Illicit Drugs, Metabolites and Adulterants in Wastewater: Monitoring Community Drug Abuse in the Brazilian Federal District during the 2014 Soccer World Cup

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A multiresidue method was developed to estimate community drug use during two weekends of the 2014 Soccer World Cup via wastewater analyses. Five of the 16 investigated substances were detected in all eight samples. Benzoylecgonine (BE), the major cocaine metabolite, was the most abundant substance (1.9 ± 0.3 to 4.2 ± 0.2 μg L⁻¹) followed by anhydroecgonine (AE) (1.5 ± 0.2 to 2.6 ± 0.2 μg L⁻¹), phenacetin (PHE) (0.62 ± 0.06 to 1.3 ± 0.1 μg L⁻¹), levamisole (LEV) (0.5 ± 0.2 to 1.23 ± 0.09 μg L⁻¹), and cocaine (COC) (0.46 ± 0.07 to 0.8 ± 0.1 μg L⁻¹). COC and BE levels suggested a human consumption origin for the investigated drugs. AE/BE ratios revealed a significant use of smoked crack/free base cocaine. Relationships between the adulterants PHE and LEV evidenced a higher use of crack cocaine in the South-Wing WWTP region. An estimative for cocaine use revealed slightly higher drug consumption during the Soccer World Cup weekends in comparison with a typical weekend in 2012.

Keywords: wastewater-based drug epidemiology, drug use estimates, cocaine adulterants, smoked cocaine, FIFA World Cup

Introduction

The analysis of wastewater for illicit drugs and their metabolites has provided alternative approaches to the traditional epidemiological tools used to understand the scenario involving the occurrence, prevalence and abuse of drugs. The wastewater-based drug epidemiology (WBDE) was implemented in Italy in 2005¹ and have been applied since then by several groups in countries such as Spain,² Belgium,³ USA,² China,² Brazil² and France.⁷

The approach is based on the quantitative analyses of urban raw sewage, containing a pool of human metabolic excretion products, some of them resulting from several illicit drug consumption.² By assessing the levels of parent compounds as well as their metabolites it is possible to back-calculate the amount used by certain population, commonly the one served by a specific urban sewer system. One of the main advantages of this approach is to provide objective, quantitative, and near real-time profiles of illicit drug consumption as well as to estimate and compare consumption patterns (e.g. g day⁻¹, doses day⁻¹, mg day⁻¹ per habitant or doses day⁻¹ per 1000 inhabitants).⁹,¹⁰

Brazil is located nearby cocaine-producing countries such as Peru, Colombia and Bolivia, thus being an important route for drug trafficking. In addition, our country has experienced an increase of smoked cocaine consumption¹¹ leading our research group to conduct WBDE studies.⁶,¹² Our group also has access to data from the Brazilian Federal Police chemical profiling program based on the quantification of coca alkaloids and pharmaceuticals used as adulterants such as caffeine, lidocaine, levamisole and phenacetin, in seized cocaine samples.¹³,¹⁴

Due to the near real-time characteristics of the WBDE approach, consumption estimates can be assessed in order to identify drug use variations according to different seasons,¹⁵-¹⁷ days of a week,¹⁶ and even recreational events.¹⁸ Gerrity et al.¹⁹ investigated temporal variability associated with the 2009 National Football League’s Super Bowl, a
significant weekend for tourism in the event area, given the potential for unusual flow patterns and mass loadings of pharmaceuticals, personal care products and illicit drugs. Although results did not indicate significant effects of the Super Bowl on the loadings of many investigated compounds, the use of WBDE approach shows a slightly increase of cocaine consumption during the Super Bowl weekend.

The occurrence of the Soccer World Cup in Brazil in 2014 was an opportunity to estimate if a major sport event would affect total illicit drugs consumption as determined by WBDE. A high income of tourists in the Brazilian Federal District (FD) was observed during the tournament when seven games were played in Brasília, with 68,000 people per game only inside the Brasília National Stadium, Mané Garrincha. The final balance of the event estimated that almost 500,000 national and international tourists arrived to Brazil and that an increase of 20% of hotels occupation was accomplished.

The aim of this work is to evaluate the occurrence of cocaine alkaloids, cocaine metabolites, cocaine adulterants, and amphetamine-type substances, in the influent sewage collected from selected wastewater treatment plants (WWTP) in the FD. The sampling was performed on two weekends synchronized with two 2014 FIFA Soccer World Cup matches. The results were compared with the ones obtained during an ordinary weekend previously investigated by our group.

**Experimental**

**Chemicals and reagents**

Cocaine (COC), anhydroecgonine methyl ester (AEME), benzoylecgonine (BE), nor-benzoylecgonine (Nor-BE) and ecgonine methyl ester (EME) were purchased from Cerilliant (Austin, USA); anhydroecgonine hydrochloride (AE) was obtained from UFRGS (Porto Alegre, Brazil); ecgonine hydrochloride (ECG), norcocaine hydrochloride (Nor-COC) and (+)-methylamphetamine hydrochloride (MAMP), were purchased from NMI (North Ryde, Australia); (+)-amphetamine (AMP), (+)-3,4-methylenedioxymethamphetamine (MDMA), (+)-3,4-methylenedioxyamphetamine (MDA), (+)-3,4-methylenedioxy-ethylamphetamine (MDEA) and (+)-3,4-methylenedioxy-ethylamphetamine (MBDB) were purchased from Lipomed AG (Arlesheim, Switzerland); levamisole (LEV) was obtained from Sigma (St. Louis, USA) and phenacetin (PHE) was purchased from TCI Chemical (Tokyo, Japan).

Working solutions were prepared by dilution of stock solutions (20 mg L⁻¹) with water:methanol, 90:10 (v/v). All working solutions and sample extracts were prepared with 0.1% HPLC-grade formic acid (Fluka, Buchs, Switzerland) prior to analysis in order to improve analytical sensitivity. These solutions were prepared weekly and stored at 4 °C, protected from light.

Acetonitrile and methanol (HPLC grade) were obtained from Tedia (Fairfield, OH, USA). Hydrochloric acid (HCl, 37%) was provided by Mallinckrodt (Paris, USA). Ultrapure water (18.2 MΩ cm) was produced in a Milli-Q Plus purification system (Millipore, USA). Nitrogen for drying (99% of purity) and for instrumental analyses (99.995% of purity) was supplied from White Martins, Brazil.

**Background**

The analytical protocol to measure all analytes was optimized through a collaborative program involving the Forensic Chemistry Service of the National Institute of Criminalistics of the Brazilian Federal Police (where the main equipment used in this work is installed) and the environmental chemistry laboratory at the University of Brasilia (where samples were prepared), with collaboration of the Federal District Company of Environmental Sanitation (where sewage was sampled).

**Study area and sampling**

This work was carried out in Brasilia, the capital of Brazil located in the Brazilian Federal District (FD), a federative unit comparable to a state. Along with the nearest satellite cities, the FD has a population of about 2.7 million inhabitants. Raw sewage collected in the FD is treated in 16 wastewater treatment plants (WWTP). In this work, raw sewage samples were collected from two selected WWTP, namely Asa-Sul (e.g. South-Wing) and Asa-Norte (e.g. North-Wing), serving an equivalent population of approximately 700 thousand inhabitants.

Brasília is a planned city and it has a number of sectors which concentrate certain types of activities, such as banking, commercial, and hospital sectors, among others. The city also has two hotel sectors, located nearby the downtown area on the inner edges of both North and South Wings. The North and South hotel sectors were strategically built close to several monuments and entertainment sectors (Figure S1, Supplementary Information). During the 2014 FIFA World Cup, virtually all hotels on both hotel sectors were occupied by tourists. The downtown area also experienced a large flow of people from the metropolitan region attracted by the activities related to the FIFA World Cup games. The National Stadium of Brasília, located at
the central area, near to the North hotel sector, hosted seven soccer matches.

The game between Argentina 1 vs. 0 Belgium took place on July 5th at 1:00 pm, while the 3rd place playoff between Brazil 0 vs. 3 Netherlands happened on July 12th at 5:00 pm. Both games were played on Saturdays. Thus, raw sewage samples were collected in both North-Wing and South-Wing WWTPs inlets during a 48-hours period in July 5-6th and 12-13th. The sampling began at 12:00 am on Saturdays, covering the period of both FIFA World Cup matches. A total of two 24 h-composite samples were collected from each WWTP using a refrigerated automatic water/wastewater sampler (Teledyne Isco, Lincoln, NE), equipped with a peristaltic pump that draws wastewater aliquots according to the influent flow into a collection bottle under refrigeration. During the sampling period, composite samples were stored in amber glass bottles in the dark at 4 °C until further analytical steps.

The reference composite sample, collected in the North-Wing WWTP during an ordinary weekend on 21-22nd April 2012 was submitted to the same sampling and sample preparation routine as the samples collected during the FIFA World Cup. However, at that time only COC and BE were investigated in this sample.

Sample preparation

Aliquots of 50 mL of unfiltered raw sewage samples (three replicates) were transferred to individual 60-mL syringe tubes connected in line to solid-phase cartridges containing 500 mg of a polystyrene divinylbenzene sorbent with both hydrophilic and lipophilic properties (Strata X®, Phenomenex, USA). To avoid clogging on the top frit of the cartridges a portion of laboratory grade glass wool (Sigma-Aldrich) was placed inside each one. Extraction parameters, namely sample pH, conditioning and eluting solvent mixtures, were optimized using a 2^4 factorial planning in order to provide the best recovery for a 0.1 or 1.0 μg L^{-1} spiked raw sewage depending on the analyte.

Prior to extraction, raw samples were acidified to pH 2.0 with a 2 mol L^{-1} HCl solution. Solid-phase extraction (SPE) was carried out using a lab-made extraction system to avoid cross contamination similar to the one described elsewhere.\(^{22}\)

The sorbent phase was conditioned with 6 mL of a methanol:acetonitrile mixture (60:40 v/v) followed by 6 mL of a pH 2.0 ultrapure water. Samples were passed through the cartridges at a flow rate of 3 mL min^{-1} in order to provide adequate contact between the analytes and the sorbent phase. Cartridges were then centrifuged at 4000 rpm for 5 min to eliminate residual water from the solid phase and dried under a constant stream of N\(_2\) for 5 min. Analytes were recovered under vacuum in a 12-port manifold (Visiprep, Supelco) with 6 mL of methanol into previously cleaned glass tubes. The eluates were gently evaporated using a dry heated nitrogen evaporator (TE-09 Tecnal). Analytes were finally redissolved in a 90:10 (v/v) ultrapure water:methanol mixture to a final volume of 5.0 mL. Under this pre-concentration factor, previous studies demonstrates minimal matrix effects during analyses using electrospray ionization (ESI).\(^6\)

Analytes determination

Sample extracts were analyzed using an Agilent 1200 liquid chromatographic system, equipped with a micro-vacuum degasser, a binary pump, an autosampler, and a thermostated column compartment, coupled to a Sciex API 3200 triple quadrupole mass spectrometer equipped with an ESI source (Sciex, Framingham, USA). A Zorbax RRHD SB-C18 column (50 × 2.1 mm i.d., 1.8 μm particle size, Agilent Technologies) was used for chromatographic separation.

Formic acid solutions (0.01% v/v) prepared in ultrapure water and methanol were used as mobile phase solvents at a flow rate of 0.3 mL min^{-1}. The initial gradient of water:methanol, 90:10 (v/v), was linearly increased to a relative methanol volume of 50% in 3 min. The mobile phase composition was changed to 100% methanol in 3.1 min and held until the end of the analysis. After readjusting to the initial conditions, the system was re-equilibrated for 7 min. The temperature in the column compartment was kept in 25 °C. The injection volume was 2.0 μL.

Analytes were ionized in the ESI Turbo Ion Spray® probe in the positive mode with nitrogen (45 psi) used as drying gas at 450 °C. The optimized gas pressures were 45 psi (nebulizer), 12 psi (curtain) and 6 psi (collision). The ion spray source voltage was set at 5500 V.

Mass spectrometric analyses were carried out in the multiple reaction monitoring (MRM) mode measuring the fragmentation products of the protonated molecular ions [M + H]^+ for all analytes. Each compound was quantified by MRM using the three most abundant precursor → product ion transitions. Instrumental parameters such as declustering potential, entrance potential, collision cell entrance potential, collision energy and collision cell exit potential were optimized for each analyte as shown in the Supplementary Information (Table S1).

All analytes were quantified by external calibration using, at least, five-point analytical curves (R^2 > 0.990). Recovery tests were carried out in raw sewage samples for all analytes and varied between 56 ± 3% (AMP) and...
107 ± 4% (COC). Only eugonine (ECG) and eugonine methyl ester (EME) were not recovered after SPE extraction. The limits of detection (LOD) and quantification (LOQ) were expressed as 3S_y/b and 10S_y/b, respectively, where S_y was the standard deviation of the linear response and b was the slope of the analytical curve.²³ Analytical figures of merit of the method are portrayed in the Table S2 of the Supplementary Information.

Results and Discussion

Drugs of abuse, metabolites and adulterants in wastewater samples

Table 1 shows the concentration of the investigated analytes in wastewater samples collected during the two 2014 FIFA World Cup match weekends, as well as the concentration of COC and BE in a typical weekend of April 2012. It is possible to observe that only BE, COC, AE, phencetin and levamisole could be detected and quantified in the 2014 samples. The other investigated substances were not detected (ND) during the LC-MS/MS (liquid chromatography tandem mass spectrometry) analysis or presented analytical signals below LOQs. The five aforementioned analytes were found in all samples collected in 2014 suggesting that they are frequently discharged in the sewer systems. Benzoylcondamine, the major cocaine metabolite, was the most prevalent substance in the investigated samples, varying from 1.9 ± 0.6 to 4.2 ± 0.2 µg L⁻¹, followed by AE whose concentrations varied between 1.5 ± 0.2 and 2.6 ± 0.5 µg L⁻¹.

The epidemic use of crack cocaine, i.e., smoked unrefined base cocaine, in Brazil²⁴ coupled to its high occurrence in police seizures in our country,¹⁴,²⁵ led us to test specific metabolites related to the consumption of different forms of the drug. The high temperature used to vaporize crack/base cocaine in the smoking process led to the production of AEME which is also consumed.²⁶ Thus, besides cocaine and its most common metabolites, the use of crack cocaine also led to the formation of other metabolites such as anhydroeugonine ethyl ester, AE and nor-AE.²⁶,²⁷ In the human plasma, AEME is degraded through both enzymatic cleavage and spontaneous chemical hydrolysis to form AE.²⁸ Thus, considering that both products will be excreted through urine, the presence of AEME and AE in the investigated sewage samples could represent strong evidence on the crack/free base cocaine use by the FD population.

In Table 1 it is noticed that AE was detected in the samples in concentrations that slightly exceeds LOQ (1.2 µg L⁻¹). On the other hand, AEME was never found in wastewater. Similar results were obtained by Castiglioni et al.²⁹ investigating wastewater samples from Milan, Como and Chicago. However, concentration of AE in these samples varied between 1.0 and 35 ng L⁻¹, whereas in our work concentrations were, at least, 40 times higher. Biscoglia et al.³⁰ found both AE (92 ± 2.9 ng L⁻¹) and AEME (15 ± 0.5 ng L⁻¹) in a sewage sample submitted do SPE extraction revealing the prevalence of the former in accordance to our results.

The quantification of AE as a “smoked crack/free base cocaine marker” could, in theory, be used to

Table 1. Concentration of the investigated analytes in WWTP influents samples in the Federal District, June 2014 and April 2012 samplings

<table>
<thead>
<tr>
<th>WWTP</th>
<th>Date</th>
<th>Concentration / (µg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>COC</td>
</tr>
<tr>
<td>South-Wing</td>
<td>7/5/14</td>
<td>0.75 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>7/6/14</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>7/12/14</td>
<td>0.49 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>7/13/14</td>
<td>0.46 ± 0.07</td>
</tr>
<tr>
<td>North-Wing</td>
<td>7/5/14</td>
<td>0.75 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>7/6/14</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>7/12/14</td>
<td>0.65 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>7/13/14</td>
<td>0.6 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>4/21/12</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>4/22/12</td>
<td>0.69 ± 0.08</td>
</tr>
</tbody>
</table>

¹LOQ: limit of quantification; ²ND: not determined; ³NA: not analyzed. WWTP: wastewater treatment plant; COC: cocaine; AEME: anhydroeugonine methyl ester; BE: benzoylcondamine; Nor-BE: nor-benzoylcondamine; Nor-COC: norcocaine hydrochloride; EME: eugonine methyl ester; AE: anhydroeugonine hydrochloride; ECG: eugonine hydrochloride; LEV: levamisole; PHE: phencetin; AMP: (±)-amphetamine; MAMP: (±)-methylamphetamine hydrochloride; MDA: (±)-3,4-methylenedioxymethamphetamine; MDMA: (±)-3,4-methylenedioxymethamphetamine; MDEA: (±)-3,4-methylenedioxyethylamphetamine; MBDB: (±)-3,4-methylenedioxy-N-methyl-butamphetamine.
estimate the rate between snorted/injected hydrochloride cocaine versus smoked cocaine, providing relevant information to public authorities dealing with health, law enforcement and educational issues concerning the abuse of that drug. Castiglioni et al.\textsuperscript{28} also propose that the ratio of AE to BE could be useful as a rough estimate of the amount of crack cocaine used in a community. In their work, it was possible to observe an AE/BE ratio three times higher in Chicago (22 \times 10^3) than in Milan (6 \times 10^3) suggesting that the percentage of crack cocaine used in the former city was higher. According to the authors, this result was corroborated by epidemiological investigations indicating the greater prevalence of crack in USA than in Italy. In our work, the AE/BE ratio ranged from 629 \times 10^3 to 853 \times 10^3 suggesting a major use of smoked crack/free base cocaine. Our results show that, in thesis, AE could be also included in WBDE studies.\textsuperscript{11} However, there are still some limitations on the use of AE to estimate the actual use of smoked cocaine in a community. Firstly, it is known that the quantity of AEME formed during the drug vaporization depends on the pyrolytic conditions as well as on the composition of the illicit cocaine. In addition, the metabolic profile of cocaine in urine and wastewater can vary strongly depending on the characteristics of the investigated community. Finally, one can consider the growing number of users of both powder and crack cocaine, evidencing the difficulty to establish different group of users.\textsuperscript{72}

The results in Table 1 also show significant concentrations of levamisole, ranged from 0.5 \pm 0.2 to 1.23 \pm 0.09 \mu g L^{-1}, and phenacetin, from 0.62 \pm 0.06 to 1.3 \pm 0.1 \mu g L^{-1}. Levamisole is an anthelmintic present in registered pharmaceutical products while phenacetin is a pain-relieving and fever-reducing drug with no pharmaceutical products registered in Brazil. The findings of the Brazilian Federal Police chemical profiling program (PeQui project) showed that in the Federal District phenacetin is the main adulterant in crack/free base cocaine, while levamisole is mainly found in cocaine hydrochloride, both on the streets as well as in international trafficking cocaine seizures.\textsuperscript{13,14,25,33} Since both adulterants do not have significant legal use in Brazil, the detection in wastewater could be mainly related to the cocaine use, providing additional indicators about different forms of use (e.g. snorted versus smoked).

As discussed by our group in a previous work,\textsuperscript{12} it is relevant to the law enforcement point of view, the investigation of different cocaine forms of presentation (hydrochloride or crack/free base cocaine), since they have distinct patterns of international trafficking, rates of purification by oxidation (refining) and adulteration. Broséus et al.\textsuperscript{34} suggested that the cocaine adulteration with pharmaceutical products tends to be done very close to the production of cocaine or in high levels in the distribution chain. Thus, the WBDE approach could also be tuned to consider typical ways of adulteration of cocaine using levamisole and phenacetin concentration in wastewater. Figure 1 shows different analyte ratios calculated from the data portrayed in Table 1 in order to better understand the potential use of phenacetin and levamisole in WBDE studies.

![Figure 1. Ratios between cocaine, benzoylecgonine, anhydroecgonine, phenacetin and levamisole concentrations in both South-Wing (mean values as open squares) and North-Wing (mean values as full squares) WWTP samples collected in 2014. The large box represents the 25th percentile, median, and 75th percentile; error bars indicate the 5th and 95th percentiles and × symbols represent minimum and maximum values.](image-url)

Ratios between COC and BE have been used in WBDE studies to investigate possible dumping of non-consumed cocaine in the sewers.\textsuperscript{15,35} van Nuijs et al.\textsuperscript{10} suggested a cut-off value of 0.75 for the evaluation of abnormal COC/BE ratios with higher values indicating the absence of in vivo metabolization when the microbial conversion of COC into BE in the sewage system is slow and gradual. In Figure 1, all COC/BE values for both South and North-Wing WWTPs samples were lower than the cut-off value suggesting that measured COC and BE resulted from human consumption. As pointed out earlier, ratios between AE and BE are higher in our study in comparison of data collected elsewhere,\textsuperscript{28} but are in the same magnitude when both investigated regions are compared. Levamisole and phenacetin concentrations were also compared with BE data in order to identify possible differences on the forms of consumed cocaine. We believe that the ratio LEV/BE could indicate the cocaine use as hydrochloride cocaine whereas PHE/BE can be useful to differentiate the consumption of crack cocaine in addition to the calculation of AE/BE ratios. The magnitude of the LEV/BE ratios was similar in both investigated regions with slightly higher values observed for the North-Wing region. On the other hand, PHE/BE ratios were slightly higher.
in the South-Wing region suggesting a major use of free base/crack cocaine in this region. Thus, our results show the potential use of LEV and PHE in WBDE approaches in order to identify possible differences on the forms of consumed cocaine.

All ratios portrayed in Figure 1 were calculated using BE as denominator considering its well-established use in WBDE approaches. However, the use of BE to differentiate different forms of consumed cocaine might not be adequate since the production of metabolized BE is expected when both crack and hydrochloride cocaine is consumed. Thus, based on the previous findings of the PeQui Project we suggest that PHE/LEV ratios in WWTP samples could also be useful to indicate the prevalent use of different forms of cocaine. It is noticed in Figure 1 significant higher values for the PHE/LEV ratio for the samples from the South-Wing WWTP, indicating a major use of crack cocaine in this southern region. The consumption of crack in FD is not restricted to a particular region, but occurs in a diffuse way. However, numerous reports have already identified the South commercial sector, at the inner edge of the South-Wing, as a location, known as “cracolândia”, i.e. crackland, where crack cocaine users make steady use of the drug. However, we cannot attest that the crack cocaine use in this region was considerably higher during the weekends of the FIFA World Cup.

We believe that this approach, i.e., the use of PHE/LEV ratios in our studies, may be capable to provide useful information about the forms of cocaine consumption. However, as pointed out by several authors, chemical and biological transformation processes during transport in sewers, sampling and preservation steps demand special attention to led to more reliable WBDE data involving this pharmaceutical products.37

### Drug use estimates

In this work, the estimative of total cocaine consumption was carried out considering the approach suggested by Zuccato et al.,1 where the concentration of BE in the sewage samples has to be multiplied by 2.33 to correlate to the amount of free base cocaine consumed. As discussed by our group in a previous work,6 to correlate to cocaine hydrochloride consumed, the BE concentration has to be multiplied by 2.61, leading to an estimative of consumed hydrochloride COC 12% higher in comparison with the free base drug.

Considering the flow rate of both investigated WWTP during the sampling periods, it is possible to estimate the quantity of cocaine (g day⁻¹) as well as the per capita use considering the population served by the two WWTPs.

Estimates for cocaine use in the Federal District during the 2014 FIFA World Cup are shown in Table 2. Concentration of BE used in the back-calculations were individually corrected based on the results obtained during recovery test carried out on each sample. Standard deviations associated with the corrected BE concentration, as well as with load and consumed data, were calculated by the uncertainty propagation approach. The average volumetric flow rate (Qv) on both investigated WWTPs during the sampling periods was provided by the Environmental Sanitation Company of the Federal District (CAESB) together with the number of inhabitants served.

### Table 2. Estimative of consumed cocaine, expressed as free base, in the Federal District during weekends of July 2014 (2014 FIFA World Cup weekends) and April 2012

<table>
<thead>
<tr>
<th>WWTP</th>
<th>Date</th>
<th>Rec. BE / %</th>
<th>BEcorr. / (μg L⁻¹)</th>
<th>Qv / (L day⁻¹) × 10⁶</th>
<th>Hab / 10⁴</th>
<th>Load / (g day⁻¹)</th>
<th>Drug use / (mg hab⁻¹ day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>South-Wing</td>
<td>7/5/14</td>
<td>88 ± 2</td>
<td>3.9 ± 0.3</td>
<td>79.2</td>
<td>512.8</td>
<td>713 ± 61</td>
<td>1.4 ± 0.1abc</td>
</tr>
<tr>
<td></td>
<td>7/6/14</td>
<td>84 ± 4</td>
<td>2.8 ± 0.3</td>
<td>71.4</td>
<td>512.8</td>
<td>473 ± 51</td>
<td>0.9 ± 0.1c</td>
</tr>
<tr>
<td></td>
<td>7/12/14</td>
<td>103 ± 10</td>
<td>1.9 ± 0.4</td>
<td>76.6</td>
<td>512.8</td>
<td>333 ± 64</td>
<td>0.7 ± 0.1c</td>
</tr>
<tr>
<td></td>
<td>7/13/14</td>
<td>111 ± 4</td>
<td>2.3 ± 0.3</td>
<td>69.1</td>
<td>512.8</td>
<td>365 ± 44</td>
<td>0.71 ± 0.09b</td>
</tr>
<tr>
<td>North-Wing</td>
<td>7/5/14</td>
<td>90 ± 6</td>
<td>4.6 ± 0.4</td>
<td>35.6</td>
<td>141.9</td>
<td>385 ± 34</td>
<td>2.7 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>7/6/14</td>
<td>92 ± 8</td>
<td>3.2 ± 0.5</td>
<td>34.0</td>
<td>141.9</td>
<td>252 ± 38</td>
<td>1.8 ± 0.3a</td>
</tr>
<tr>
<td></td>
<td>7/12/14</td>
<td>92 ± 7</td>
<td>2.7 ± 0.3</td>
<td>53.8</td>
<td>141.9</td>
<td>340 ± 44</td>
<td>2.4 ± 0.3a</td>
</tr>
<tr>
<td></td>
<td>7/13/14</td>
<td>101 ± 5</td>
<td>3.1 ± 0.6</td>
<td>58.2</td>
<td>141.9</td>
<td>417 ± 83</td>
<td>2.9 ± 0.6a</td>
</tr>
<tr>
<td></td>
<td>4/21/12</td>
<td>98 ± 2</td>
<td>3.0 ± 0.3</td>
<td>41.3</td>
<td>139.6</td>
<td>287 ± 31</td>
<td>2.1 ± 0.2ab</td>
</tr>
<tr>
<td></td>
<td>4/22/12</td>
<td>98 ± 2</td>
<td>2.3 ± 0.3</td>
<td>42.1</td>
<td>139.6</td>
<td>226 ± 26</td>
<td>1.6 ± 0.2ab</td>
</tr>
</tbody>
</table>

WWTP: wastewater treatment plant; aRecovery (100 μg L⁻¹ of benzoylecgonine (BE)) calculated using three authentic replicates for each collected sample; bcorrected concentration of benzoylecgonine according to recovery results; c volumetric flow data (Qv) and number of inhabitants were compiled by the Environmental Sanitation Company of the Federal District (CAESB); dmeans followed by equal letters do not differ by the Tukey test, at 5% probability.
Results on Table 2 revealed that, during the World Cup, cocaine consumption was significantly lower in the region served by the South-Wing WWTP (from 0.7 ± 0.1 to 1.4 ± 0.1 mg hab\(^{-1}\) day\(^{-1}\)) when compared to the North-Wing WWTP region, where cocaine use varied between 1.8 ± 0.3 and 2.9 ± 0.6 mg hab\(^{-1}\) day\(^{-1}\)). These results are coherent with previous data collected in the both served regions as a result of the significant higher population served by the South-Wing WWTP. It is also possible to observe that the consumption of cocaine was apparently higher in both FIFA World Cup weekends in comparison with the ordinary reference weekend. With the exception of the result obtained on July 6\(^{th}\), 2014, i.e., the day after the Argentina vs. Belgium match, all estimates obtained for cocaine use were significantly higher during the Soccer World Cup in comparison with the 2012 weekend. Despite the higher per capita cocaine consumption in the north region during the Soccer World Cup, Table 2 also shows that cocaine loads were consistently higher in both investigated regions during this major event in comparison to the ordinary reference weekend.

Figure 2 shows average cocaine loads in both investigated WWTPs in 2012 and 2014, as well as the average cocaine per capita consumption in the regions served by the WWTPs. The values showed in Figure 2 were based on the geometric means of the data portrayed in Table 2 since it indicates more efficiently the central tendency or the typical value for a given set of numbers.

Figure 2 evidenced that cocaine loads, expressed in grams per day, were higher in the South-Wing WWTP in comparison with the data collected in the North-Wing WTP, corroborating previous findings of our group. When both data related to the North-Wing WWTP is compared, i.e., from 2012 and 2014, it is possible to infer the influence of a major sport event on the abuse of drugs. In this case, we estimate a 25% increase of cocaine use in 2014 (mean of 343 g day\(^{-1}\)) in comparison with 2012 (mean of 255 g day\(^{-1}\)). When consumption loads are normalized with the number of inhabitants served by each WWTP it is possible to observe that the per capita use of cocaine is significantly higher in the North region, as expected. Again, an increase of approximately 25% regarding the consumed cocaine during the 2014 FIFA World Cup was noticed when compared with per capita data from 2012.

**Conclusions**

An analytical method based on SPE-LC-MS/MS was implemented to quantify cocaine alkaloids, metabolites, adulterants and amphetamine-type substances in the influent sewage collected in two wastewater treatment plants in the Brazilian Federal District (FD). The method was applied to investigate community drug use during two consecutive weekends with two 2014 FIFA World Cup matches. The results were compared with the ones obtained during an ordinary weekend in 2012.

Only benzoylecgonine (BE), anhydroecgonine (AE), phenacetin (PHE), levamisole (LEV), and cocaine (COC) could be quantified in the investigated samples while the other analytes, mostly cocaine related and amphetamine-type substances, could not be determined.

The major detection of AE, more than 300 times higher in comparison with previous results in the literature, reinforces the viability of the WBDE approach to tackle the use of different cocaine presentation forms, since AE/BE ratios revealed a significant use of smoked crack/free base cocaine in the FD.

COC and BE levels suggested a human consumption origin for the investigated analytes while the relationships between the adulterants PHE and LEV clearly indicate different patterns of cocaine use, either via smoked crack/free base cocaine in the southern region or in the form of snorted/injected cocaine in the northern region.

Estimates for cocaine use in the FD during the 2014 FIFA World Cup were obtained using benzoylecgonine levels followed by typical back-calculations applied in the WBDE approach. It was possible to infer a 25% increase of cocaine use in 2014 (mean of 343 g day\(^{-1}\)) in comparison with 2012 (mean of 255 g day\(^{-1}\)) in the region covered by the North-Wing WWTP.

This work also revealed the importance of technical partnerships and collaborations between academic and forensic institutions, since the aggregation of forensic
chemical profiling results to the WBDE could provide new analytical targets that may contribute to the better understanding of the illicit drug scenario.

**Supplementary Information**

Supplementary data are available free of charge at http://jbcs.sbq.org.br.

**Acknowledgments**

This work has been supported by the National Institute for Advanced Analytical Science and Technology (INCTAA/FINEP/MCT 01.09.0275-00) and by the National Council of Technological and Scientific Development (CNPq 402783/2010-7). The authors also thank to the forensic experts Élvio Dias Botelho and Lucio Paulo Lima Logrado for the support during the LC-MS/MS method development.

**References**