janeiro. A systematic use of cocaine and cannabis was identified by detection in all samples analysed. This study corroborates with other findings related to the methodological approach which can be largely applied to monitor the use of drug of abuses, giving important subsidies to both environmental and health control systems.

Keywords: Drug of Abuse; Wastewater-Based Epidemiology; Environmental Detection; Public Health.

Drogas de abuso y metabolitos en aguas residuales urbanas brutas: un estudio de caso, municipio del Rio de Janeiro, Brasil

Resumo - O uso de drogas de abuso é uma preocupação para aspectos sociais e de saúde pública, mas também para a contaminação ambiental. O estudo destes poluentes torna-se importante, pois a magnitude, natureza e consumo podem ser avaliados através de compostos detectados em estações de tratamento de esgoto (ETEs). A epidemiologia baseada em esgoto depende do princípio de que os vestígios de compostos aos quais uma população é exposta ou consume são excretados inalterados ou como metabolitos na urina e/ou nas fezes e acabam na rede de esgotos. Para tanto, utilizou-se o método analítico de cromatografia líquida de alta eficiência acoplada à espectrometria de massas sequencial (HPLC-MS/MS) para avaliar as concentrações de duas drogas ilícitas e metabolitos (cocaína e cannabis) em amostras de influentes das ETEs da Penha e Ilha do Governador, Município do Rio de Janeiro. Foi identificado um uso sistemático de cocaína e cannabis, por detecção em todas as amostras analisadas. Este estudo corrobora com outros achados relacionados à abordagem metodológica que pode ser aplicada para monitorar o uso de drogas ilícitas, fornecendo importantes subsídios para os sistemas de controle ambiental e de saúde.

Palavras-chave: Drogas de Abuso; Epidemiologia Baseada em Esgoto; Detecção Ambiental; Saúde Pública.

DROGAS DE ABUSO Y METABÓLITOS EN LAS AGUAS RESIDUALES URBANAS BRUTAS: UN ESTUDIO DE CASO, MUNICIPIO DEL RIO DE JANEIRO, BRASIL

Resumen - El consumo de drogas de abuso es una preocupación para los aspectos sociales y de salud pública, pero también para la contaminación ambiental. La epidemiología basada en análisis de aguas residuales constituye una herramienta
importante para la estimación del consumo local a través de la investigación de los flujos de masa de drogas de abuso inalteradas o de sus metabolitos en las estaciones depuradoras de aguas residuales (EDARs). La cuantificación de residuos metabólicos objetivo en aguas residuales urbanas brutas permite identificar la exposición o el uso de sustancias de interés en una comunidad. Para ello, se utilizó el método analítico HPLC-MS/MS para evaluar las concentraciones de dos drogas ilícitas y metabolitos (cocaína y cannabis) en muestras de influentes de las EDARs de Penha y de Ilha do Governador, en el Municipio de Río de Janeiro. Se identificó un uso sistemático de cocaína y cannabis, por detección en todas las muestras analizadas. Este estudio corrobora con otros hallazgos relacionados con el enfoque metodológico que puede ser aplicado para monitorear el uso de drogas de abuso, proporcionando importantes subsidios para los sistemas de control ambiental y de salud.

PALABRAS CLAVE: Drogas de Abuso; Epidemiología Basada en las aguas residuales brutas; Detección Ambiental; Salud Pública.

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” (WHO 1987).

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 (Thomas et al. 2012). 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 (Zuccato et al. 2008; van Nuijs et al. 2011; Thomas et al. 2012; Nefau et al. 2013).

With the increase of drug consumption in recent decades, in particular cocaine and cannabis, the social impact on society has also propagated (EMCDDA 2015). 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 (Banta-Green and Field 2011; Castiglioni et al. 2014).

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 (2001) and developed by Zuccato et al. (2005). 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 (Gomes et al. 2008; Castiglioni et al. 2013; Plósz et al. 2013) allowing, more recently, for the undertaking of international comparative studies (Thomas et al. 2012; Ort et al. 2014). 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) (Gerrity; Trenholm; Snyder 2011; Lai et al. 2013), as well as within smaller communities (e.g. prisons) (Schröder; Gebhardt; Thevis 2010, Postigo; Alda; Barceló 2011). 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 (Hall et al. 2012).

Drug of abuse are recognised as environmental pollutants of emerging concern to both human and ecological health, whose impact on ecosystems is still poorly understood (Yadav et al. 2017). 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 (van Nuijs et al. 2011).

Regarding the relationship of drugs to health, the World Drug Report 2017 (UNODC 2017) 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 (UNODC 2016).

Campos (2014) detached which in Brazil, in particular on 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 (OPS 2015). 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 (UNODC 2017). Around 31 million people who use drugs of abuse suffer from their disorders, which means 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 (UNODC 2017).
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 of the State of Rio de Janeiro, Brazil. 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. The present study constitutes the largest one conducted in WWTPs for the first time in this Municipality/State.

**Material and Methods**

*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.

*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.

**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/floculation 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.

**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).

**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 autosamplers 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.
**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 TH-COOH.

**Analytical methodology**

**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 $2 \times 5$ mL MeOH and $2 \times 5$ mL ultra-pure water. Samples were percolated at a flow rate of 2 mL/min. The SPE cartridges were then washed using $2 \times 5$ mL ultra-pure water and dried for 30 min. Analytes were eluted with $2 \times 5$ 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 analysis in a high-performance liquid chromatography - tandem mass spectrometry (HPLC-MS/MS).

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>
<thead>
<tr>
<th>Table 1. Analytical validation data.</th>
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<tbody>
<tr>
<td><strong>Molecules</strong></td>
</tr>
<tr>
<td>COC</td>
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<tr>
<td>BZE</td>
</tr>
<tr>
<td>EME</td>
</tr>
<tr>
<td>NOR</td>
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<tr>
<td>CET</td>
</tr>
<tr>
<td>THC-COOH</td>
</tr>
</tbody>
</table>

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
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.

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 Europe. On the other hand, the values found for COC and BZE are significantly lower than those found in another Brazilian study (WHO, 2018), so that the 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.

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

<table>
<thead>
<tr>
<th>Compound analysed</th>
<th>Sample collection</th>
<th>Compound concentration (ng/L)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>COC</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>102.2</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>111.8</td>
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<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>120.4</td>
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<tr>
<td></td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>101.5</td>
</tr>
<tr>
<td>BZE</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>242.7</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>247.9</td>
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<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>288.8</td>
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<tr>
<td></td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>211.6</td>
</tr>
<tr>
<td>THC-COOH</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>39.6</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>42.8</td>
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<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>38.2</td>
</tr>
<tr>
<td></td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>44.9</td>
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</tbody>
</table>

SD - Standard Deviation; CI - confidence interval; IL- inferior limit; UL - upper limit

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 (van Nuijs et al. 2011). 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 nature 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.
Table 3. Concentrations (ng/L) of drug of abuses detected in replicate samples from the WWTP - Ilha do Governador, Rio de Janeiro, 2018.

<table>
<thead>
<tr>
<th>Compound analysed</th>
<th>Sample collection</th>
<th>Compound concentration (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>COC</td>
<td>143.4</td>
<td>8.52177</td>
</tr>
<tr>
<td></td>
<td>141.6</td>
<td>13.61692</td>
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<tr>
<td></td>
<td>192.6</td>
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<tr>
<td></td>
<td>155.4</td>
<td>20.39633</td>
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<td>396.3</td>
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<td></td>
<td>399.5</td>
<td>50.16016</td>
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<td>381.1</td>
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</tr>
<tr>
<td></td>
<td>403.7</td>
<td>41.35421</td>
</tr>
<tr>
<td>BZE</td>
<td>396.3</td>
<td>55.37131</td>
</tr>
<tr>
<td></td>
<td>399.5</td>
<td>50.16016</td>
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<tr>
<td></td>
<td>403.7</td>
<td>41.35421</td>
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<tr>
<td>THC-COOH</td>
<td>57.3</td>
<td>10.13916</td>
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<td></td>
<td>65.4</td>
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<td></td>
<td>45.1</td>
<td>5.99382</td>
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<td></td>
<td>39.7</td>
<td>4.65916</td>
</tr>
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</table>

SD - Standard Deviation; CI - confidence interval; IL- inferior limit; UL - upper limit

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 (Whiteford et al. 2010).

WHO (2018) pointed that 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 negligeciated interconnectedness of human actions and activities influence the environment in countless ways. These impacts ascending from specially deposition of drugs of abuse 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.

Consumption of drugs of abuse 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.
CONCLUSION

Using wastewater-based epidemiology, this study provides the first objective snapshot of local drug use in a population of Municipality of Rio de Janeiro, Brazil. These results prove that wastewater-based epidemiology is a useful additional tool for drug consumption assessment offering information for a defined area that usual surveys population investigation and policy statistics are unable to give.

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.

The research on the presence of drugs of abuse, particularly as active compounds in the environment, is vital to improving 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.

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 every single step. This way, 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 develop methods to integrate wastewater analysis with established methods of drug epidemiology.

ACKNOWLEDGMENTS

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