frontiers | Frontiers in Analytical Science

ORIGINAL RESEARCH
published: 23 June 2022
doi: 10.3389/frans.2022.930480

Understanding Illicit Drug Use Trends During the Carnival Holiday in the Brazilian Capital Through Wastewater Analysis

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Cocaine and cannabis consumption during and after the 2019 Carnival holiday were assessed using the wastewater-based epidemiology (WBE) in the capital of Brazil, Brasília. The substances 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), cocaine (COC), benzoylecgonine (BE), and cocaethylene (COE) were monitored in composite samples (24 h) collected in the entrance of North-Wing (NW) and South-Wing (SW) wastewater treatment plants (WWTP) for 15 consecutive days, including the Carnival holiday. Aliquots (100 ml) were enriched with isotope-labeled standards, solid-phase extracted and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Results reveal higher cocaine consumption during the Carnival (average of 2.8 ± 0.7 g/1000inh/day) compared to the subsequent period (average of 1.7 ± 0.3 g/1000inh/day). Cannabis (THC) use was also higher during the holiday (14 ± 5 g/1000inh/day) but differences were not significative (unpaired *t*-test, 95%) compared to the following days (11 ± 3 g/1000inh/day), where consumption remained relatively constant corroborating that cannabis overall consumption is less affected by occasional abuse. Regarding cocaine, an unusual low consumption was noticed in the weekend immediately after the Carnival Holiday, indicating lower demand or supply issues. Higher cocaine and cannabis use was observed throughout the entire sampling period in the area covered by NW-WWTP, probably due to the higher proportion of young people. This investigation brings the first data on cannabis use in Brazil by WBE and confirms this strategy as a well consolidate tool for estimating illicit drug use and abuse.

Keywords: illicit drugs, sewage, wastewater-based epidemiology, carnival holiday, cocaine, cannabis, temporal trends

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Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Environmental Analysis, a section of the journal Frontiers in Analytical Science

Received: *28 April 2022*
Accepted: *01 June 2022*
Published: *23 June 2022*

Citation:
Sodré FF, Freire DdJS, Alcântara DB and Maldaner AO (2022) Understanding Illicit Drug Use Trends During the Carnival Holiday in the Brazilian Capital Through Wastewater Analysis. Front. Anal. Sci. 2:930480. doi: 10.3389/frans.2022.930480

INTRODUCTION

Residues from licit and illicit drugs, personal care products, hormones, pathogens, and a variety of substances associated with consumer products have been investigated in environmental samples, considering their relation to lifestyle in modern societies (Pal et al., 2014). Although wastewaters are an important source of these contaminants of emerging concern to the environment, their investigation may also reveal population exposure, behavior and habits (Gracia-Lor et al., 2017; Choi et al., 2019).

Recently, wastewater-based epidemiology (WBE) emerged as a monitoring strategy to assess population exposure do SARS-CoV-2 worldwide (Ahmed et al., 2021). However, it has also been used for the past decade to estimate population exposure to pharmaceuticals, pesticides and, most notably, illicit drugs (Choi et al., 2018; Lorenzo and Picó, 2019). Illicit drug use estimates using wastewater analysis allow dynamic and rapid investigations into the temporal and geospatial evolution of consumption (Choi et al., 2018). In addition, WBE can provide important information about illicit drug use at special events, such as National holidays and popular festivals.

Several studies show an increase in the concentration of drug residues in wastewater collected during holidays, festive and sport events in different regions of the world (Sodré et al., 2017; Verovšek et al., 2020; Montgomery et al., 2021). Music event attendees, for example, may consume illicit substances more often in comparison with ordinary people of the same age group. Such festive events are therefore opportune for monitoring illicit drug use (Van Havere et al., 2011). Rushing and Burgard (2019) reported higher loads of cocaine and its major metabolite, benzoylecgonine, in wastewaters collected during the Pride festival in Washington (United States). In Queensland (Australia) Lai et al. (2013b) monitored residues of drugs and their metabolites in wastewaters and found that cannabis and cocaine use steadily increased throughout a six-day annual music festival. Mackuľak et al. (2019) showed that there was preferential use of cocaine at dance and multi-genre themed festivals, while cannabis was the drug of choice at a pop-rock festival, evidencing that the abuse of drugs is closely associated with specific musical preferences. These findings show that the illicit substance market is rapidly expanding into special music events where attendees appear to be willing to abuse drugs.

A significant increase in cocaine loads was also observed during the Independence Day in US (Foppe et al., 2018), the Easter holiday in France (Thiebault et al., 2019), the Chinese National Day (Zhang et al., 2019), and the Brazilian Carnival (da Silva et al., 2018). Lai et al., 2013a) and Huerta-Fontela et al. (2008) found that cannabis and cocaine use on Christmas and New Year's Eve increased in urban areas of Australia and Spain, respectively. The concomitant use of cocaine and alcohol was assessed by da Silva et al. (2018) through the high content of cocaethylene in a wastewater sample collected during the 2018 Carnival Holiday in Brazil. The authors suggested that cocaine use could be even higher, as the ingestion of ethanol with cocaine leads to the excretion of cocaethylene instead of benzoylecgonine, the main metabolite used to estimate consumption.

As the previous findings assessing cocaine consumption during the Brazilian Carnival were obtained through the analysis of a single sample collected in the nation capital, Brasilia (da Silva et al., 2018), the goal of this study is to assess, for the first time, cannabis and cocaine consumption (including the analysis of cocaethylene) in wastewater samples from two treatment plants, collected throughout the entire Carnival holiday and in subsequent days, totalizing 15 consecutive days of sampling. This complementary work aims to provide a better understand on the illicit drug use trends as influenced by a major special event such as the Carnival in Brazil. To the best of our knowledge this is also the first study assessing cannabis consumption using WBE in Brazil. In addition to the data produced involving cocaine use, the results can help authorities to map hot spots of drug use and associate them with the behavior of illicit drug users during special events and regular days of the week.

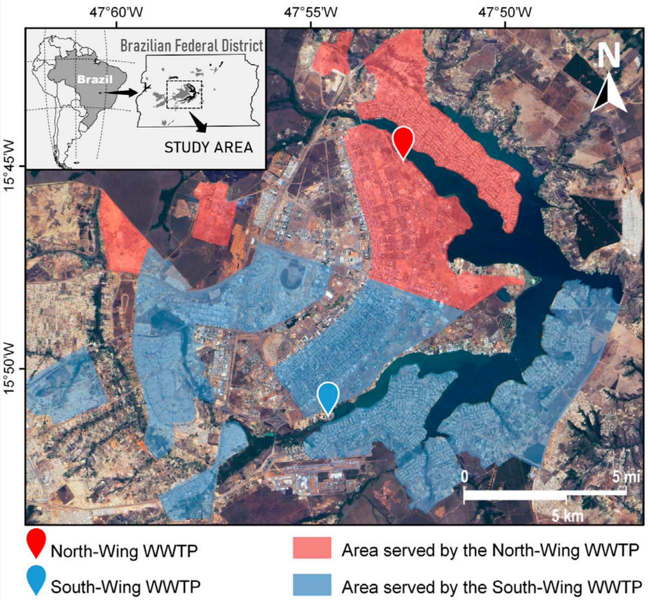

FIGURE 1 | Map showing the location of Brasília, in the Brazilian Federal District (Brazil), and the areas served by the North-Wing and South-Wing wastewater treatment plants.

MATERIALS AND METHODS

Chemicals and Reagents

Cocaine (COC) and its metabolites benzoylecgonine (BE) and cocaethylene (COE) were purchased from Cerilliant (Round Rock, TX, United States) as solutions of 1 mg/ml in acetonitrile (ACN). The main metabolite of tetrahydrocannabinol, 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) was also obtained from Cerilliant as a 0.1 mg/ml methanol (MeOH) solution. Deuterated compounds were used as surrogate standards for analytical quantitation. Cocaine-D3 (COC-D3), benzoylecgonine-D3 (BE-D3), cocaethylene-D3 (COE-D3), and THC-COOH-D3 were supplied by Cerilliant. Mixed solutions were prepared with MeOH whereas working solutions were prepared before analysis by successive dilutions of the mixed solution with MeOH and stored at −20°C in the dark.

Formic acid (98%) MS-grade, hydrochloric acid (HCl, 37%) and MeOH (LC LiChrosolv grade) were purchased from Merck (Darmstadt, Germany). Ultrapure water was produced in a Milli-Q Academic system (Millipore, Bedford, MA, United States).

Characterization of the Study Area

This work was carried out in the Brazilian capital, Brasília. Along with other satellite cities and administrative regions, Brasilia is part of the Brazilian Federal District (FD), a relatively small three million inhabitants' unit, politically equivalent to the other 26 Brazilian states. With an airplane shape, Brasília was specifically planned and build from the scratch in the 1960s to host the executive, legislative and judicial branches. The so-called Pilot Plan is organized in sectors that concentrate certain types of activities, such as banking, financial, commercial, hospital, entertainment and residential sectors. Wastewater produced in the FD is collected and treated in 15 wastewater treatment plants (WWTP), but in the present work only two, located in the south and north wings of the Pilot Plan, were selected (**Figure 1**). The South-Wing (SW) WWTP serves around 525,000 people residing in ten administrative regions of the FD while North-Wing (NW) WWTP covers an area with approximately 145,000 inhabitants from five administrative regions. Together, they serve the entire population of Brasília (Pilot Plan) and around 22% of the entire FD.

Characterization of the Carnival Holiday in Brazil

Carnival is the most popular festival holiday in Brazil. Celebrations occur between February and March and usually begin on Friday afternoon, ending on the Ash Wednesday at noon, marking the beginning of Lent, the forty-day period before Easter, according to Catholic tradition. During this period, several festive events take place throughout Brazil, from North to South. The southeast cities of Rio de Janeiro and São Paulo host the most famous carnival parades, while in the northeast cities of Salvador, Recife and Olinda, more emphasis is given to the street events. In practice, the country is virtually unified for almost a week, in day and night festivities, accompanied by lots of music, dancing and drinking throughout the holiday. In Brasília, as in the vast majority of Brazilian cities, there are daytime activities on the streets as well as night parties in sport clubs, associations and nightclubs. The Carnival holiday studied was in 2019 and started on Friday, March 1st and ended on Wednesday, March 6th, although related events may also have occurred few days before or after this period.

Sampling and Sample Preparation

Raw sewage influents from the two WWTPs were collected daily, over two consecutive weeks, from March 1st to 15th 2019, with the exception of the 08/03 sample which was not collected at SW-WWTP due to logistical issues. This period corresponded to the Carnival holiday and the following days. On each day, 24-h flow proportional composite samples were obtained from each WWTP using refrigerated automatic wastewater samplers (AS950 AWRS, Hach, Loveland, CO, United States). Sampling began 12 midnight (12.00 a.m.) and ended at the same hour on the next day. During this period, aliquots were kept in the dark at 4°C. The composite samples were then taken to the laboratory in amber glass bottles every day at 7:00 a.m. and analyzed on the same day.

Sample preparation was carried out according to the protocol proposed by Causanilles et al. (2017a). Briefly, 100-ml wastewater aliquots were pH-adjusted (7.0), enriched with 4 ng/ml of the surrogate deuterated standards, passed through 1.2 μm GF/C glass microfiber filters (Whatman, Maidstone, United Kingdom) followed by 0.45 μm cellulose acetate membranes (Whatman, Maidstone, United Kingdom). Then, they were transferred to individual syringe tubes connected in line to disposable cartridges for solid-phase extraction (SPE) containing 500 mg of a polystyrene divinylbenzene sorbent with both hydrophilic and lipophilic groups (HLB Oasis, Waters, Milford, CT, United States). The sorbent was conditioned with 10 ml of MeOH and 10 ml of pH 7.0 ultrapure water and samples percolate cartridges at a flow rate of 3 ml/min in order to provide adequate contact between analytes and the solid phase. Cartridges were then centrifuged at 4,000 rpm for 8 min to eliminate residual water and stored at −20°C until the elution step (Senta et al., 2014; Causanilles et al., 2017a). Immediately before analysis, the analytes were eluted from the cartridges with 10 ml of MeOH and recovered into previously cleaned glass tubes. The eluates were individually evaporated under vacuum in a Syncore Analyst system (Buchi, Flawil, Switzerland) in order to obtain a volume of approximately 0.2 ml and the extract was diluted to a final volume of 1.0 ml using a 0.1% (v/v) formic acid solution prepared in ultrapure water. Before injections, extracts were filtered through a 0.22 μm PVDF syringe membranes (Merck Millipore, Billerica, MA, United States) in order to avoid residual solids.

Quantification of the Target Analytes

Extracts were analyzed by liquid chromatograph (1200 Series, Agilent, Santa Clara, CA, United States) coupled to triple-quadrupole (QqQ) mass spectrometer (QTRAP 3200, Sciex, Toronto, Canada) with electrospray ionization (ESI) interface operating at 550°C and 5500 V, using nitrogen as curtain gas at 10 psi and as auxiliary and nebulizing gas at 40 and 45 psi, respectively.

Separation was performed at 30°C using a Kinetex C18 column (2.1 mm × 50 mm, particle size of 1.3 μm, Phenomenex, Torrance, CA, United States) with gradient elution (0.2 ml min^{-1}) using water and ACN as mobile phases containing 0.1% (v/v) of formic acid, used to improve ionisation and the intensity of analytical signals. The gradient was achieved by maintaining a relative ACN concentration of 20% for 1 min, followed by the increase to 95% in 5 min, and held constant for 4 min. After readjusting to the initial conditions, the system was re-equilibrated for 5 min. The injection volume was 10 μL.

Mass spectrometric analyses were carried out using the multiple reaction monitoring (MRM) mode in order to identify and quantify the target analytes by measuring the fragmentation products of the protonated molecular ions $[M + H]^+$ for all analytes. Each analyte was monitored using the two most abundant precursor → product ion transitions. Instrumental parameters, such as declustering potential (DP), entrance potential (EP), collision cell entrance potential (CEP), collision energy (CE) and collision cell exit potential (CXP) were optimized for each analyte as shown in **Table 1**.

All analytes were quantified by internal standard calibration using 8-points analytical curves (1.0–350 μg/L for the cocainics and 5.0–350 μg/L for THC-COOH) prepared in tap water in a matrix-matched approach. Curves ($R^2 > 0.995$) were previously tested for the homogeneity of variances by the Cochran test. Limits of quantification of the method (LOQ) were expressed by

TABLE 1 | Mass spectrometer parameters used during the analysis, chromatographic retention times and limits of detection and quantification of the target analytes.

Analyte	DP (V)	EP (V)	CEP (V)	MRM transitions (*m/z*)	CE (eV)	CXP (V)	RT (min)	LOD (ng/L)	LOQ (ng/L)
BE	37	4.0	12	290.1→168.4[1]	27	3.0	1.14	3	10
				290.1→105.2	41	3.6			
COC	37	4.0	12	304.2→182.3[1]	24	3.7	1.93	3	10
				304.2→105.2	48	3.0			
COE	39	4.0	12	318.2→196.2[1]	46	2.3	2.79	3	10
				318.2→82.1	27	7.7			
THC-COOH	36	4.0	12	345.3→327.4[1]	21	4.3	5.70	15	50
				345.3→299.1	27	12			
BE-D3	35	5.0	21	293.1→171.4[1]	27	5.0	1.15	n/a	n/a
				293.1→105.3	46	4.0			
COC-D3	31	5.0	21	307.2→185.3[1]	22	3.0	1.95	n/a	n/a
				307.2→105.3	44	3.0			
COE-D3	31	5.0	22	321.1→199.3[1]	23	3.5	2.81	n/a	n/a
				321.1→85.2	44	2.3			
THC-COOH-D3	50	5.0	24	348.2→330.2[1]	16	6.7	5.74	n/a	n/a
				348.2→302.4	23	5.5			

[1]Transition used for quantification. DP, declustering potential; EP, entrance potential; CEP, collision cell entrance potential; CE, collision energy; CXP, collision cell exit potential; RT, retention time; LOD, limit of detection of the method; LOQ, limit of quantification of the method; n/a, not applicable.

the lower standard concentration of the analytical curves divided by the pre-concentration factor of 100 times. Limits of detection (LOD) were calculated using LOQ divided by the factor of 3.3. Recoveries from a wastewater spiked solution (500 ng/L) ranged from 77% (THC-COOH) to 102% (BE) (RSD < 9.9%). The software Analyst was used to instrument control and data acquirement, whilst data treatment was performed using the SignalFinder1 algorithm in the Multiquant platform, both integrated to the Analyst software.

Illicit Drug Use Estimates

Cocaine and cannabis consumption estimates (C), in mg/1000inh/day, were calculated according to **Eq. 1**, where c, in mg/L, is the concentration of the biomarker (BE for cocaine and THC-COOH for cannabis estimates), Q_v is the volumetric flow of the influent wastewater (L/day), f is a correction factor associated to the excretion of the biomarker in relation to the drug consumed and *hab* is the number of inhabitants served by the WWTP.

$$C = \frac{c \times Q_v \times f}{inhab} \tag{1}$$

For cocaine use estimates, a f value of 4.19 has been used in Brazil (da Silva et al., 2018; Sodré et al., 2018), reflecting a weighted urine excretion factor of 25% of BE as influenced by pharmacokinetics studies (Castiglioni et al., 2013) as well as by the average route of administration of cocaine in Brazil with a prevalence of intranasal (cocaine hydrochloride) adult users (70%) in comparison with crack (free-base) users (30%) (Laranjeira et al., 2014). For cannabis (THC), an average excretion of 0.5% of THC-COOH in urine is considered when cannabis is administered *via* the pulmonary route. Therefore, considering a THC/THC-COOH molar ratio of 0.91, the f value is 182 (Gracia-Lor et al., 2016).

For all intents and purposes, in this work, estimated cannabis consumption refers to THC, the main active ingredient in any of the very broad families of cannabis products available (marijuana, hashish, extracts, amid others).

In addition to the carnival period, two other periods were analyzed in the present work. Wastewater samples from Monday to Thursday were considered weekdays, while those from Friday to Sunday were considered weekends. As sampling starts at midnight, user contribution during Friday night social activities can be computed. This approach has been used elsewhere (Celma et al., 2019; Bijlsma et al., 2021; Kuloglu Genc et al., 2021), probably because users may abuse alcohol and other drugs during promotions such as "happy hours," which usually take place on Thursday, Friday and Saturday nights. In addition, Dázio et al. (2016) investigate the use of alcohol and controlled drugs among male university students in Brazil and show that preferred days to use controlled substances are Fridays and Saturdays.

RESULTS AND DISCUSSION

Concentration of the Target Analytes in Wastewater

Table 2 shows the concentration of cocaine, benzoylecgonine, cocaethylene and carboxi-THC obtained during 15 consecutive days in wastewater samples collected in the North-Wing and South-Wing WWTP.

The concentrations of cocaine and benzoylecgonine were similar to those found in previous studies carried out in both WWTPs (Maldaner et al., 2012; Sodré et al., 2017, 2018; da Silva et al., 2018). However, concentrations of cocaethylene in this work, ranging from 3 to 53 ng/L, were lower than those observed by da Silva et al. (2018) for samples collected at the same sampling points in a regular week of 2017 (19–188 ng/L). Regarding THC-COOH, data shown in **Table 2** are the first obtained in wastewater samples from the study region and, to the best of our knowledge, from the entire Brazilian territory. **Figure 2** portrays the concentrations ratios of the target analytes.

TABLE 2 | Daily concentrations, in ng/L, of the investigated substances in the wastewater samples and average volumetric flow at the sampling points during the sampling period.

Date	BE	COC	COE	THC-COOH	Qv (L/s)
North-wing WWTP					
03/01 (F)	2945	1161	15.2	244.6	617.0
03/02 (S)	2453	1092	37.4	446.3	624.4
03/03 (S)	2436	822.8	26.4	351.4	623.3
03/04 (M)	1704	867.2	10.4	327.7	536.8
03/05 (T)	2844	779.4	20.0	375.2	510.1
03/06 (W)	2181	676.9	<LOQ	304.0	534.8
03/07 (T)	2126	610.7	<LOQ	313.4	544.9
03/08 (F)	2036	778.1	<LOQ	269.1	523.3
03/09 (S)	1538	1094	53.3	303.7	450.2
03/10 (S)	1752	943.6	45.3	243.1	482.5
03/11 (M)	1427	646.9	25.3	251.4	492.6
03/12 (T)	1283	718.5	13.2	280.8	568.5
03/13 (W)	1211	631.1	<LOD	311.2	565.5
03/14 (T)	1424	543.8	12.6	256.4	544.3
03/15 (F)	1904	730.1	<LOQ	279.8	574.9
Mean	*1951*	*806.4*	*25.9*	*303.9*	*546.1*
Median	*1904*	*778.1*	*22.7*	*303.7*	*544.0*
South-wing WWTP					
03/01 (F)	2088	838.5	<LOQ	106.9	1545
03/02 (S)	3680	1190	29.5	279.8	1362
03/03 (S)	1902	1013	17.5	190.9	1446
03/04 (M)	1387	730.0	<LOQ	218.3	1371
03/05 (T)	1972	753.6	<LOQ	163.6	1392
03/06 (W)	1566	606.9	<LOD	108.9	1199
03/07 (T)	1538	616.0	<LOD	149.9	1265
03/08 (F)	N/A	N/A	N/A	N/A	1278
03/09 (S)	1264	825.7	14.5	172.7	1250
03/10 (S)	1201	576.8	13.8	168.2	1130
03/11 (M)	1182	572.8	13.9	165.5	1059
03/12 (T)	1036	500.2	<LOD	196.9	1251
03/13 (W)	1395	491.4	<LOQ	167.6	1261
03/14 (T)	1812	711.9	<LOQ	139.8	1414
03/15 (F)	2238	688.2	<LOQ	208.3	1378
Mean	*1733*	*722.5*	*17.8*	*174.1*	*1307*
Median	*1552*	*700.1*	*14.5*	*167.9*	*1278*

BE, benzoylecgonine; COC, cocaine; COE, cocaethylene; THC-COOH, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol; Q_v, volumetric flow; WWTP, wastewater treatment plant; LOD, limit of detection; N/A, not analyzed.

The values for COC/BE ratios in **Figure 2**, ranging between 0.27 and 0.71 (average 0.43 ± 0.09), are in agreement with previous studies (da Silva et al., 2018; Sodré et al., 2018) and indicate the predominance of human consumption to explain the origin of cocaine in the samples. Higher ratios were observed for samples collected during the weekdays in both investigated WWTP.

Although van Nuijs et al. (2009) have suggested a cut-off value of 0.75 to indicate sources unrelated to cocaine consumption, such as transport and handling in streets or clandestine facilities, the proportions of parental cocaine and its metabolites in excreted urine may vary significantly depending on the time of use, route of administration and co-consumption with other substances (Shimomura et al., 2019; Isenschmid, 2020). Moreover, transformations may also occur in sewage or samples if proper preservation routines are not implemented during analysis (McCall et al., 2016).

In-sewage transformations of cocaine, benzoylecgonine and cocaethylene were previously estimated considering ambient conditions of the Brasilia wastewaters (pH 7.5 and temperature of 23°C) during 24 h (da Silva et al., 2018). Considering the results, where a relative decrease of 14.6% for cocaine and an increase of 9.0% for benzoylecgonine was observed, the COC/BE ratios should become smaller and smaller over time. Therefore, monitoring COC/BE rations should still be promising to investigate sources unrelated to illicit drug use. One can consider that high COC/BE ratios could be potentially useful to indicate the disposal of non-consumed drugs into sewer systems. However, conclusions about the origin of the drug using diagnostic ratios must be made with care, even if they are promising to support decision-making related to public safety in countries such as Brazil, which borders the three largest cocaine producers—Colombia, Peru, and Bolivia—and is a major transit point for cocaine smuggling to Europe through Central and West Africa (Miraglia, 2015). Nonetheless, COC/BE ratios between 0.76 and 25.83 were reported in samples collected in Bursa—Turkey indicating that not all cocaine loads were from human consumption (Kuloglu Genc et al., 2021). According to the authors, cocaine was widely seized in Europe during 2017, leading to a new smuggling route from Turkey to Europe.

It is important to point out that low rates of metabolic conversion of cocaine to benzoylecgonine could also be a consequence of the concurrent use of cocaine with other substances such as ethanol and clinical drugs (Shimomura et al., 2019; Abbott et al., 2020). The combination of cocaine and ethanol, in addition to forming cocaethylene, dramatically alter the molar ratios of cocaine metabolites. Harris et al. (2003) found that ethanol ingestion before cocaine administration decreased urinary benzoylecgonine levels by 48% and increased urinary cocaethylene and ecgonine ethyl ester levels. In this case, COC/BE ratios may become higher due to the use of cocaine concomitantly with alcohol, with the consequent formation of cocaethylene. It is interesting to note that, in fact, the highest COC/BE ratios observed on weekdays (**Figure 2**) are followed by high COE/BE ratios (cocaethylene/benzoylecgonine), notably for samples collected at the North-Wing WWTP.

In the present work, the COE/BE ratios were strangely higher during the ordinary weekdays, while high values are normally expected during the weekends and at the Carnival, as shown earlier (da Silva et al., 2018). In view of the low cocaethylene values determined in the samples, the COE/BE ratios were also below those reported in other studies, where values ranged between 0.025 and 0.055 (Cami et al., 1998; Harris et al., 2003). On the 2018 Carnival holiday, for example, da Silva et al. (2018) reported values around 0.07 for samples collected in Brasília, while in the present work, COE/BE ratios ranged between 0.002 and 0.034.

Concomitant use of alcohol and cocaine is quite common at festive events and during regular weekends (Van Havere et al., 2011). This is a potentially harmful combination, since cocaethylene is an active substance, with a stimulant action similar to that of cocaine, while benzoylecgonine is a non-active metabolite (Shimomura et al., 2019). Cocaethylene excretion is less than 1%, with higher values directly associated with the proportion of alcohol and cocaine ingested (Harris et al.,

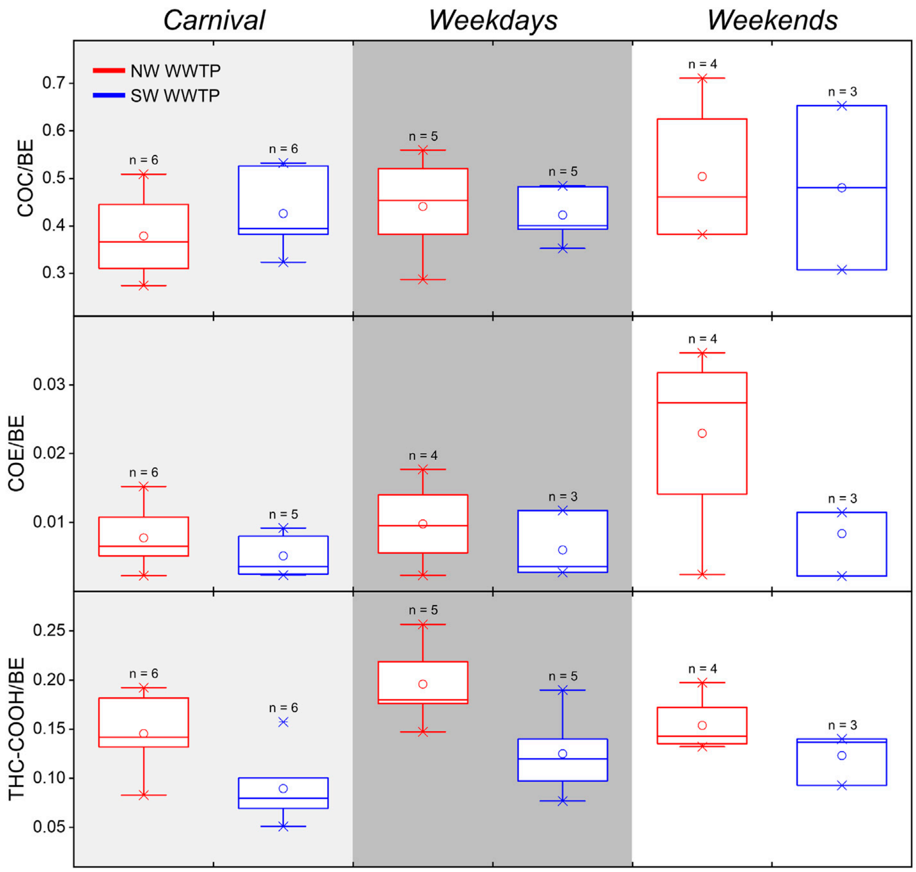

FIGURE 2 | Concentration ratios of the investigated substances. Data from the North-Wing WWTP in red and from the South-Wing WWTP in blue. For COE/BE ratios, COE concentrations between LOD and LOQ were represented by LOQ/2. Values below LOD were not included.

2003; Herbst et al., 2011; Occupati et al., 2017). With this information, it is theoretically possible to assess whether there was significant co-consumption of cocaine with alcoholic beverages by estimating COE/BE ratios. Rodríguez-Álvarez et al. (2015) compiled pharmacological data concerning the administration of both substances and suggest a cut-off value of 0.039. None of the COE/BE ratios obtained in the present work was higher than the reference value.

Ratios between the metabolites used to estimate cannabis and cocaine use, THC-COOH and benzoylecgonine, respectively, are also shown in **Figure 2**. Higher THC-COOH/BE values were constantly observed for samples collected in the North-Wing WWTP, independently of the sampling period. Although such diagnostic ratio is not commonly used in the literature, it may be useful to compare the prevalence of cannabis consumption between different regions under investigation.

Illicit Drug Use Estimates During the Sampling Period

In **Figure 3**, illicit drug use estimates, in mg/1000inh/day, are shown for the investigated regions during the 2019 Carnival holiday and the subsequent days.

Cocaine use during the Carnival holyday was higher than in the subsequent period, a result in agreement with previous findings (da Silva et al., 2018). Considering both investigated regions, an average cocaine consumption of 2754 ± 686 mg/1000inh/day (mean ± confidence interval, 95%) was noticed during the Carnival holyday period (from the 1st to the 6th of March), whereas for the subsequent period, from the 7th to the 15th of March, a consumption of 1739 ± 327 mg/1000inh/day, significantly lower (unpaired sample *t*-test, $t = 3.05 > t_{P=0.05} = 2.05$), was observed. Higher cocaine consumption was observed in the region covered by the North-Wing WWTP compared to the region served by the South-Wing WWTP, with the exception of three isolated days. This result is consistent with findings from the last 10 years that have always shown higher cocaine use in the northern region of Brasília (Maldaner et al., 2012; Sodré et al., 2017, 2018; da Silva et al., 2018; González-Mariño et al., 2019).

During the Carnival period, it is possible to notice a gradual decrease in cocaine consumption in the last days of the holiday (except for 03/05), as well as shortly after, between the 6th and 12th of March. This behavior is intriguing, as it indicates a relatively lower cocaine use on the weekend following the Carnival period, although several studies have shown that

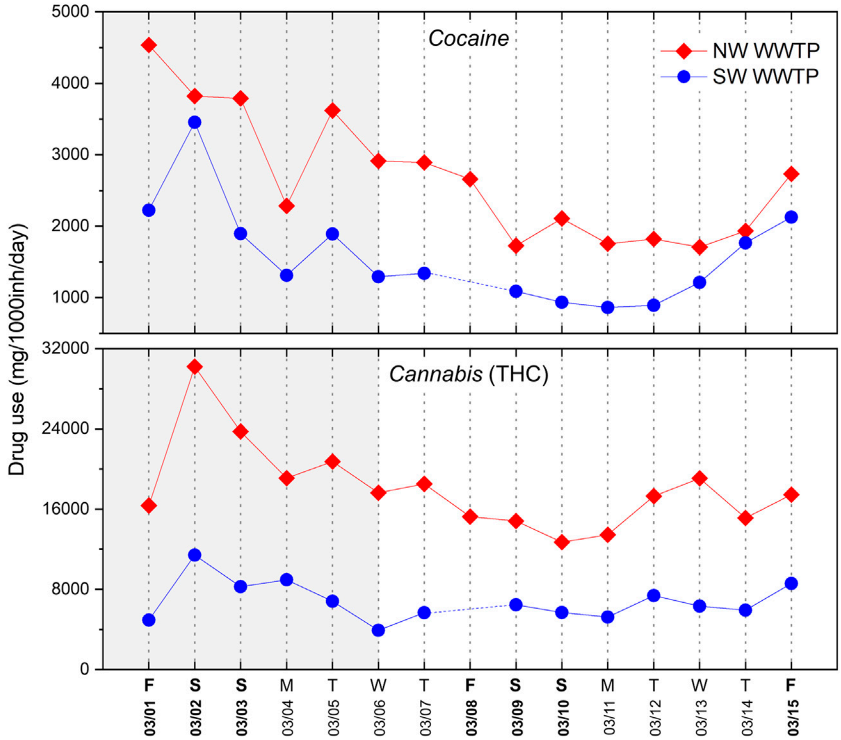

FIGURE 3 | Cocaine and cannabis (THC) *per capita* consumption estimated in the regions covered by North-Wing (NW, in red) and South-Wing (SW, in blue) WWTPs. The gray area represents the Carnival period and dates in bold represent the days of the weekend.

there is a higher use of cocaine, as well as other stimulant drugs, on weekends compared to weekdays. Therefore, three hypotheses may be raised to understand this behavior. The first considers that the high use of cocaine during the carnival holiday, almost 60% higher than the subsequent period, results in an immediate reduction in drug use on the following weekend, as if users were facing a kind of hangover from cocaine. Actually, this behavior is typical of binge users, i.e., episodic users who consume repeated doses of cocaine followed by a period of abstinence (Vosburg et al., 2010).

The second hypothesis is based on the fact that cocaine is a drug whose intense use is more often related to recreational and social activities (Zuccato et al., 2008; Tscharke et al., 2016; Verovšek et al., 2020). Thus, the cocaine hangover may have been motivated both by excessive use during Carnival and by the relative low frequency of parties and recreational activities on the weekend immediately after the festivities. Finally, the third hypothesis considers a possible decrease in the supply or a reduction of purity of cocaine on the streets, precisely because of the excessive demand during the Carnival. In this case, the low consumption on the weekend after holiday may be related to the dynamics of drug trafficking, affected by the high demand during the festivities. Although there is a lack of elements to support any of the hypothesis, future work can be carried out in coordination with the activities of the National Secretariat for Drug Policies (SENAD), one of the funders of wastewater-based epidemiology studies in Brazil.

In **Figure 3**, it is also possible to observe that cocaine consumption in both investigated areas rose sharply from March 13th, suggesting that drug use increases in the next weekend as expected.

Regarding cannabis, the first notable aspect in **Figure 3** is the higher consumption in the region served by the North-Wing WWTP compared to the southern region of Brasília. Considering that drug use is more frequent among adolescents, young adults and adults (UNODC, 2021), the difference in the consumption profile in the investigated regions may be associated to the relative higher proportion of young people in administrative regions served by North-Wing WWTP (CODEPLAN, 2016). Also, the northern part of the Brasília Pilot Plan is characterized by a high number of colleges and universities, including main *campus* of the University of Brasília. Higher THC-COOH concentrations were also reported by Mackuľak et al. (2014) in areas with increased concentration of young people and artists in the Slovak Republic.

Apparently, cannabis consumption in **Figure 3** was higher during the Carnival holiday compared to the subsequent period. A deeper look at the data reveals an average consumption of 14,342 ± 5232 mg/1000inh/day (mean ± confidence interval, 95%) during the Carnival period (from the 1st to the 6th of March), while for the subsequent period, from the 7th to the 15th of March, a consumption of 11,471 ± 2679 mg/1000inh/day was noticed. Although a higher cannabis consumption is observed for the Carnival period, a statistical comparison (unpaired sample

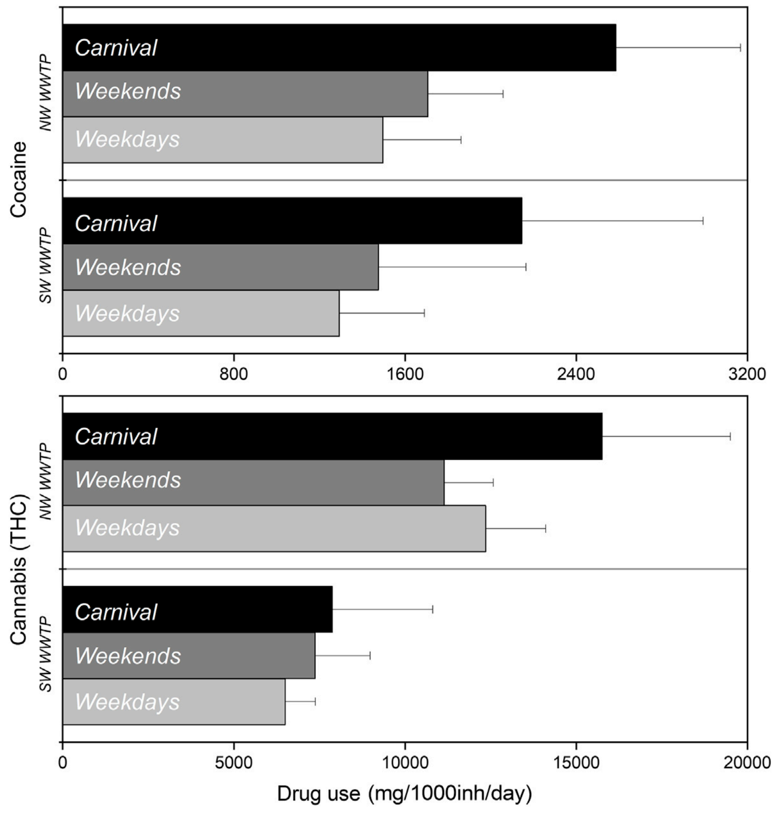

FIGURE 4 | Cocaine and cannabis (THC) consumption in the regions covered by North-Wing (NW) and South-Wing (SW) WWTPs in different periods during March 1st to 15th, 2019.

t-test, $t = 1.11 < t_{P=0.05} = 2.05$), revealed that there is no significant difference between the tested data. Therefore, there are indications to believe in a higher consumption of cannabis during the Carnival holiday, but the results presented here are not conclusive.

Our results concerning cannabis use during a major summer event are in agreement with other reports in the literature. Nefau et al. (2013) observed a lack of weekend effect on cannabis use in French cities, suggesting that cannabis is not a typical festive drug such as cocaine or MDMA. Mackuľak et al. (2014) investigated drug use at two important Slovak music events and could not confirm the increase in the number of cannabis users during these summer festivals. No observable effect on illicit drug use was also reported by Devault et al. (2020) during the World Music Day in Bordeaux, a French festival with massive public participation in the streets. Foppe et al. (2018) verified that THC use in a community of Western Kentucky (US) was not influenced by special events, such as the Independence Day and the first week of semester, compared to a regular week. However, in another community, THC consumption was 78% higher during the Independence Day festivities (3rd, 4th, and 5th of July) compared to a typical week. Mackuľak et al. (2019) analyzed wastewater samples during seven music festivals in the Czech and Slovak Republics evidencing that cocaine and methamphetamine loads increased during rock and dance music festival, whereas cannabis was consumed independently on the music genre preferences. Bijlsma et al. (2020) investigated illicit drug use at six music festivals across Europe and observed higher THC-COOH loads (approximately 10 times) during a pop/rock festival in Portugal compared to a regular period. The authors also observed higher levels of traditional illicit drugs in the investigated samples (wastewater and pooled urine) compared to new psychoactive drugs (NPS). All mentioned events, including the Brazilian carnival, are notably popular among young people, and could explain the increase in THC consumption, as youth tends to underestimate cannabis dangers (UNODC, 2021).

After the Carnival holiday, cannabis consumption remained relatively constant throughout the week, a behavior that is well documented in the literature (Lai et al., 2011; Tscharke et al., 2016; Zuccato et al., 2016). Therefore, it is possible to infer that in Brasília/Brazil, differently from cocaine, cannabis is not a drug of occasional abuse, exhibiting a usage profile similar to that of tobacco, for example.

Figure 4 shows the average illicit drug use considering different periods, i.e., the Carnival holiday (March 1st to 6th), weekends (Fridays, Saturdays and Sundays between March 7th and 15th), and weekdays (remaining days from March 7th to 15th). As previously reported, cocaine consumption is directly related to social and special events. Thus, higher values are observed for the Carnival holiday, followed by weekends, and weekdays, as expected, in both regions studied. Even though a period of decrease in consumption was observed immediately after Carnival, the compilation of data from the entire sampling period was still able to show a slightly higher consumption during weekends compared to weekdays.

Estimates of cocaine consumption corroborate the high exposure of the Brazilian population to this illicit drug. The estimated average consumption in the regions served by the North-Wing and South-Wing WWTPs after Carnival, 2149 ± 479 and 1279 ± 453 mg/1000inh/day, respectively, are similar to estimations produced in Reus and Castellón—Spain (2.8 and 1.1 g/1000inh/day, respectively) (Bijlsma et al., 2021), Medellín—Colombia (3022 mg/1000inh/day) (Bijlsma et al., 2016), Reykjavik—Iceland (2660 mg/1000inh/day) (Löve et al., 2022), Fort de France—Martinique (2420 mg/1000inh/day) (Devault et al., 2014), El Roble—Costa Rica (2390 mg/1000inh/day) (Causanilles et al., 2017b), and higher than values reported in New South Wales, Victoria, and Queensland—Australia (893, 719, and 640 mg/1000inh/day) (Wilkins et al., 2018), San Luis Río Colorado—Mexico (370 mg/1000inh/day) (Cruz-Cruz et al., 2021), Athens—Greece (213 mg/1000inh/day) (Gatidou et al., 2016), Istanbul—Turkey (201 mg/1000inh/day) (Asicioglu et al., 2021), Kotka—Finland (54 mg/1000inh/day) (Kankaanpää et al., 2016), and Diyarbakır—Turkey (47 mg/1000inh/day) (Daglioglu et al., 2021).

Figure 4 shows slightly differences between cannabis use in the three studied periods, especially in the area covered by the South-Wing WWTP, where the average consumption was 7 ± 2 g/1000inh/day. In the region served by the North-Wing WWTP, average uses of 21 ± 5 and 16 ± 2 g/1000inh/day were calculated for the Carnival holiday and for the subsequent period, respectively. Unlike cocaine, cannabis use in Brasilia was lower than that reported in several other regions, such as Western Kentucky—US (62 and 81 g/1000inh/day in two communities) (Foppe et al., 2018), Fort de France—Martinique (38 g/1000inh/day) (Devault et al., 2014), Barcelona—Spain (38 g/1000inh/day aging 15–64) (Mastroianni et al., 2017).

Due to the low excretion of the metabolite used in the back-calculation for cannabis, consumption data are often reported in terms of THC-COOH loads. Therefore, in the present work, average THC-COOH loads for the regions served by South-Wing and North-Wing WWTPs were 38 ± 11 and 99 ± 24 mg/1000inh/day, respectively. Comparing with other regions, THC-COOH loads were lower than in Amsterdam—Netherlands (192 mg/1000inh/day), Paris—France (124 mg/1000inh/day) (Thomas et al., 2012), Innsbruck—Austria (109 mg/1000inh/day) (Reinstadler et al., 2021), and more similar to Bogota—Colombia (33 mg/1000inh/day), Medellin—Colombia (47 mg/1000inh/day) (Bijlsma et al., 2016), and Petržalka—Slovakia (35 mg/1000inh/day) (Mackuľak et al., 2014).

According to the most recent National Survey on Drug Use by the Brazilian Population, (FIOCRUZ, 2017), prevalence of cocaine and cannabis consumption was 3.1 and 7.7% respectively. These data corroborate the higher relative incidence of the per capita consumption of cannabis and confirm that WBE is a consolidated strategy for estimating drug use and abuse, producing responses in agreement with those obtained by other strategies.

One should also bear in mind that WBE data usually provides results for a drug consumed as a 100% purity product and do not consider the real composition of the drug purchased on the streets and actually consumed by the user. From a law enforcement perspective, it is mandatory to have up-to-date information on the actual composition of drugs to convert WBE estimates into information that helps investigators assess how much of each drug has been consumed and trafficked. This issue has been raised in previous works, notably for cocaine (Maldaner et al., 2012; Ort et al., 2014; Sodré et al., 2018). However, there is still no consensus in the WBE literature regarding the terms and units used to express the estimation of drug consumption, especially cannabis.

Uncertainties and Limitations

As with other estimates, data collected using WBE are accompanied by uncertainties and limitations. They can be related to the different steps of the analytical sequence as well as to the parameters used in the back-calculation. Sampling uncertainties have been minimized with composite samples obtained by automated refrigerated samplers programmed to collect flow or time-proportional aliquots during 24 h.

Errors in chemical analysis are partially addressed through the use of deuterated internal standards as surrogate. During sampling, transport, storage and sample preparation, the stability of biomarkers is a matter of concern and has been addressed elsewhere (da Silva et al., 2018; Sodré et al., 2018). Briefly, benzoylecgonine is considered a relatively stable biomarker, while concentrations of cocaine, cocaethylene and carboxi-THC may decrease over time, specially at ambient temperatures (van Nuijs et al., 2012; Bisceglia and Lippa, 2014; Devault et al., 2017). Other uncertainties include assessing the wastewater volumetric flow and population variations related to the regions covered by the ETEs, especially during the carnival period. In the literature, strategies to addresses these uncertainties include using a high-frequency flow-proportional sampling strategy coupled to conscious calibration of flowmeter sensors (Lai et al., 2011; Subedi, 2019) and the monitoring of (bio) markers associated with the population such as caffeine, nicotine, ammonia and BOD, as well as the use of mobile phone networks and population censuses (Lai et al., 2011, 2015; Been et al., 2014; Senta et al., 2015; Rico et al., 2017; Thomas et al., 2017; Tscharke et al., 2019).

Despite the uncertainties, WBE is a well-established and widely used strategy to complement estimates produced by classical methods, which are also surrounded by uncertainties. It is worth mentioning that the WBE provides anonymous and objective answers, not only about drug consumption, but also about the spatiotemporal exposure of communities to different chemical and biological hazardous substances, and with relatively low costs, considering the size of the investigated population.

CONCLUSION

The findings obtained confirm that the use of drugs is directly related to social and special events since the consumption of cocaine and cannabis increased during the Carnival holyday. Due to the difference in the young population served by the WWTPs, was noted higher consumption of cannabis and cocaine in the administrative region of North-Wing. While for cannabis the consumption showed relatively constant throughout the post Carnival days, cocaine had its consumption profile significatively decreased after Carnival, indicating its recreational use, usually on weekends or in social events. This work shows that WBE is a consolidated strategy to estimate illicit drug use, producing near real-time information and allowing dynamic investigations concerning the temporal evolution of consumption.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

FS was responsible for writing the original manuscript, data curation, conceptualization, project management and student supervision; DF was in charge of visualizing, producing and processing the data and writing the first original draft; DA was responsible for writing, reviewing and editing the original manuscript; and AM was in charge of data curation, project management, student supervision and reviewing the original manuscript.

FUNDING

This work was supported by the Federal District Research Foundation (FAPDF) under Grant 19300000121/2019-13; the Brazilian National Council for Scientific and Technological Development (CNPq) under Grant 429858/2018-3; the National Institute of Advanced Analytical Science and Technology (INCTAA) under Grant 465768/2014-8, and the Ministry of Justice and Public Security under Grant TED/UnB/SENAD 04/2020.

ACKNOWLEDGMENTS

The authors acknowledge the Environmental Sanitation Company of the Federal District (CAESB) and its employees, especially Ana Maria do Carmo Mota, for their support in obtaining the samples.

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