Population drug use in Australia: A wastewater analysis

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Abstract

Accurate information on drug use in communities is essential if health, social and economic harms associated with illicit drug use are to be addressed efficiently. In most countries population drug use is estimated indirectly via surveys, medical presentations and police and custom seizures. All of these methods have at least some problems due to bias, small samples and/or long time delays between collecting the information and analysing the results. Recently the direct quantification of drug residues in wastewater has shown promise as a means of monitoring drug use in defined geographical areas. In this study we measured 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine and benzoylecgonine in sewage inflows in metropolitan and regional areas of Australia and compared these data with published European data. Cocaine use was small compared to European cities ($p < 0.001$) but was compensated for by much greater consumption of methamphetamine ($p < 0.001$) and MDMA ($p < 0.05$). MDMA was

more popular in regional areas ($p < 0.05$) whereas methamphetamine and cocaine were mainly consumed in the city ($p < 0.05$). Greater than 5-fold increases in MDMA use were detected on weekends ($p < 0.001$). This approach has the potential to improve our understanding of drug use in populations and should be further developed to improve prevention and treatment programs.

Key words: Geographical drug monitoring; Wastewater analysis; Cocaine; Benzoylecgonine; Methamphetamine; MDMA

1. Introduction

Accurate information on drug use in communities is essential if health, social and economic harms associated with illicit drug use are to be addressed efficiently. In most countries population drug use is estimated indirectly via surveys, medical presentations and police and customs seizures. All of these methods have at least some problems due to bias, small samples and/or long time delays between collecting the information and analysing the results. Surveys are also very costly, limiting their use, and are unable to provide sufficient resolution in small regional population areas.

There are several important consequences of these limitations in current methods. One such consequence is that direct measurement of changes in drug use as a result of a public health campaigns is difficult. A relatively inexpensive method that could provide near real-time measures would be needed for such evaluation. A second consequence is that international comparisons between countries based on self-reported drug use (e.g. World Drug Report 2009) are limited. Differences in questions, survey methods, etc. limit comparability. International comparisons have important ramifications for the implementation and development of global strategies to combat illicit drug use, and a more accurate method of comparison would be of value.

Recently, the measurement of illicit drugs in wastewater as a means of direct and quick assessment of drug use in a community has been explored in a number of countries [1]. The advantages of developing this technology to improve information on illicit drug use have been recognised [2, 3]. The first reported study was conducted in Italy and sampled from the River Po as well as four wastewater treatment plants servicing medium-sized Italian cities [4]. Data showed that benzoylecgonine, the major human metabolite of cocaine, was present in the samples. Subsequent studies by this group and others have extended these findings to a number of different geographical locations in Europe and North America [1, 5]. The markers for a number

of additional illicit drugs including methamphetamine, heroin and cannabis have also now been assessed [6-8]. Calculations of *per* capita drug consumption have then been made based on populations served by the wastewater treatment plant, daily volumes of wastewater produced in the areas, and excretion rate of each drug. Although there are a number of technical issues outstanding for drugs with unstable metabolites, it is clear that this approach provides an identifiable method to objectively quantitate illicit drug use on a continual basis.

Traditionally, information on population drug use in Australia is mainly obtained from a project named National Drug Strategy Household Survey (NDSHS), which is carried out by the government once every three years [9]. The reports derived from the survey are very informative, but limited data on weekly fluctuation and geographic difference of the drug use is provided, also up-to-date information is unavailable. It is also reported that the use pattern of illicit stimulants in Oceania differs from Europe, with methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) more popular in Australia and cocaine users equally distributed in these two continents [10]. However, these differences are based on survey, seizure and anecdotal evidence, and we hypothesised that wastewater analysis data of Australia and Europe would provide a more objective comparison.

Hence, in this study we applied this novel approach to wastewater samples collected in the State of South Australia from a number of metropolitan and regional wastewater treatment plants on midweek and weekend days, confined our analysis to the stimulant drugs (methamphetamine, MDMA and cocaine), and then compared our results with previously published data from Europe.

2. Materials and methods

2.1. Sample collection

From April 2009 to October 2009, 1.2-L samples were taken from sewage inlet pipes of metropolitan and regional wastewater treatment plants immediately after sewage has passed through screens during which large solids were removed. The metropolitan samples were obtained from three independent plants servicing the Adelaide greater metropolitan area using auto-samplers which collected 24-hour composite samples flow-dependently. Regional samples were grabbed from 10 regional plants throughout the State of South Australia and ranged in the populations they serviced from 370 to 23,300 (Table 1). No more than one sample was collected from one plant in 1 day. The samples were stored frozen until analysis.

2.2. Drug analysis

Samples were thawed to room temperature and mixed by inverting several times, and then filtered under vacuum using glass microfiber filters GF/A 1.6µm (Whatman, Kent, U.K.). 200µL of deuterated internal standards of MDMA, methamphetamine and benzoylecgonine were added to 300mL of duplicate samples to give resultant concentrations of 33.3, 33.3 and 166.7ng/L, respectively. Acetic acid (2.5%) was added to lower the pH of the samples to 4.5 - 5. The acidified samples were loaded onto pre-conditioned mixed-mode solid phase extraction (SPE) cartridges (UCT™ XRDAH; 500mg/6mL). Cartridges were successively washed with 6mL of pH 5.7 acetate buffer, 2mL of 0.1M acetic acid and 6mL of methanol. Analytes were eluted with a mixture of 96% dichloromethane: i-propanol (80:20) / 4% ammonia and evaporated to dryness. The dry residue was reconstituted with 20µL of methanol and then mixed with 180µL of 0.1% formic acid. A set of diluted extracts was prepared by transferring 40µL of the original extract to new vials and diluting each with 160µL of 0.1% formic acid. Both sets were analysed by liquid chromatography / tandem mass spectrometry (LC/MS/MS).

Chromatographic separation was carried out using an Agilent 1200 series liquid chromatograph with a Phenomenex^{TM} Luna PFP column (3µm, 50mm × 4.6mm)

connected to a PFP guard column (5µm, 4mm × 2.0mm). The mobile phase consists of methanol (solvent A) and 0.1% formic acid (solvent B) with a flow rate of 0.5mL/min. The gradient started with 95% B for 1 min. Then it was brought down to 5% B in the next 14 min and kept there for 1 min. Finally, the gradient was brought back to 95% B in 0.1 min and kept there for 2 min. Sample injection volume was 10µL. Mass spectra were obtained using a 4000 Q-Trap™ (Applied Biosystems, Toronto, Canada) system equipped with an electrospray ionisation source. Mass spectrometric analysis was performed in positive mode via multiple-reaction monitoring (MRM). The optimum MS/MS parameters for the detection of our analytes were as follows: nitrogen was used as the nebulizer and auxiliary gas, the ion spray voltage (IS) was maintained at 4.0kV and the source temperature (TEM) was 650°C, the curtain gas (CUR), gas 1 (GS1), gas 2 (GS2) and collision gas (CAS) were set at 30, 70, 70 and 'medium', respectively. Three transitions were used for each analyte and the most responsive one was used for quantitation. The most responsive transition of each internal standard was also monitored for quantitation. Settings for compound-dependent parameters are summarised in Table 2.

2.3. Validation

The limits of quantitation (LOQ) were established as the concentration of the analytes in distilled water that gives rise to peak height with an S/N of 10. They were set at 2ng/L for methamphetamine and MDMA, and 10ng/L for benzoylecgonine. Using the above LC/MS/MS conditions, methamphetamine and MDMA were linear up to 500ng/L, and benzoylecgonine to 2500ng/L ($r^2 > 0.998$ for all analytes). The absolute recovery of the extraction method for MDMA, methamphetamine and benzoylecgonine were found to be 86.5 -92.0%, 80.5 -85.7%, and 53.7 -61.2%, respectively. Using internal standards, the relative recovery was 97.9 -102.1% for MDMA, 97.6 -102.4% for methamphetamine and 98.8 -101.2% for benzoylecgonine. Reproducibility of the method was evaluated by analysing one wastewater sample for

10 times on each of 2 days. The RSD% was found to be 3.66% for MDMA, 1.07% for methamphetamine, and 4.08% for benzoylecgonine, respectively.

2.4. Data analysis

The concentrations of methamphetamine and MDMA were calculated by multiplying the concentration of spiked internal standard by the ratio of analyte peak area to internal standard peak area of the diluted set. The most responsive transitions were used for quantitation, and the other transitions were used for identification of the analytes (see Table 2). The concentration of benzoylecgonine was determined in the same way using the undiluted set.

Drug consumption estimation was based on the recent reported method [11], in which drug excretion *per* 1000 of the population was calculated from the known concentration of drug measured in sewage, daily flow of sewage to the wastewater treatment plant and population served by the plant.

For temporal comparison, only data from metropolitan samples were used. Considering a 24-hour composite sample is a mixture of samples collected flow-dependently from 8:00 am of the day before sample collection day to 8:00 am of the sample collection day, the metropolitan samples collected on Tuesday, Wednesday or Thursday were grouped as mid-week samples, and those collected on Saturday, Sunday or Monday were grouped as weekend samples.

Similarly, geographic comparison was based on metropolitan samples collected on Wednesdays and regional samples grabbed on Tuesday, considering the fact that the Wednesday composite samples were collected from Tuesday morning to Wednesday morning.

All samples collected from metropolitan plants were used for international comparisons. Data were analysed using the following procedure: first, average daily disposition of drug residues on each day of a week of each of the three plants was

estimated; then daily disposition on each day of the week in Adelaide was calculated as the average of the three plants; finally, daily excretion of the drug residues in Adelaide was expressed as the average of seven days. The final data were then used for comparison with published data in European cities.

Data were analysed by GraphPad Prism^{TM} software. Means and standard error of the means were calculated. One-way ANOVA with Tukey's post hoc tests or unpaired two-tailed t-tests were applied as appropriate.

3. Results

MDMA, methamphetamine and benzoylecgonine were detected in all of the samples taken from metropolitan plants (Fig. 1). When samples collected midweek were compared to weekend collections, higher concentrations of stimulants in weekend samples were observed ($p < 0.05$). In particular, weekend use of MDMA was five times higher than mid-week use ($p < 0.001$).

A direct comparison of drug amount between metropolitan and regional samples demonstrated geographical differences (Fig. 2). MDMA use in regional areas was twice as high ($p < 0.05$) as in Adelaide. In contrast, methamphetamine use was higher in metropolitan areas ($p < 0.05$). Only low concentrations of cocaine metabolite were detected in regional samples whereas it was easily found in samples from the city ($p < 0.001$).

Table 3 shows a comparison of the three analytes of interest reported in the literature and compared with the values measured in this study. Our data was calculated as *per* the previous paper of Zuccato et al [11] to directly compare with the previous published results. Very large differences were observed. Cocaine use is approximately 30 times greater in Milan and London compared to Adelaide ($p < 0.001$), but the latter city has a 10-fold higher use of MDMA ($p < 0.05$) and a 30-fold higher use of methamphetamine ($p < 0.001$) when compared with the European cities.

4. Discussion

Our analysis of wastewater has consistently detected illicit stimulant drugs and shown geographical as well as time of the week differences in drug concentrations. We have no way of identifying if these differences are due to the number of drug users or the daily dose consumed or a combination of both. This would require some adjunct survey data. However, data derived from wastewater analysis with the unit mg/d/1000 people may be regarded as a unique indicator related to drug prevalence that is comparable among different situations [2].

The results suggest that weekend use of drugs was more than midweek use. This change is similar to those reported by other authors. Zuccato et al [11] showed increased concentrations of cocaine and MDMA on the weekend wastewater samples in Italy. van Nuijs et al [12] found higher concentration of cocaine and its metabolites in Belgian weekend samples. Cocaine and MDMA consumption on weekends and during a music festival were higher than weekdays in France [13]. Occurrence of methamphetamines, MDMA as well as cocaine and its metabolites showed an increase on weekends in Spain [14, 15]. Benzoylecgonine and MDMA concentrations were higher in weekend samples in the study carried out in Croatia [16]. Estimated consumption of cocaine and amphetamines is also higher on weekends in Canadian cities [5].

This study found that cocaine was more popular in urbanised areas, which agreed with the studies in Europe [12, 13, 17] and North America [5] [18]. However, the influence of urbanity on MDMA and methamphetamine use was complicated. Our results showed more MDMA use in regional areas and higher prevalence of methamphetamine use in urban areas, while the study carried out in the US found higher MDMA use in urban centres and no geographic difference of methamphetamine prevalence. Metcalfe et al. analysed wastewater samples from three cities in Canada and found that the highest prevalence of amphetamines occured

in the biggest one [5], while Postigo et al. reported that the highest prevalence of MDMA and methamphetamine were found in one of the smallest urban area investigated in Spain [17].

In this article we compared stimulant use in Australia with that in UK and Italy, though wastewater epidemiological studies were also carried out in other European countries such as France [13], Spain [17], Germany [19], Belgium [12], Switzerland [11] and Croatia [16]. Detailed drug profiles were different from country to country, but the levels were of the same order in western European countries, with the exception that cocaine use in Croatia was several times lower [16]. Our study has shown large difference in stimulant use between Australia and the European countries. This finding agrees with the World Drug Report data [10] showing higher use of MDMA and methamphetamine in Australia compared to all other countries surveyed. However, the present study showed that methamphetamine and MDMA prevalence in Australia were approximately 10-40 times higher than European countries, which was much greater than expected from international survey data [10]. Besides, the World Drug Report suggested that the proportion of cocaine users was similar in Europe and Oceania [10], while the wastewater analysis data showed that in Europe the prevalence of cocaine use might be approximately thirty times higher than Australia. van Nuijs et al [12] calculated cocaine consumption in Belgium and by extrapolation estimated that the prevalence of cocaine users in Belgium was 0.8%, which agreed with the data provided by the United Nations Office on Drugs and Crime [20]. According to a study carried out by Postigo et al [17] in Spain, the order of drug abundance in wastewater was slightly different from the official profile. The observations of Zuccato et al [4] for benzoylecgonine in Italian wastewater indicated that use of cocaine was about 40,000 doses *per* day, which was much higher than officially estimated. The procedures used in these wastewater epidemiology studies were similar, but while some of their results were similar to the survey data, others

showed large differences. One possible reason is the bias inherent in self-report surveys since they were nation-dependent in design and protocol.

It would be ideal if all the samples analysed in this study were composite samples collected flow-dependently for 24 hours. However, since the auto samplers were not available in regional wastewater treatment plants, only grab samples could be obtained from these sources. Thus, comparison between drug use in metropolitan and regional area were based on comparison of composite and grab samples. Little is known about the difference between a composite and a grab sample from a regional plant, hence whether the grab samples could represent actual drug use in these regional areas or not were uncertain. One possible solution to this issue so far would be increasing the sample size to minimize the random error, which was what we tried to do in this study.

The issue of the analytes of interest being the result of non-illicit drug use was considered. Selegiline, a prescription drug, is used in the treatment of Parkinson's disease. Approximately 37% of oral administered selegiline is excreted as *l*-methamphetamine in the urine [21]. This amount of *l*-methamphetamine from selegiline use may contribute to the methamphetamine concentration in the wastewater because the current LC/MS/MS method was not able to differentiate *l*-methamphetamine from its isomer, the illicitly used *d*-methamphetamine. However, it was estimated that from 2006 to 2008, for every 1000 people in Australia about 0.5mg of selegiline was consumed daily [22]. Hence, selegiline use only causes about $0.5 \times 37\% = 0.185$ mg/d/1000 people of methamphetamine excretion in the community, which is far below the level of methamphetamine detected in this study. Use of famprofazone (an analgesic and antipyretic agent) and benzphetamine (an anorectic agent) may also result in excretion of methamphetamine [23, 24], but these two medications are not prescribed in Australia. Hence, the methamphetamine detected in this study was mainly from the illicit use of methamphetamine. No

pharmaceutical drug is metabolised to MDMA or benzoylecgonine, hence the only source of these residues in wastewater is the illicit use of MDMA and cocaine.

In this study only the use of cocaine, MDMA and methamphetamine were monitored. However, we are currently fine-tuning our analysis procedures in order to determine a larger range of illicit drug residues and utilize this method to observe other illicit drug use in Australia. Also, it should be noted that our results were only from one state and may not accurately reflect Australia's drug use as a whole. Expanding the sampling sites to other states of Australia to get a full picture of Australian drug use is planned. Moreover, some technical issues that we are examining may improve the reliability of the estimates. These include degradation of analytes in wastewater and employing a population biomarkers to normalize the drug concentrations for a measured population [25].

This study shows the utility of analytical chemistry in the field of drug use monitoring. Zuo et al. also demonstrated another novel way to monitor drug use, in which GC-MS was employed to detect cocaine on US paper currency [26]. These studies together with other similar ones indicated that development of methods based on modern analytical chemistry could be a new trend in drug use monitoring.

This study estimated the stimulant use in Australia using wastewater epidemiology and compared the data with the other recent studies in Europe. This report along with the others indicates that wastewater analysis can be used to identify geographic and temporal changes in drug use in urban and regional communities. The method provides objective and timely data that cannot be gleaned from traditional epidemiological methods. Therefore, it has great potential application in objectively measuring outcomes when time- and area- specific campaigns are to be planned and evaluated by police and health care agencies. Continued development of this approach to achieve a standardised method for a range of drugs which could be applied globally would have huge benefits to all agencies involved in drug issues.

Conflict of interest statement

All authors declare that they have no conflicts of interest with other people or organisation that could inappropriate influence this work.

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Table 1
Concentration of MDMA, methamphetamine and benzoylecgonine in wastewater samples collected from metropolitan and regional treatment plants in South Australia.

Plant type	Plant name	Average flow rate (kL/d)	Population served	Sample type	Sampling day of week	No of samples	Concentration of residues (ng/L) (mean ± SEM or single value)		
							MDMA	Methamphetamine	Benzoylecgonine
Metropolitan	Bolivar	141243	820000	Composite	Sun	2* Δ	208 ± 124	346 ± 75	55 ± 8
					Mon	2* Δ	265 ± 138	4108 ± 1118	52 ± 8
					Tue	3* Δ	39 ± 20	216 ± 35	27 ± 8
					Wed	4*# Δ	26 ± 8	224 ± 17	30 ± 7
					Thu	1* Δ	63	287	31
					Fri	1 Δ	72	338	40
					Sat	2* Δ	85 ± 51	311 ± 103	50 ± 14
	Christies Beach	27520	150000		Sun	2* Δ	706 ± 264	640 ± 116	74 ± 1
					Mon	1* Δ	321	445	35
					Tue	3* Δ	76 ± 26	388 ± 116	31 ± 14
					Wed	4*# Δ	62 ± 21	367 ± 45	17 ± 8
					Fri	1 Δ	192	527	30
					Sat	2* Δ	330 ± 81	553 ± 12	58 ± 1
	Glenelg	52550	200000		Sun	1* Δ	326	357	118
					Mon	4* Δ	209 ± 83	368 ± 67	64 ± 13
					Tue	3* Δ	66 ± 12	277 ± 18	38 ± 4
					Wed	3*# Δ	51 ± 23	368 ± 58	47 ± 5
					Thu	1* Δ	114	524	67
					Fri	1 Δ	136	505	69
					Sat	1* Δ	392	586	103
Regional	Angaston	364	1900	Grab	Tue	2#	20 ± 19	69 ± 30	ND
	Finger Point	5226	23300		Tue	2#	183 ± 16	160 ± 31	ND
	Mt. Burr	121	370		Tue	2#	5 ± 3	12 ± 0	12 ± 0
	Nangwarry	134	480		Tue	1#	ND	305	ND
	Naracoote	900	4780		Tue	1#	ND	2	ND
	Pt. Augusta East	1323	5000		Tue	1#	167	218	ND
	Pt. Augusta West	627	3500		Tue	1#	200	507	ND
	Pt. Lincoln	2842	12660		Tue	4#	268 ± 50	105 ± 36	14 ± 4
	Pt. Pirie	3414	13260		Tue	2#	31 ± 20	85 ± 47	ND
	Whyalla	4139	21270		Tue	7#	134 ± 27	282 ± 77	ND

ND: Not detected.
Δ Samples used for international comparisons.
* Samples used for comparisons of midweek days and weekend days.
# Samples used for comparisons of metropolitan and regional areas.

Table 2

Selected mass spectrometric parameters used in the analysis of 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine and benzoylecgonine for Applied Biosystems 4000 Q-Trap™.

Transition	**Q1 m/z**	**Q3 m/z**	**Dwell time (ms)**	**DP[a] (V)**	**EP[b] (V)**	**CE[c] (V)**	**CXP[d] (V)**
MDMA 1[#]	194	163	60	50	10	20	30
MDMA 2	194	105	40	50	10	30	30
MDMA 3	194	135	40	50	10	35	30
MDMA-d_5*[#]	199	165	60	50	10	20	30
Methamphetamine 1[#]	150	91	60	50	10	25	12
Methamphetamine 2	150	119	40	50	10	25	12
Methamphetamine 3	150	65	40	50	10	44	12
Methamphetamine-d_5*[#]	155	92	60	50	10	25	12
Benzoylecgonine 1[#]	290	168	120	130	10	30	15
Benzoylecgonine 2	290	105	80	130	10	45	15
Benzoylecgonine 3	290	77	80	130	10	80	15
Benzoylecgonine-d3*[#]	293	171	120	130	10	30	15

[a] Declustering potential.

[b] Entrance potential.

[c] Collision energy.

[d] Collision cell exit potential.

[#] Transitions used for quantitation.

* Internal standards.

Table 3

A comparison of amount of analytes detected in wastewater inflows.

	Adelaide	**Milan** [#]	**London** [#]
MDMA	39.3 ± 7.7	4.2 ± 0.1**	3.4 ± 0.5*
Methamphetamine	85.1 ± 7.2	4.5 ± 0.4***	2.4 ± 0.2***
Benzoylecgonine	11.5 ± 1.7	390 ± 13***	296 ± 9.0***

Data expressed as mg/day/1000 people, mean ± SEM. Adelaide: n = 20. Milan: n = 21 for Benzoylecgonine, 14 for MDMA and methamphetamine. London: n = 4. [#] Data derived from Zuccato et al [11].

*$p < 0.05$ (1-way ANOVA with Tukey's post hoc test).

** $p < 0.01$ (1-way ANOVA with Tukey's post hoc test).

***$p < 0.001$ (1-way ANOVA with Tukey's post hoc test).

Figure legends

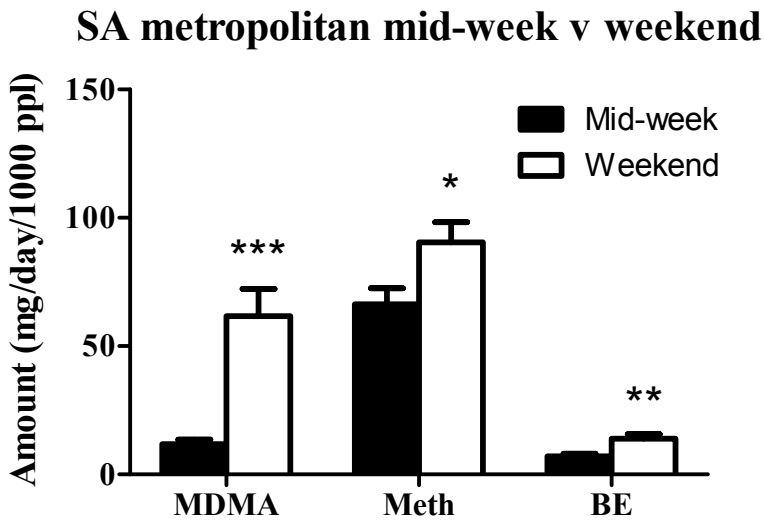
Fig. 1.

A comparison of amount of analytes detected in sewage inflows collected midweek and at the weekend expressed as mg/day/1000 people, mean ± SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ two-tailed t-test; n = 20. Meth = methamphetamine and BE = benzoylecgonine.

Fig. 2.

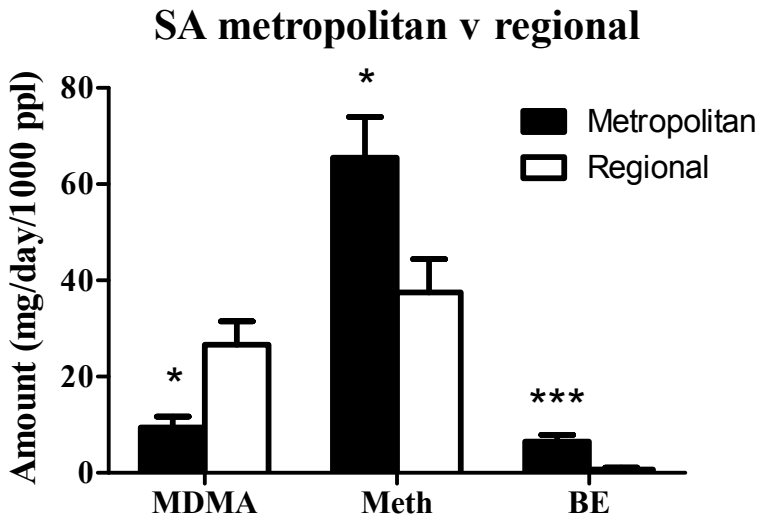
A comparison of amount of analytes detected in sewage inflows collected in metropolicatn and regional centers expressed as mg/day/1000 people, mean ± SEM. *$p < 0.05$ and ***$p < 0.001$ two-tailed t-test; n = 10. Meth = methamphetamine and BE = benzoylecgonine.

Fig. 1.



A comparison of amount of analytes detected in sewage inflows collected midweek and at the weekend expressed as mg/day/1000 people, mean ± SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ two-tailed t-test; n = 20. Meth = methamphetamine and BE = benzoylecgonine.

Fig. 2.



A comparison of amount of analytes detected in sewage inflows collected in metropolicatn and regional centers expressed as mg/day/1000 people, mean ± SEM. *$p < 0.05$ and ***$p < 0.001$ two-tailed t-test; n = 10. Meth = methamphetamine and BE = benzoylecgonine.