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Opioids, stimulants and depressive drugs consumption in fifteen Mexican cities: A wastewater-based epidemiological study

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Abstract:	Background: We examined temporal and geographical patterns in the use of cocaine, methamphetamine, amphetamine, ecstasy, marijuana, heroin, and the potential emergence of ketamine and fentanyl in Mexican cities through wastewater-based epidemiology (WBE). We evaluated the potential of WBE to identify population-level correlations in the use of drugs. Methods: 105 daily composite wastewater samples were collected from sewage treatment plants from fifteen Mexican cities. We quantified drug residues using liquid chromatography–high resolution mass spectrometry. The consumption of selected drugs was estimated by back calculations from the loads. Within-city correlations were estimated to identify drugs whose consumption was linearly related. Results: Eight drugs and/or their metabolites were identified in at least one sample across the cities. The drugs with the highest median consumption were marijuana, methamphetamine and cocaine. The weekly median consumption range for marijuana was between 147 and 20,364 mg/day/1000 inhabitants across cities, whereas the weekly median consumption for methamphetamine ranged between 5 and 3,628 mg/day/1000 inhabitants. Cocaine consumption was found in levels between 2 and 370 mg/day/1000 inhabitants. A higher consumption of methamphetamine and amphetamine was observed in Tijuana and San Luis Río Colorado, cities in the US border. Heroin, ecstasy, ketamine and fentanyl were more frequently consumed on weekends, whereas marijuana, cocaine and amphetamine were consumed during the whole week. Drugs pairs with the highest correlation, suggesting simultaneous use in these cities, were cocaine-ketamine, cocaine-amphetamine, ecstasy-methamphetamine, ketamine-fentanyl and ketamine-heroin. Conclusion: Our study provides the first report of fentanyl, norfentanyl and ketamine in wastewater in Mexico. Results indicate a higher level of consumption along known drug traffic routes, demonstrating that WBE can help identify areas of high drug consumption and assist governments in developing policies to reduce drug use and harm in the communities.
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Mexico City, Mexico, July 17, 2020

Dr. Alison Ritter
Editor in Chief of International Journal of Drug Policy
University of New South Wales Social Policy Research Centre, Sydney, Australia

Dear Dr. Ritter

Please find attached our manuscript entitled: "Opioids, stimulants and depressive drugs consumption in fifteen Mexican cities: A wastewater-based epidemiological study"; for consideration towards publication in the *International Journal of Drug Policy*. We aimed to monitor drug use over a whole week in 15 cities of Mexico through wastewater analysis, including drugs frequently consumed in the country, such as cocaine and marihuana, as well as emerging drugs, such as ketamine and fentanyl. We found drug levels in wastewater that were concordant with recent population surveys, regarding cocaine, marihuana and meta-amphetamine; we also observed geographical patterns indicating higher levels of consumption in Mexican cities bordering the US. Interestingly, fentanyl and norfentanyl were found in levels above the limit of detection and quantitation in two cities, although traces were observed in 11 cities for fentanyl and 14 cities for norfentanyl.

We believe our manuscript will be of interest to your readers because it represents the first formal evaluation of drug use through wastewater analysis in Mexico. This analysis allowed us to estimate weekend and weekday drug consumption patterns, estimate the correlation of drug use within each city, and to identify consumption along well-known routes of drug trafficking. Finally, we were able to identify the potential emergence of fentanyl use in the country, a finding that had been previously suggested by local and anecdotal evidence, but for which a country-wide assessment was lacking. All authors worked jointly on the manuscript and approved its contents. No author declares conflict of interest. I certify that the content of this paper has not been published elsewhere and does not overlap or duplicate already published work. Thank you for your time and consideration.

Yours sincerely,

Tonatiuh Barrientos Gutierrez

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

This study was approved by the Ethics, Research and Biosafety Committees at the National Institute of Public Health (CI:1476)

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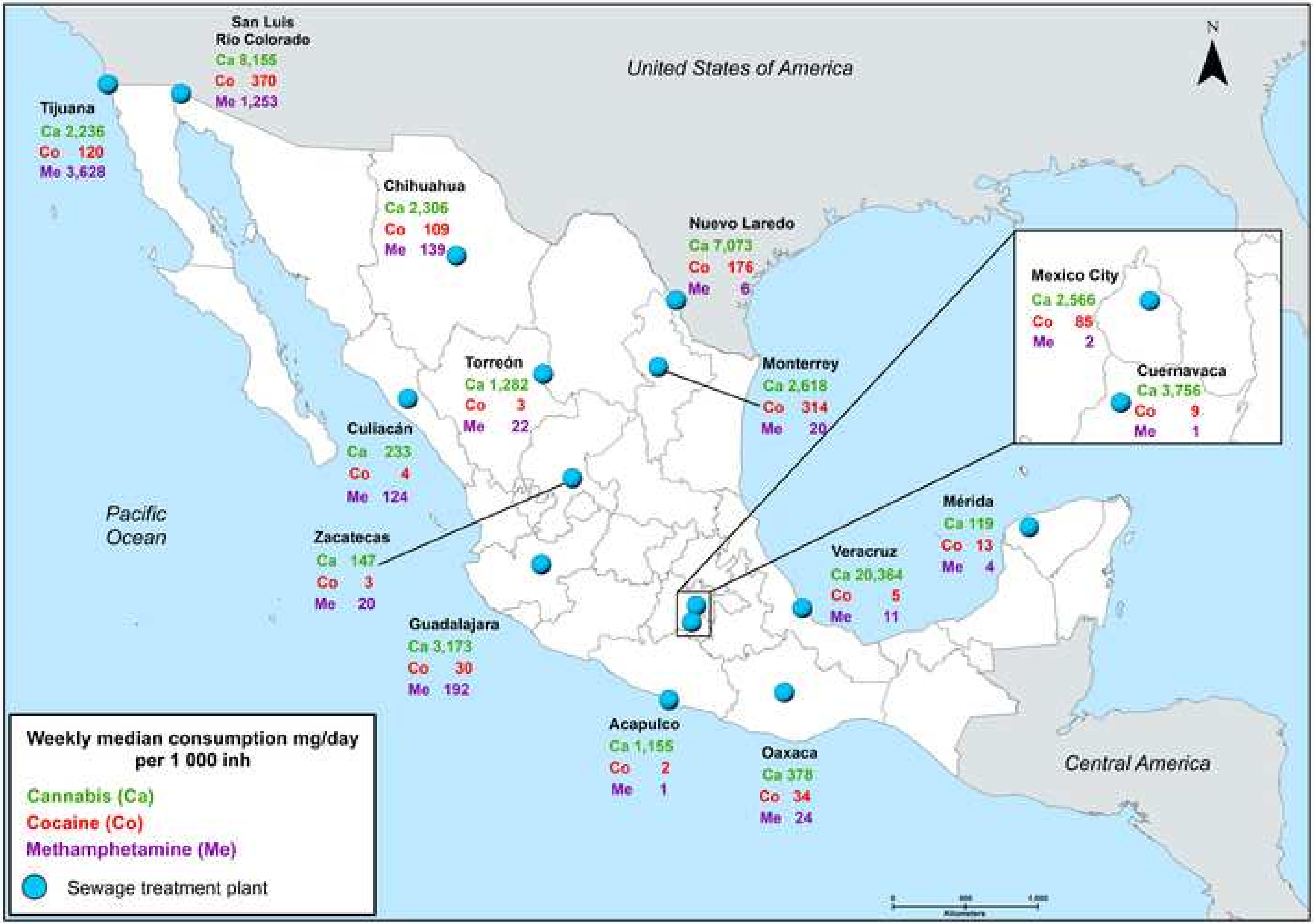
Graphical Abstract (for review)



Opioids, stimulants and depressive drugs consumption in fifteen Mexican cities: A wastewater-based epidemiological study

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Declarations of interest: none

Opioids, stimulants and depressive drugs consumption in fifteen Mexican cities: A wastewater-based epidemiological study

ABSTRACT

Background: We examined temporal and geographical patterns in the use of cocaine, methamphetamine, amphetamine, ecstasy, marijuana, heroin, and the potential emergence of ketamine and fentanyl in Mexican cities through wastewater-based epidemiology (WBE). We evaluated the potential of WBE to identify population-level correlations in the use of drugs.

Methods: 105 daily composite wastewater samples were collected from sewage treatment plants from fifteen Mexican cities. We quantified drug residues using liquid chromatography–high resolution mass spectrometry. The consumption of selected drugs was estimated by back calculations from the loads. Within-city correlations were estimated to identify drugs whose consumption was linearly related.

Results: Eight drugs and/or their metabolites were identified in at least one sample across the cities. The drugs with the highest median consumption were marijuana, methamphetamine and cocaine. The weekly median consumption range for marijuana was between 147 and 20,364 mg/day/1000 inhabitants across cities, whereas the weekly median consumption for methamphetamine ranged between 5 and 3,628 mg/day/1000 inhabitants. Cocaine consumption was found in levels between 2 and 370 mg/day/1000 inhabitants. A higher consumption of methamphetamine and amphetamine was observed in Tijuana and San Luis Río Colorado, cities in the US border. Heroin, ecstasy, ketamine and fentanyl were more frequently consumed on weekends, whereas marijuana, cocaine and amphetamine were consumed during the whole week. Drugs pairs with the highest correlation, suggesting simultaneous use in these cities, were cocaine-ketamine, cocaine-amphetamine, ecstasy-methamphetamine, ketamine-fentanyl and ketamine-heroin.

Conclusion: Our study provides the first report of fentanyl, norfentanyl and ketamine in wastewater in Mexico. Results indicate a higher level of consumption along known drug traffic routes, demonstrating that WBE can help identify areas of high drug consumption and assist governments in developing policies to reduce drug use and harm in the communities.

Keywords: drug use, Mexico, wastewater-based epidemiology, back-calculation, fentanyl, drug target residue

INTRODUCTION

Drug use is a public health problem linked to direct negative health effects and associated with violence and insecurity (Csete et al., 2016). In Mexico, drug use is a persistent health and social problem. The prevalence of any illicit drug use in the previous year in Mexico increased from 1.5% in 2011 to 2.7% in 2016; the two drugs most frequently consumed were marijuana and cocaine(INPs, 2017). A key challenge to the rapidly changing landscape of drug consumption is the need of responsive monitoring systems(Zuccato, et al, 2008) capable of detecting fast changes in consumption patterns and identifying emerging drugs, such as synthetic opioids, that have produced a large death toll in the US but whose impact in Mexico is still unknown(Scholl, et al, 2017).

Wastewater analysis has emerged as an innovative approach to estimate drug use. This method involves identifying and quantifying the excreted drugs and their metabolites in wastewater collected at sewage treatment plants (STP), to then back-calculate the amount of drugs consumed in the community by taking into account the flow rate of wastewater received and the size of the population served by the STP (Lancaster, et al, 2019). Compared to surveys, wastewater analysis can provide information on drug consumption patterns, including time and spatial trends, in a shorter time period (EMCDDA, 2016). These analyses are an accessible and inexpensive source of quasi real-time, pooled epidemiologic information, which could be included in the drug-use monitoring activities of countries (Werschler & Brennan, 2019).

Wastewater analysis has been recognized by governments and international organizations as a promising tool to monitor the impact of demand and supply reduction strategies, identify emerging trends of drug use, and support the allocation of resources to priority areas (EMCDDA, 2016). International efforts have been made to standardize methods and procedures, improving the comparability of results across locations and over time(Castiglioni, et al, 2014) (Bijlsma, et al, 2016). Wastewater analysis has been implemented in a network of 70 European cities, showing important geographical and temporal differences(EMCDD, 2019). In Mexico, our team conducted a wastewater analysis pilot study in 2015 obtaining a single-day sample from 31 STP, to assess the feasibility of measuring cocaine, marihuana, meta-amphetamine and amphetamine. Nuevo Laredo, Culiacan and Torreon had the highest consumption of all substances, concluding that wastewater analysis was feasible and informative(Cruz-Cruz et al., 2019).

Considering the need to extend our strategies to monitor drug consumption, we aimed to monitor the weekly drug consumption in 15 cities in Mexico using the WBE approach. The sampling strategy included daily measurements to capture variations in drug use during the week and to investigate population-level correlations in drug use over the week. In addition to drugs that are frequently consumed in Mexico, such as cocaine and marihuana, we aimed to assess the emergence of fentanyl and ketamine use in Mexico.

METHODS

Sampling sites

Influent wastewater samples were collected from STP located in fifteen cities in Mexico. Cities were selected to represent high, medium and low levels of drugs consumption based on the survey results reported in the 2016 National Survey of Drug, Tobacco and Alcohol Use (ENCODAT-Mexico) and the results of the pilot study conducted in 2015 and to represent all five Mexican regions (Supporting Information Table S1)(INPs, 2017) (Cruz-Cruz et al., 2019). The cities included were Northwestern: Tijuana, San Luis Río Colorado (SLRC), Culiacan and Chihuahua; Northeastern: Torreon, Monterrey and Nuevo Laredo; Western: Zacatecas and Guadalajara; Central: Mexico City, Cuernavaca and Acapulco; and Southeastern: Veracruz, Oaxaca and Mérida. From each city, the STP servicing the largest number of people in the city was selected to obtain the most representative data for each city and improve the comparability of results.

Characteristics of the sewage treatment plants

Information about the sewage treatment plants (STP) was obtained through a standardized questionnaire, as previously used by other studies (Ort et al., 2014) (Causanilles et al., 2017). The information collected included catchment properties, population served, location, wastewater flow control, and facility characteristics (questionnaire available in Supporting Information, Figure S1). Additionally, the wastewater daily flowrates were recorded at each STP during the sampling campaign.

Sampling

Wastewater samples were obtained from influents of each STP during seven consecutive days. Samples were collected during the dry season, between November 2017 and February 2018. Table S2(Supporting

Information) provides information on the STPs location, population served, flow rate, and sampling dates. Samples were collected using a manual-sampler with 1-hour intervals, across 24 hours. Samples were stored in polyethylene bottles at 4°C during the 24 hours sampling. Afterwards, samples were frozen at -60°C to avoid analyte degradation and sent frozen to McGill University (Montreal, Canada) for chemical analysis.

Analytical method

Wastewater samples were analyzed for eight drugs (heroin, amphetamine, methamphetamine, ecstasy, ketamine, fentanyl, cocaine, marijuana) and their major metabolites (6-acetylmorphine-heroin, norfentanyl-fentanyl, benzoylecgonine-cocaine, 3,4 Methylenedioxy methamphetamine (MDMA-Ecstasy) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH)-marijuana). The chemical analysis was done using a solid phase extraction followed by identification and quantitation through liquid chromatography–high resolution mass spectrometry (LC–HRMS) system. The methods were adapted from our previously published methods(Yargeau, et al, 2014)(Jacox et , 2017) as briefly described in the following sections.

Sample extraction

Wastewater samples were vacuum filtered using 1.5 μm glass fiber filters to remove suspended solids and spiked with 100μL of 400 ng/mL of a mixture of labelled compounds representative of each of the target analyte. Using a Supelco Visprep vacuum manifold with 6 mL-150mg LP Oasis MCX cartridges (Waters, Milford, USA), the samples were subjected to solid phase extraction. The extraction method used was based on a previous method developed at McGill(Yargeau et al., 2014), which was adapted based on Jacox, et al. (Jacox et al., 2017). Briefly, the cartridges were pre-conditioned consecutively with 6mL of methanol, 6mL milli-Q water and 6mL 0.1% HCl milli-Q water. Samples (110 mL) were acidified to pH 2.5 by adding 550µL HCl and then loaded onto the cartridges at a rate of 5 mL/min. Sample bottles were consecutive rinsed with 5mL of milli-Q water and 0.1% HCl milli-Q water that are then loaded on the cartridges. Cartridges were aspired to dryness and eluted with 8 mL of dichloromethane: Isopropanol: ammonium hydroxide (78:20:2, v/v/v). Collected eluents were evaporated to dryness using a nitrogen evaporation System N-EVAP - 112 (Organomation, Berlin, MA) and reconstituted in a volume of 0.4 mL of a water-methanol solution (9:1 V/V).

Quantification of the drugs by chemical analysis

25 µL of reconstituted extract was injected into the liquid chromatography–high resolution mass spectrometry (LC–HRMS) system for analysis. Positive controls (to verify the detection of each compound during each analytical run) and blanks (to verify the absence of contamination) were pre-concentrated using the same method and run along the samples for quality controls. The chromatographic separation of the target compounds was done using the method described in Rodayan et al. 2016(Rodayan, et al, 2014). LC–HRMS using an Accela LC system coupled to an LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA) was used to measure analyte concentrations. Ionization was done in positive mode using heated electrospray ionization (HESI) source with the following parameters: sheath gas flow 35 arbitrary units, auxiliary sheath gas flow 10 arbitrary units, capillary temperature 275 °C, capillary voltage 4.0 V, and tube lens 110 V. Two acquisition modes were performed; full scan mode (50–600 m/z) at high resolution (FTMS resolution @ 30,000) and simultaneous MS2 scan on linear ion trap for structural product ion confirmation. Analyte quantification was carried out by extracting the ion of interest using an m/z range of ±5ppm accuracy and confirmation by MS/MS spectra. A six-point calibration curve generated for each compound in the range of 2–150

μg/L and in the same solvent as the reconstituted samples was used for quantification with a constant deuterated stable isotope surrogate concentration of 100 μg/L use to determine recovery and matrix effect in each sample. The linear correlation coefficients were at least 0.990 for all analytes studied. The limits of detection (LODs) and limits of quantification (LOQs) based on samples for the analytes in wastewater were in the range of 4.31 to 7.09 ng/L and 14.35 to 23.64 ng/L. The LODs and LOQs for each compound, as well as the labelled surrogates used for each compound and their sources, are provided in the Supplemental Materials (Supporting Information, Table S3). These were determined by a signal to noise ratio of 3*S/N for the LODs and 10*S/N for the LOQs and method validation was done using spiked samples of wastewater.

Back calculation of drug consumption

The community drug consumption was estimated through back-calculation, according the Zuccato method(Zuccato et al., 2008). First, the amount of drug target residues (DTR) discharged (mg/day) in each STP was obtained by multiplying the concentration of the target drug quantified in the 24-h composite samples of untreated wastewater (ng/L) by the corresponding flow of sewage (L/day). Concentrations between the Limit of Quantification (LOQ) and the Limit of Detection (LOD) were replaced with a value equal to (LOQ+LOD)/2 and concentrations below the LOD were replaced with half the LOD.(Rousis et al., 2020)(Luo Yuzhou et al., 2012). Data were then standardized to the population served at each city (mg/day/1000 inhabitants) and multiplied by the correction factor for each drug, as reported in supplementary material (Supporting Information, Table S4). Correction factors are based on the known fraction of the consumed parent drug normally excreted as DTR, and the parent drug-to-DTR molar mass ratio(EMCDDA, 2016). The back-calculation method is widely-used on the following assumptions: i) no sewage loss in the sewer system, ii) no relevant transformation and negligible adsorption to particulate matter of targeted substances, iii) no direct drug disposal into the sewage system, and iv) no substantial changes of population over the period of the study.

Statistical Analysis

Data analysis of drug consumption was performed using descriptive statistical techniques. For each city, we estimated the percentage of wastewater samples in which drugs and drug metabolites were detected. Drug consumption was expressed as the median value in mg/day/1000 inhabitants. The results from the daily samples were used to evaluate variations in drug consumption over the week. Spearman correlations (Rs) and pairwise p-values were calculated to estimate the correlation of consumption of all pairs of drugs over the days of the week within each city, considering a correlation above 0.70 to be high,(Hinkle Dennis E, 2009) indicating that the consumption of two drugs was linearly related at the population level over the week. Statistical analyses were performed in STATA v.13 (College Station, TX). Our study was approved by the Ethics, Research and Biosafety Committees at the National Institute of Public Health (CI:1476) .

RESULTS

Table 1 presents the percentage of samples at each STP in which DTR were detected. Amphetamine, methamphetamine, MDMA, benzoylecgonine, cocaine and THC-COOH were detected in all STPs; we found detectable levels of ketamine in 10 STPs and traces (detected but <LOD) in 5 STPs, while 6-acetylmorphine was detected in 6 STPs, with traces in 7 STPs and no detection in two. We detected fentanyl in Culiacan, with traces in 11 other STPs and no detection in Chihuahua, Nuevo Laredo and Acapulco. Norfentanyl was detected in Veracruz, with traces in all other STPs. Tijuana and San Luis Río Colorado (SLRC) were the cities with the highest number of samples with drugs detected (100% of

samples with THC-COOH, cocaine, benzoylecognine, methamphetamine, amphetamine and methamphetamine.

---------------------- Table 1. Percentage of wastewater samples with drug detection---------------------

Figure 1 summarizes the weekly median levels of marijuana, cocaine, methamphetamine, ecstasy, amphetamine, ketamine, heroin and fentanyl consumption (mg/day/1000 inhabitants) by STP location. Marijuana consumption was calculated using 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) as its DTR. The highest levels of marijuana consumption were observed in Veracruz (20,363 mg/day/1000 inhabitants), followed by SLRC (8,155 mg/day/1000 inhabitants); lowest levels of consumption were observed in Merida (119 mg/day/1000 inhabitants) and Zacatecas (147 mg/day/1000 inhabitants). The consumption levels of cocaine were high in SLRC (370 mg/day/1000 inhabitants), followed by Monterrey (314 mg/day/1000 inhabitants); lowest levels of consumption were observed in Zacatecas (3 mg/day/1000 inhabitants) and Acapulco (2 mg/day/1000 inhabitants).

The highest levels of methamphetamine consumption were found in Tijuana (3,628 mg/day/1000 inhabitants) and SLRC (1740 mg/day/1000 inhabitants) and lowest levels were observed in Merida (4 mg/day/1000 inhabitants) and Nuevo Laredo (7 mg/day/1000 inhabitants). The highest levels of ecstasy consumption were found in Tijuana (70 md/day/1000 inhabitants) and Mexico City (52 mg/day/1000 inhabitants); the lowest consumption levels were estimated in Mérida and Cuernavaca (0.4 mg/day/1000 inhabitants each one). The highest levels of amphetamine consumption were found in SLRC (156 md/day/1000 inhabitants) and Guadalajara (86 mg/day/1000 inhabitants); the lowest consumption levels were estimated in Oaxaca (4 mg/day/1000 inhabitants) and Veracruz (7 mg/day/1000 inhabitants). The highest levels of ketamine consumption were found in SLRC (4 md/day/1000 inhabitants) and Veracruz (3.9 mg/day/1000 inhabitants). The highest levels of heroin and fentanyl consumption were found in Monterrey (15822 and 1.1 md/day/1000 inhabitants, respectively) and SLRC (141 and 0.99 mg/day/1000 inhabitants, respectively).

-----Figure I Weekly median levels of drug consumption (mg/day per 1,000 inhabitants) per city----

Table 2 summarizes the evaluation of correlations between pairs of drugs within each city over the week, which are indicative of drugs possibly consumed simultaneously at the population level. Ketamine-fentanyl, cocaine-ketamine, ketamine-heroine, ecstasy-methamphetamine, cocaine-amphetamine were the drugs with the highest number of correlations through Mexican cities (n=8, n=5, n=4, n=4 and n=3, respectively). The cities with the highest number of significant correlations were Culiacan (n=8), Veracruz (n=7) and Zacatecas (n=6) In Culiacan, we found a correlation between ketamine and fentanyl consumption (Rs=0.764, $p<0.05$), ecstasy and amphetamine consumption (Rs=0.855, $p<0.05$); ecstasy and heroin (Rs=0.784, $p>0.05$); ecstasy and marijuana (Rs=0.809, $p<0.05$), marijuana and heroin (Rs=0.844, $p<0.05$); methamphetamine and heroin (Rs=0.777, $p<0.05$); amphetamine and heroin (Rs=0.905, $p<0.05$); amphetamine and marijuana (Rs=0.864, $p<0.05$).

---------------Table 2. Significative correlations between drugs consumption by city--------------

The median levels of marijuana, cocaine and amphetamine consumption showed a stable trend during the week. However, patterns were different across cities; for example, Tijuana, SLRC Monterrey and Veracruz had a higher consumption of marijuana and cocaine on the weekend, while amphetamine consumption in Culiacan was higher during workdays. Methamphetamine was mostly consumed during the weekdays, particularly in Culiacan, Tijuana and SLRC. Ecstasy and opioids as heroin, ketamine and fentanyl were more heavily consumed during the weekends (Supporting Information, Figure S5)

DISCUSSION

We aimed to estimate drug consumption in 15 cities in Mexico, analyzing daily and weekly patterns of known and emerging drugs. We identified target illicit drugs and their metabolites in at least one sample of each STP. THC-COOH was detected in 100% of samples in 10 cities; acetylmorphine was detected in samples from six STPs, all from Northern cities. Fentanyl and norfentanyl were found in levels above the limit of detection and quantitation in two cities (Culiacan and Veracruz), and as traces in 11 cities for fentanyl and 14 cities for norfentanyl. The drugs with the highest median consumption were marijuana (range 147 and 20,363 mg/day/1000 inhabitants), methamphetamine (range 5 and 3,628 mg/day/1000 inhabitants) and cocaine (range 2 and 370 mg/day/1000 inhabitants). The drug pairs with the highest number of correlations indicating simultaneous use in the cities were ketamine and fentanyl. All drugs had different consumption patterns and varied across cities, some were highly consumed during the week and others on weekends. To put the concentrations found in Mexico in the international context we searched for comparable studies using a similar methodology. In comparison with Medellin, Colombia-2015, Mexican cities as Veracruz showed high levels of marijuana consumption (8,372 vs 20,363 mg/day/1000 inhabitants, respectively) (Bijlsma et al., 2016). SLRC showed higher levels of cocaine use than South East Queensland, Australia-2015 (370 vs 327 mg/day/1000 inhabitants, respectively) (Lai, O'Brien, Bruno, et al., 2016), and Tijuana showed higher levels of methamphetamine use than Midwestern, USA-2015 (3,628 vs 1,740 mg/day/1000 inhabitants, respectively) (Skees, Foppe, Loganathan, & Subedi, 2018)

Drug use and abuse is known to be highly variable, not only in magnitude but in types of substance consumed (Csete et al., 2016; INPs). Our results showed that marijuana, methamphetamine, ecstasy and cocaine were the most frequently detected drugs, which is in agreement with the results from the 2016 National Survey of Drug, Tobacco and Alcohol Use (ENCODAT-Mexico), where marijuana was the most used drug (2.1% prevalence of use in the past year), followed by cocaine (0.8% prevalence of use in the past year) (INPs, 2017). Cities in the north of the country showed higher concentrations of these drugs, which also agrees with the geographic distribution of drug use according to the 2016 ENCODAT survey. Mexican cities that are close to the US border, such as Tijuana, are on the route of Mexican drug cartels to the U.S., and have experienced the fastest increases in drug use over the last decade(INPs, 2017). Another example is cocaine, that is typically transported to the US following the Pacific coast of Mexico (Beittel, 2018) (DEA, 2017) (Dell, 2011). In both cases, the drug transit through Mexico seems to stimulate the domestic markets and consumption in cities in the drug traffic route are higher compared to other locations in the country. It was unexpected to detect fentanyl and norfentanyl considering that fentanyl use had not been reported in Mexico at the time the wastewater samples were collected. But since then, reports of fentanyl use have started to emerge as seized shipments (Guillermo, 2020), potential illegals labs (Sánchez D, 2019), and consumption through lacing heroin with fentanyl (Fleiz C, et al., 2019). The detection of fentanyl in wastewater of several cities should increase the awareness of the Mexican government towards the potential illegal use of the substance in the country.

Drug consumption is expected to vary over the days of the week and our results revealed different profiles of consumption amongst the Mexican cities. The median consumption of drugs like marijuana and cocaine was stable throughout the week. The lack of temporal trends over a week for marijuana is similar to trends observed in other locations but other studies reported that cocaine tends to peak during the weekends(van Nuijs et al., 2011) which was not observed in the present study. The consumption of ecstasy started to rise on Thursday and peaked on Saturday, which is consistent with another studies (Tscharke, 2015). Ecstasy is known to be consumed by people who visit clubs, parties, music festivals and dance events and these events occur mainly during the weekends (Mackulak et al., 2016; Reid, et al., 2011). Methamphetamine consumption presented the same pattern of elevated drug consumption from Friday and throughout the weekend. Our results showed that the consumption of heroin, fentanyl and ketamine increased over the weekend, particularly in two border cities (Monterrey and SLRC). This problem has been previously reported in border cities that represent a major route for drug trafficking and "spillover" from shipments had created a strong market for heroin, cocaine and methamphetamine (Kankaanpää, et al., 2014; Miller et al., 2009).

The use of drugs is complex, it is not only growing but some consumers use more than one drug at a time. Reasons to co-use drugs include to enhance desirable effects or diminish undesirable effects (Barrett, 2006). While we could not directly evaluate co-use of drugs, we found some important correlations between the use of some drugs at the community level. This suggest that drugs might be consumed at the same time and in proportional amounts in cities where correlations were significant. The highest number of correlations were found for fentanyl-ketamine, cocaine-ketamine and heroine-ketamine in Culiacan, Veracruz and Zacatecas; these results could be attributed to polydrug use, which has been reported from studies realized under other methodologies (Michael, 2005) Ketamine and fentanyl are medical drugs, that can be used alone or in combination for anesthetic purposes (Singh, Goyal, & Sharma, 2012), which could explain the high correlation observed between these two drugs; however, we consider this explanation unlikely, given that these two drugs were found at higher concentrations during the weekends than in weekdays. Cocaine was the drug with a major number of correlations with other drugs as ketamine, amphetamine, fentanyl and heroin, it could be explained for adulteration and/or co-consumption. Cocaine is commonly related to diluting agents, contaminants and adulterants, pharmacologically active substances developed for medical use with the purpose of increasing profits due to their low cost and availability, but also to augment the stimulant and analgesic effect of cocaine (Gameiro, 2019). Also, correlation could be to indicate co-consumption, the correlation between cocaine-amphetamine and cocaine-ecstasy consumption in SLRC is also interesting, and it would be valuable to investigate if both drugs are being used by the same target population. Marijuana is an illicit drug in Mexico and is considered a “gateway drug” since many individuals that initiate marijuana use go on to experimenting with other drugs (Barrett et al., 2006; EMCDDA, 2016; Taylor et al., 2017). The analysis of drugs from a community perspective opens up new venues of understanding that could help design better interventions for each specific community by taking into consideration the likelihood of simultaneous consumption of various drugs.

In comparison with Medellin, Colombia-2015, Mexican cities as Veracruz showed high levels of marijuana (8,372 vs 20,363 mg/day/1000 inhabitants, respectively) (Bijlsma et al., 2016), this finding does not agree with the ENCODAT survey, in which the state of Veracruz was found to be a low consumption area. This discrepancy could be explained by the lack of ability of household surveys to capture the consumption of tourists and visitors (Veracruz is a highly touristic city), or some other unusual behavior that is not easily monitored using surveys. Northern cities as SLRC and Tijuana showed high levels of cocaine and methamphetamine consumption. SLRC showed higher levels of cocaine use than South East Queensland, Australia-2015 (370 vs 327 mg/day/1000 inhabitants, respectively) (Lai, O’Brien, et al., 2016) and Tijuana showed higher levels of methamphetamine use than Midwestern, USA-2015 (3,628 vs 1,740 mg/day/1000 inhabitants, respectively) (Skees et al., 2018), it could be explained for the availability to both drugs because the cities are major transit points for cocaine and methamphetamine trafficking from Colombia to the United States (Beittel, 2018; Lai, O'Brien, Thai, et al., 2016).

Monterrey showed higher levels of heroin consumption than Kentucky-2018 and two midwestern cities-2017, USA (15,822 vs 1810-1294 mg/day/1000 inhabitants, respectively) (Croft, et al., 2020) (Gushgari, et al., 2019)); which is surprising considering the lack of prior evidence of high heroin consumption in this city. However there is evidence of the presence of illegal labs in the city, which could explain these results for Monterrey (Daniel, 2019; Goodman-Meza et al., 2018). SRLC showed higher levels of amphetamine consumption than Barcelona Spain-2015 and Oslo Norway-2016 (156 vs 120-100 mg/day/1000 inhabitants, respectively(Mastroianni et al., 2017) (Löve et al., 2018). Four Mexican cities as Tijuana, Mexico City, Zacatecas and SLRC showed higher levels of ecstasy consumption than Stockholm Sweden-2016 (70, 52, 46, 34 vs 30 mg/day/1000 inhabitants, respectively) (Löve et al., 2018).

According to our results, opiods as ketamine and fentanyl are consumed in low levels in Mexican cities. For example SLRC, Veracruz and Tijuana (4.0 ,3.9 ,3.8 mg/day/1000 inhabitants, respectively) showed consumption levels lower than Asiatic cities as Guangzhou China-2017 and Kuala Lumpur Malasya-2017 (357 and 256 mg/day/1000 inhabitants, respectively) (Du et al., 2020; Zhang et al., 2019). Fentanyl is a big problem in United States, the level consumption reached 169 mg/day/1000 inhabitants in Eastern Kentucky-2018, 18 mg/day/1000 inhabitants in two Midwestern cities 2015-2018; and Midwestern USA-2017 1.27 mg/day/1000 inhabitants(Croft et al., 2020; Gushgari et al., 2019; Skees et al., 2018), while Monterrey and SLRC showed a level consumption around 1.0 mg/day/1000 inhabitants. (Supporting Information, Figure S5)

Limitations

Our study has certain limitations that must be acknowledged. When the analysis is based on the drug itself rather than a metabolite, it is not possible to distinguish consumption of the drug from possible disposal of the drug in the sewers. The wastewater-based drug consumption estimation also relies on estimates of urinary excretion rates, which add a degree of uncertainty regarding the exact amount of drug used. However, this uncertainty should not impact the interpretation of the usage trends over time or across the cities. These estimates cannot be extrapolated to the state-level as they are based on the analysis of only some STPs in certain cities and only about 58% of wastewater produced in these urban centers are sent to a STP (National Water Commission of Mexico, 2017). Also, the information about population dynamics is limited; for instance, some cities could have a floating weekend population or the STP could have an input from tourism or industrial sectors. Future studies should focus on improving the sampling strategy to increase the coverage of the population in the cities selected. Third, we only sampled for seven consecutive days between November and February, which excludes the summer season, where drug consumption could be higher due to summer vacations and the influx of tourists to some of the cities.

CONCLUSION

Wastewater-based epidemiology promptly provides objective data on the population drug use through the measurement of the excreted drug residues. Our results provided valuable information about population drug use in Mexico, identified the potential use of WBE results to investigate possible co-use of drugs and to map drug transit routes. Accurate and timely information of the scale and dynamics of drug consumption in a population is important to assess the required actions of law enforcement and public health services. This information, along with population surveys, can improve our understanding of drug use and the population needs for drug addiction treatments.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Table(s)

Table 1. Percentage of wastewater samples with drug detection

Drug target residue	Tijuana	SLRC	Nuevo Laredo	Torreon	Monterrey	Culiacan	Chihuahua	Zacatecas	Guadalajara	Mexico City	Cuernavaca	Acapulco	Veracruz	Oaxaca	Merida
	n=7	n=7	n=7	n=7	n=7	n=7	n=7	n=7	n=7	n=7	n=7	n=7	n=7	n=7	n=7
THC-COOH	100	100	100	100	100	42.9	100	85.7	100	71.4	100	100	85.7	100	85.7
Cocaine	100	100	100	85.7	100	100	85.7	14.3	71.4	85.7	100	71.4	42.9	100	100
Benzoylecgonine	100	100	100	85.7	85.7	28.6	100	57.1	100	85.7	100	57.1	<LOD	100	85.7
Methamphetamine	100	100	100	100	100	100	85.7	100	100	71.4	71.4	85.7	85.7	100	71.4
Amphetamine	100	100	14.3	57.2	14.3	71.5	71.5	14.3	71.4	14.3	14.3	57.1	14.3	85.7	85.7
MDMA	100	100	42.9	71.5	71.4	57.1	100	100	100	85.7	71.4	85.7	57.1	100	85.7
Ketamine	57.1	14.3	14.3	<LOD	<LOD	<LOD	14.3	57.1	14.3	<LOD	14.3	28.6	<LOD	14.3	28.6
6-acetylmorphine	<LOD	42.9	<LOD	14.3	57.1	42.9	<LOD	<LOD	14.3	ND	ND	<LOD	<LOD	28.6	<LOD
Fentanyl	<LOD	<LOD	ND	<LOD	<LOD	28.6	ND	<LOD	<LOD	<LOD	<LOD	ND	<LOD	<LOD	<LOD
Norfentanyl	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	14.3	<LOD	<LOD

<LOD, below the limit of detection in at least one sample
ND, Not detected
SLRC, San Luis Río Colorado-Sonora
MDMA, 3,4 Methylenedioxy methamphetamine, ecstasy
THC-COOH, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol

Figure 1 Weekly median levels of drug consumption (mg/day per 1,000 inhabitants) per city

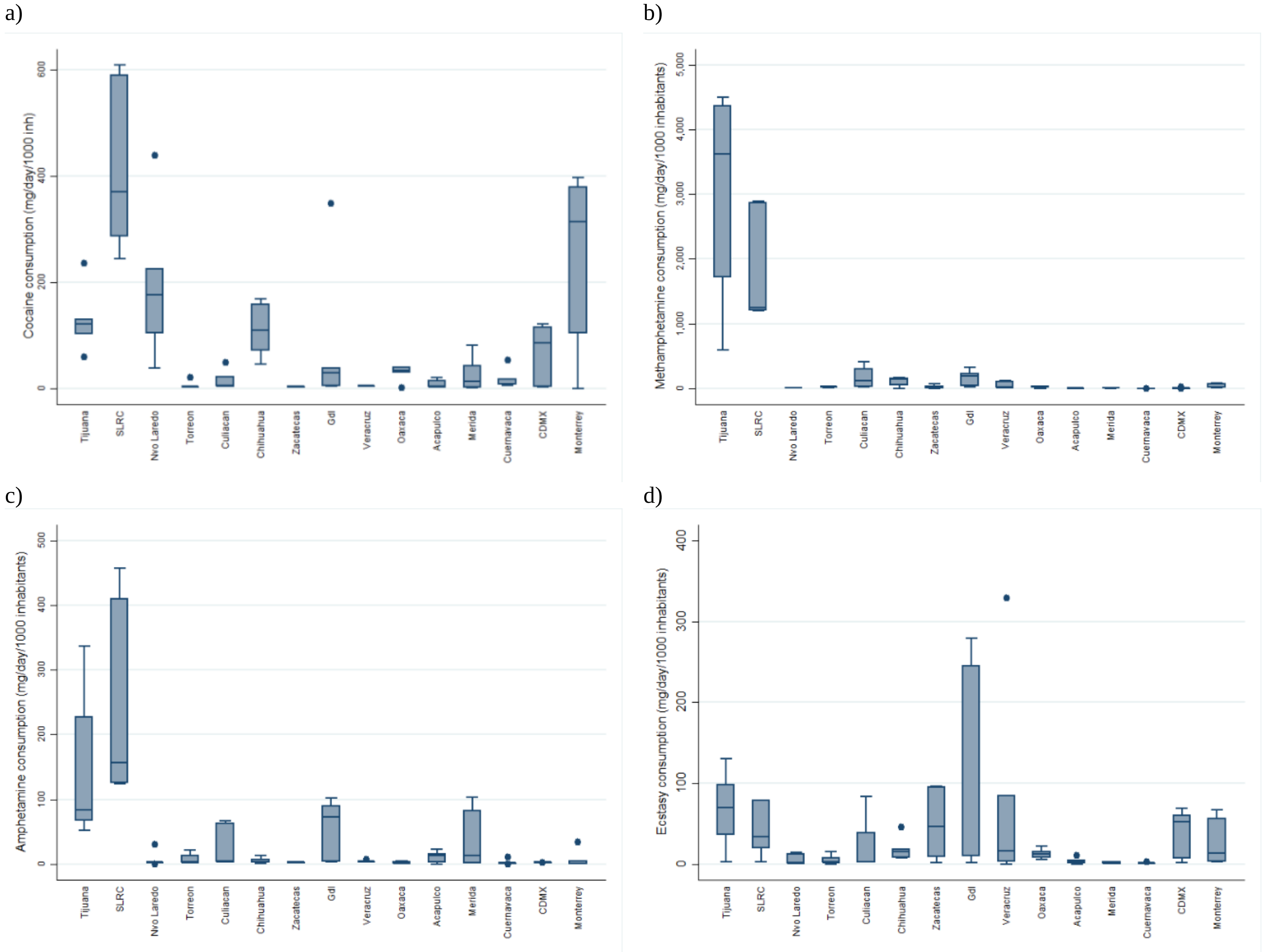
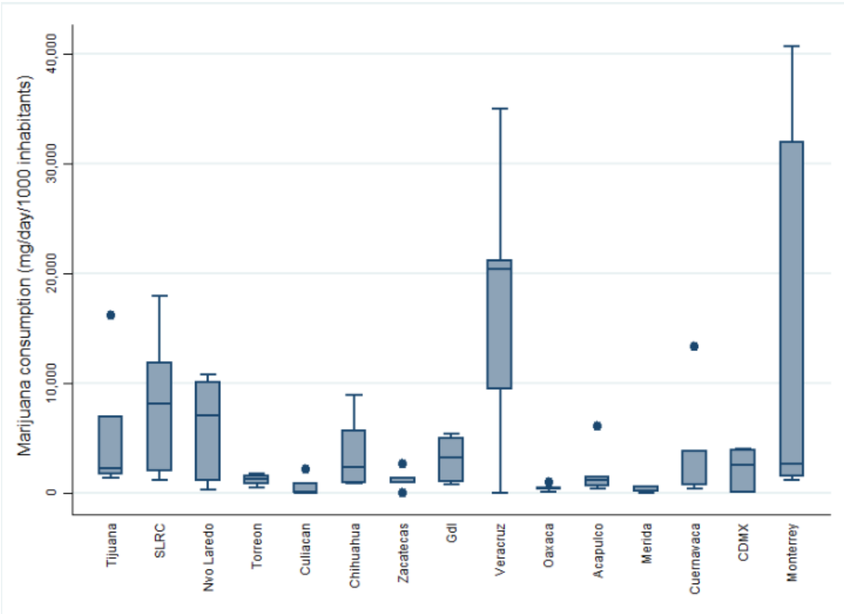
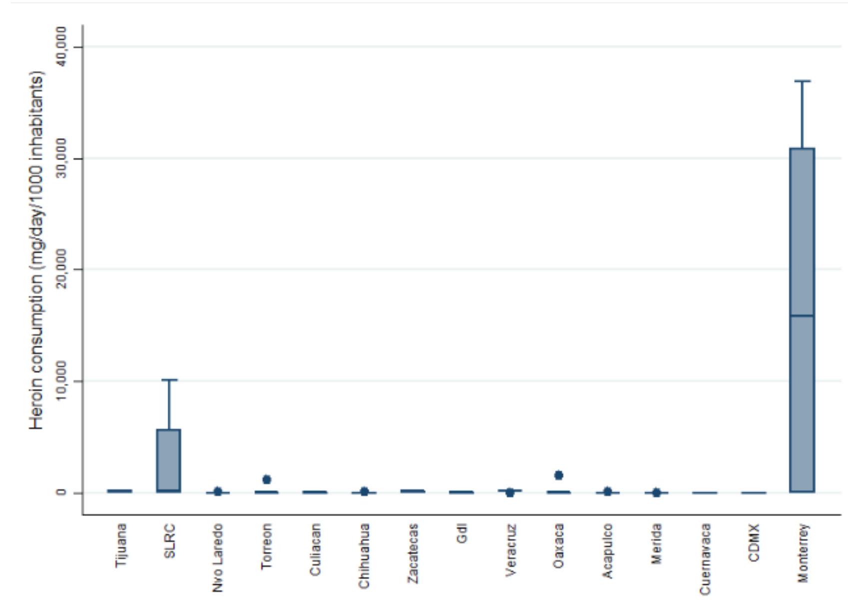


Figure 1 Weekly median levels of drug consumption (mg/day per 1,000 inhabitants) per city

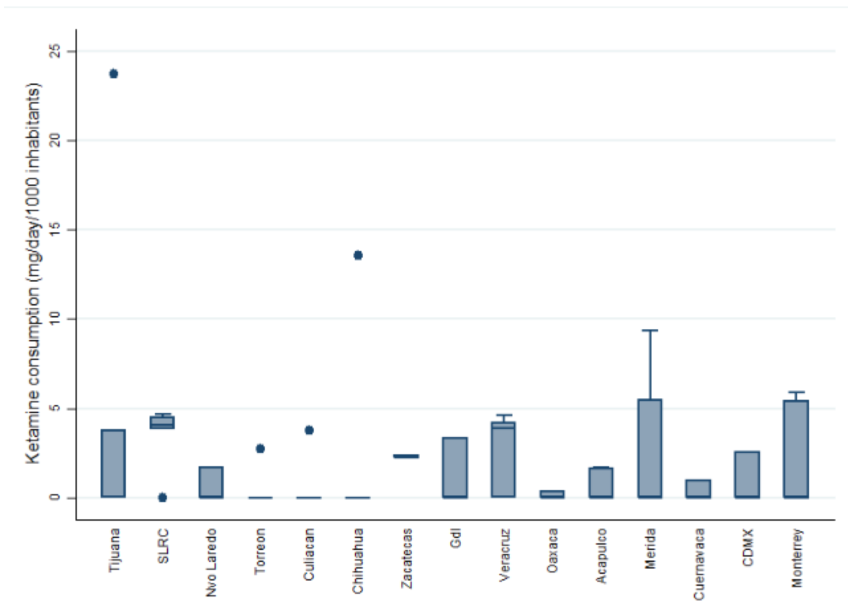
e)



f)



g)



h)

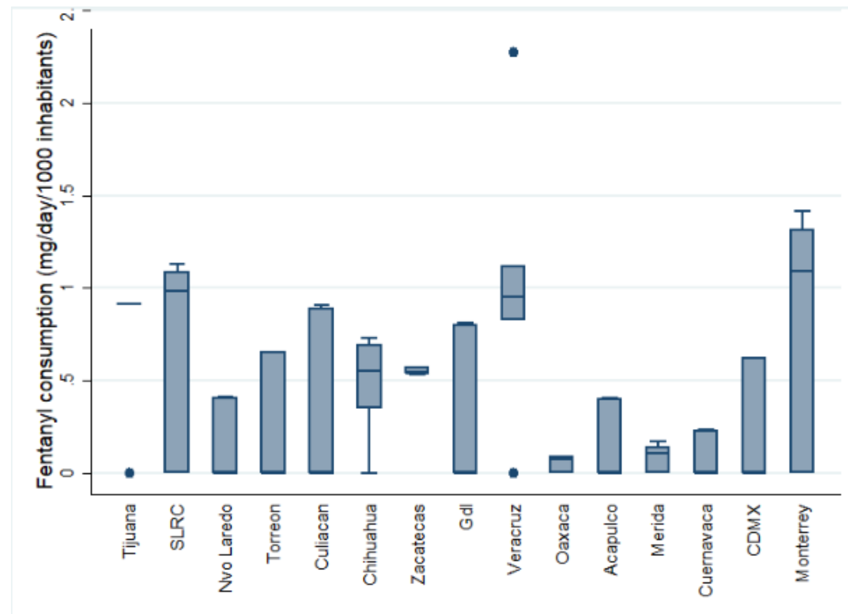


Table 2. Significative correlations between drugs consumption by city

Correlated drugs	Culiacan	Veracruz	Zacatecas	Oaxaca	Monterrey	SLRC	Tijuana	Guerrero	Cuernavaca	Nuevo Laredo	Mexico City	Chihuahua	Yucatán
KETA-Fentanyl	0.764	0.893	0.786	0.818	0.941	0.829	-	-	0.944	1	-	-	-
KETA-COC	-	0.793	1	0.867	-	-	-	0.809	0.757	-	-	-	-
KETA-HERO	-	0.847	0.786	-	0.797	-	0.935	-	-	-	-	1	-
Ecstasy-METH	-	0.893	0.786	0.893	-	-	0.929	-	-	-	-	-	-
COC-AMPH	-	0.786	-	-	-	0.857	-	-	-	0.906	-	-	-
COC-Fentanyl	-	0.857	0.786	-	-	-	-	-	0.802	-	-	-	-
COC-HERO	-	0.964	0.786	-	-	-	-	-	-	-	-	-	-
COC-METH	-	-	-	-	-	-	-	0.875	-	-	-	-	0.786
Marijuana-COC	-	-	-	0.929	-	-	0.857	-	-	-	-	-	-
Marijuana-METH	-	-	-	-	0.893	-	-	-	-	-	0.890	-	-
Marihuana-KETA	-	-	-	0.906	-	-	-	-	-	-	0.797	-	-
Ecstasy-AMPH	0.855	-	-	-	-	0.821	-	-	-	-	-	-	-
Ecstasy-HERO	0.784	-	-	-	0.815	-	-	-	-	-	-	-	-
Ecstasy-COC	-	-	-	-	-	0.964	-	-	-	-	-	-	-
Ecstasy-Marijuana	0.809	-	-	-	-	-	-	-	-	-	-	-	-
Marijuana-HERO	0.844												
METH-HERO	0.777	-	-	-	-	-	-	-	-	-	-	-	-
AMPH-HERO	0.905	-	-	-	-	-	-	-	-	-	-	-	-
AMPH-Marijuana	0.864	-	-	-	-	-	-	-	-	-	-	-	-
AMPH-Fentanyl	-	-	-	-	-	-	-	0.757	-	-	-	-	-

$p<0.05$, spearman´s rank correlation.

Supporting Information

Table S1 Comparative results from Study 2015 and ENCODAT 2016-2017 results

		Marijuana		Cocaine		Amphetamine	Methamphetamine	Amphetamine-type stimulants
Regions	State	Study 2015 (mg/day/1000 inh)	ENCODAT 2016-2017 Survey (prevalence)	Study 2015 (mg/day/1000 inh)	ENCODAT 2016-2017 Survey (prevalence)	Study 2015 (mg/day/1000 inh)	Study 2015 (mg/day/1000 inh)	ENCODAT 2016-2017 (cumulative incidence, %)
Northwestern	Tijuana, Baja California		3.8		0.6			3.1
	SLRC, Sonora							
Northwestern	Marte, Sonora	834	2.8	8	0.9		34	1.4
	Pueblo Yaqui, Sonora	1190		32			46	
	Esperanza, Sonora	1669		49			78	
Northwestern	Juarez City Chihuahua	19810	2.8	50				1.3
	Chihuahua Sur	11190		380	1.1	94	83	
Northwestern	Sinaloa, Culiacan	9159	2.5	860			1817	2
	Coahuila, Torreón	33360	3.2	273	1		383	0.4
Northeastern	Nuevo Laredo, Tamaulipas	14175	1.6	2366	0.6	240	612	0.4
Northeastern	Monterrey, Nuevo Leon		2.2		0.9			0.6
Western	Zacatecas		2		0.9			1.2
Western	Guadalajara, Jalisco		2.9		1.9			2
	Tepatitlan, Jalisco			131			30	
Southeastern	Chilpancingo, Guerrero		1.6	64	1.1			0.6
	Acapulco, Guerrero	3054		132			14	
	Cuernavaca, Morelos							
Central	Jiutepec, Morelos		1.9	221	0.3		22	0.3
	Temixco, Morelos	1145		66				
	Iztapalapa, Mexico City	9257		615			25	
	Xochimilco, Mexico City	5997		190			15	
	Iztacalco, Mexico City	4981		286			9	
Central	Tláhuac, Mexico City	2501	2.5	110	0.6			0.8
	Mexico City 1	916		76				
	Mexico City 2	785		45				
Central	Ecatepec, EDOMEX	2538	2.1	168	0.6			0.3
	Veracruz			34		7	8	
Southeastern	Perote, Veracruz	111	1.3	1	0.5			0.5
	Playa, Veracruz			44				
	Jalapa, Veracruz	5177		41				
Southeastern	Oaxaca		1		0.7	4		0.3
Southeastern	Villahermosa, Tabasco			1				
	Dos Montes, Tabasco		1.9	3	1.4			0.5
Southeastern	Tabasco Sur	2630		80				
Southeastern	Cancun Quintana Roo 1	3404	2.9	298	1.6	18		0.6
	Cancun Quintana Roo 2	1260		120				
Southeastern	Merida		1.6		0.6			0.4

Information based on (Cruz-Cruz et al., 2019; Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz; Instituto Nacional de Salud Pública, Comisión Nacional Contra las Adicciones, 2017)

Figure S1

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WASTEWATER TREATMENT PLANT CHARACTERISTICS

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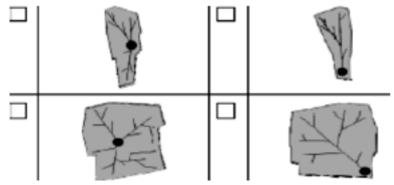
Instructions: Fill in the blanks in each section, the information you are given here is very important to know the feasibility of sampling for a wastewater analysis project.

GENERAL INFORMATION	
1.- Wastewater Treatment Plant (WWTP) name and type of treatment	Name: Type of treatment ( ) Aerated lagoons ( ) Muds activated ( ) Stabilization Lagoons ( ) Other ______________________________
2.- WWTP localization	Latitude ____° ______' _______'' ( ) N ( )S Longitude ____° ______' ______'' ( )E ( )W
3.- WWTP contact	Name ______________________________________ Phone ______________________________________ Email ______________________________________
CATCHMENT PROPERTIES	
4. – How much population serve your WWTP?	Number or range of inhabitants ______________
5. – How much wastewater discharges receive WWTP daily?	_________________ L/ inhabitants /Day ( ) Unknown
6. - ¿ What is the average daily flow receive at the WWTP?	Dry day ____________________ l/s Rainy day ____________________ l/s Monday ____________________ l/s Tuesday ____________________ l/s Wednesday ____________________ l/s Thursday ____________________ l/s Friday ____________________ l/s Saturday ____________________ l/s Sunday ____________________ l/s
7.- Does WWTP receive wastewater from the chemical industry, food industry or turistic sector)?	**( ) Yes** ( ) Chemical ( ) Food ( ) Turistic/Hotel **( ) No**

8. - Of the shape and locations of WWTP shown on the right side, which one best represents your WWTP, considering it the black dot?	

SEWER SYSTEM

9. - Is it possible to wastewater losses from the drainage lines prior to the entrance to the WWTP?	**( ) Yes** how much (as % of total inflow)?_______ **( ) No**
10. – How long wastewater travel from the nearest and remote home to the WWTP?	a) Shortest travel distance _______ Km b) Longest travel distance _______Km

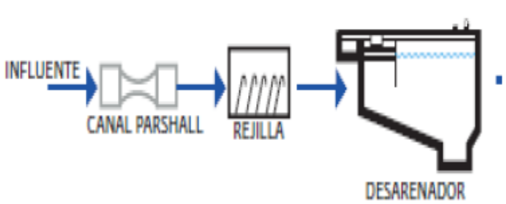
WASTEWATER FLOW CONTROL

11.- In the WWTP, Is there any special infrastructure (e. g. retention reservoirs, pumps) to regulate the flow of wastewater entering the WWTP?	**( ) Yes** **( ) No**
12.- Does WWTP have a measure system of wastewater inflow?	**( ) Yes** What type?____________________ ______________________________ **( ) No**
13.- Which do physicochemical tests in WWTP?	**( ) Yes** ( ) pH ( )Temperature ( ) Biochemical Oxygen Demand ( ) Other ____________________ **( ) No**

SAMPLING POINT

14Based on the schemes shown below, which best represents the input distribution of the influent to the WWTP? If not, can you make a simple outline of the influential entrance to the WWTP and point out the best point to sample residual water after removing macroscopic garbage but before going through another pre-treatment?

( )



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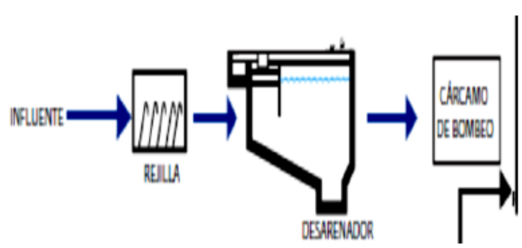

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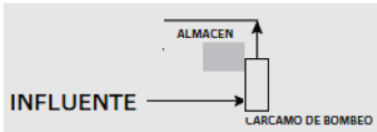

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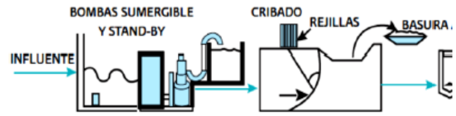

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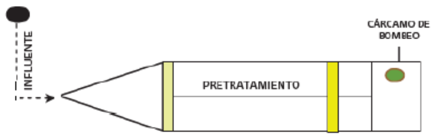

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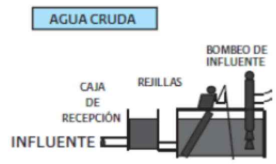

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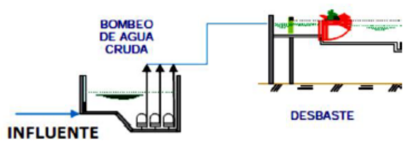

( )

15.- How long point sampling?	Depth	________m
	Long	________m
	Width	________m

PERSONNEL REQUIREMENTS	
16.- Do they have 24-hour staff available to conduct hourly wastewater sampling?	**( ) Yes** **( ) No**
17.- Number of staff available?	___________
18.- Could the above-mentioned staff be available, over a period of 15 days? Between 10 October and 10 December 2017	**( ) Yes** **( ) No**
19.- If there are only 2 workers, could they work shifts of 24X24 hours (one full day of sampling, and the next one to rest)?	**( ) Yes** **( ) No**
FACILITIES REQUIREMENTS	
20.- The WWTP Does it have an area of approximately 6 m^2 to protect a box with samples of waste water?	**( ) Yes** **( ) No**
21.- How far is the above-mentioned area from the sampling point?	___________

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22.- The above mentioned area has:	
Constant ventilation	**( ) Yes ( ) No**
Protection of sunlight.	**( ) Yes ( ) No**
Deck or table available for 7 days	**( ) Yes ( ) No**

Table S2. General characteristics of sampling sites, 2017

STP Location	Type of treatment	Inhabitants served[1]	Average Flow rate on sampling day (l/s)	Sampling date
Tijuana- Baja California Norte	Aerated lagoons	167,200	450	Nov 30 to Dec 7
SLRC-Sonora	Stabilization pond	130,200	403.5	Nov 30 to Dec 7
Chihuahua-Chihuahua	Activated sludge	740,000	1498.2	Nov 30 to Dec 7
Torreon-Coahuila	Stabilization pond	693,340	1435	Nov 29 to Dec 6
Monterrey-Nuevo Leon	Activated sludge	1,708,190	5941.7	Nov 28 to Dec 5
Nuevo Laredo-Tamaulipas	Activated sludge	475,785	606.5	Nov 29 to Dec 6
Culiacan-Sinaloa	Physicochemical	678,427	1825	Dec 7 to 14
Zacatecas-Zacatecas	Activated sludge	58,860	93.6	Dec 4 to 11
Guadalajara-Jalisco	Activated sludge	900,000	2131.21	Dec 7 to 14
Cuernavaca-Morelos	Activated sludge	338,650	237.4	Dec 5 to 12
Mexico City	Activated sludge	1,000,000	1830	Dec 7 to 14
Veracruz-Veracruz	Activated sludge	296,068	832.4	Dec 7 to 14
Oaxaca-Oaxaca	Activated sludge	400,000	101.8	Dec 6 to 13
Acapulco-Guerrero	Activated sludge	779,328	927.9	Dec 7 to 14
Merida-Yucatán	Activated sludge	20,000	10.2	Dec 8 to 15

SLRC, San Luis Río Colorado-Sonora
[1] Data provided by the Sewage Treatment Plant (STP)

Table S3 Target compounds and stable isotope surrogates surveyed in the present study, limit of detection (LOD) and limits of quantification (LOQ), back calculated for the samples (not the extracts).

Analyte	Source of target analyte	LOD; LOQ (ng/L)	Labelled surrogates	Source of labelled surrogates
Cocaine	Cerilliant	4.42; 14.72	Cocaine-d3	Cerilliant
(+/-) Methamphetamine	Cerilliant	4.79;15.96	Methamphetamine-d9	Cerilliant
(+/-) Amphetamine	Cerilliant	4.57; 15.25	Amphetamine-d8	Cerilliant
(+/-) MDMA (Ecstasy)	Cerilliant	4.32; 14.40	MDMA-d5	Cerilliant
Heroin	Cerilliant	6.01; 20.03	Heroin-d9	Lipomed
Ketamine HCl	Cerilliant	7.09; 23.64	Ketamine-d4	Cerilliant
Fentanyl	Cerilliant	4.75; 15.85	Fentanyl-d5	Cerilliant
Norfentanyl Oxalate	Cerilliant	4.91; 16.37	Norfentanyl-d5	Cerilliant
Benzoylecgonine	Cerilliant	4.31; 14.35	Benzoylecgonine-d3	Cerilliant
6-Acetylmorphine	Cerilliant	4.61; 15.37	6-Acetylmorphine-d3	Cerilliant
THC-COOH	Cerilliant	4.96; 16.55	THC-COOH-d3	Cerilliant

Table S4. Parameters used to estimate drug use

Drug	Drug Target Residue (DTR)	Percentage of drug excreted as DTR	Correction Factor
Cocaine	Benzoylecgonine	29[a]	3.59[a]
Amphetamine	Amphetamine	36.3 ± 8.4[c]	2.77[c]
Methamphetamine	Methamphetamine	43 [a]	2.3 [a]
Ecstasy	3,4 Methylenedioxy methamphetamine (MDMA)	22.5 [c]	1.65 [c]
Marijuana-Cannabis (Δ9-tetrahydrocannabinol (THC))	11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH)	0.5 ± 0.1 [c]	182 [c]
Heroin	6-acetylmorphine	1.3 [c]	86.9 [c]
Ketamine	Ketamine	3 [c]	3.3 [c,e]
Fentanyl	Norfentanyl	91.08	1.6[f]

a Castiglioni, S (Castiglioni et al., 2013)
b Zuccato E (Zuccato, Chiabrando, Castiglioni, Bagnati, & Fanelli, 2008)
c Gracia Lor E (Gracia-lor, Zuccato, & Castiglioni, 2016)
d Mastrioianni N (Mastroianni et al., 2017)
e Yargeau V(Yargeau, Taylor, Li, Rodayan, & Metcalfe, 2014)
f Gushgari AJ (Gushgari, Venkatesan, Chen, Steele, & Halden, 2019)

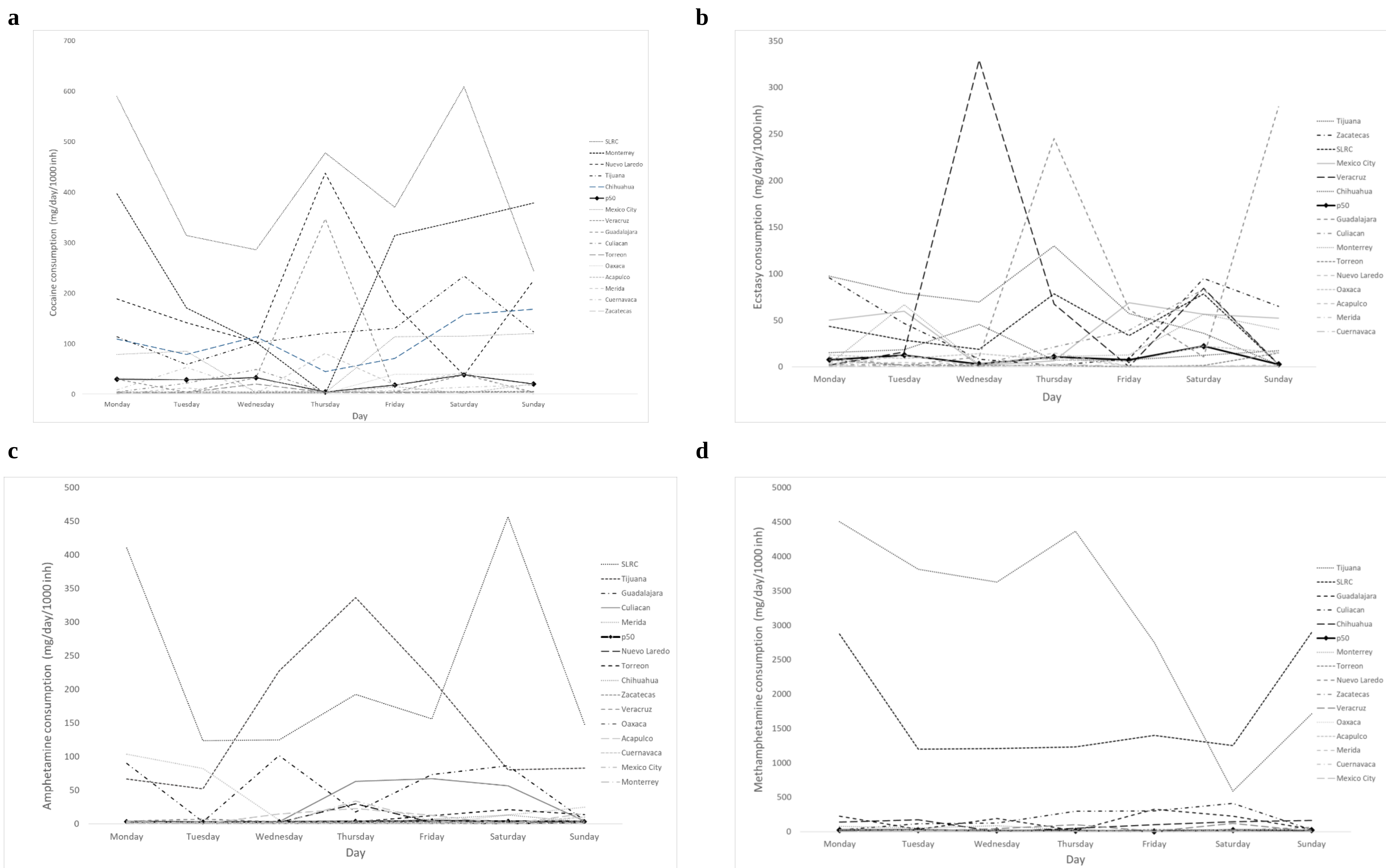

Figure S5 Weekly profile of drugs consumption in Mexican cities (mg/day/1000 inh). a) Cocaine consumption profile, b) ecstasy consumption profile, c) Amphetamine consumption profile, d) Methamphetamine consumption profile. Black line represents the whole week median (continuous line); gray and discontinuous line represent consumption profile by city.

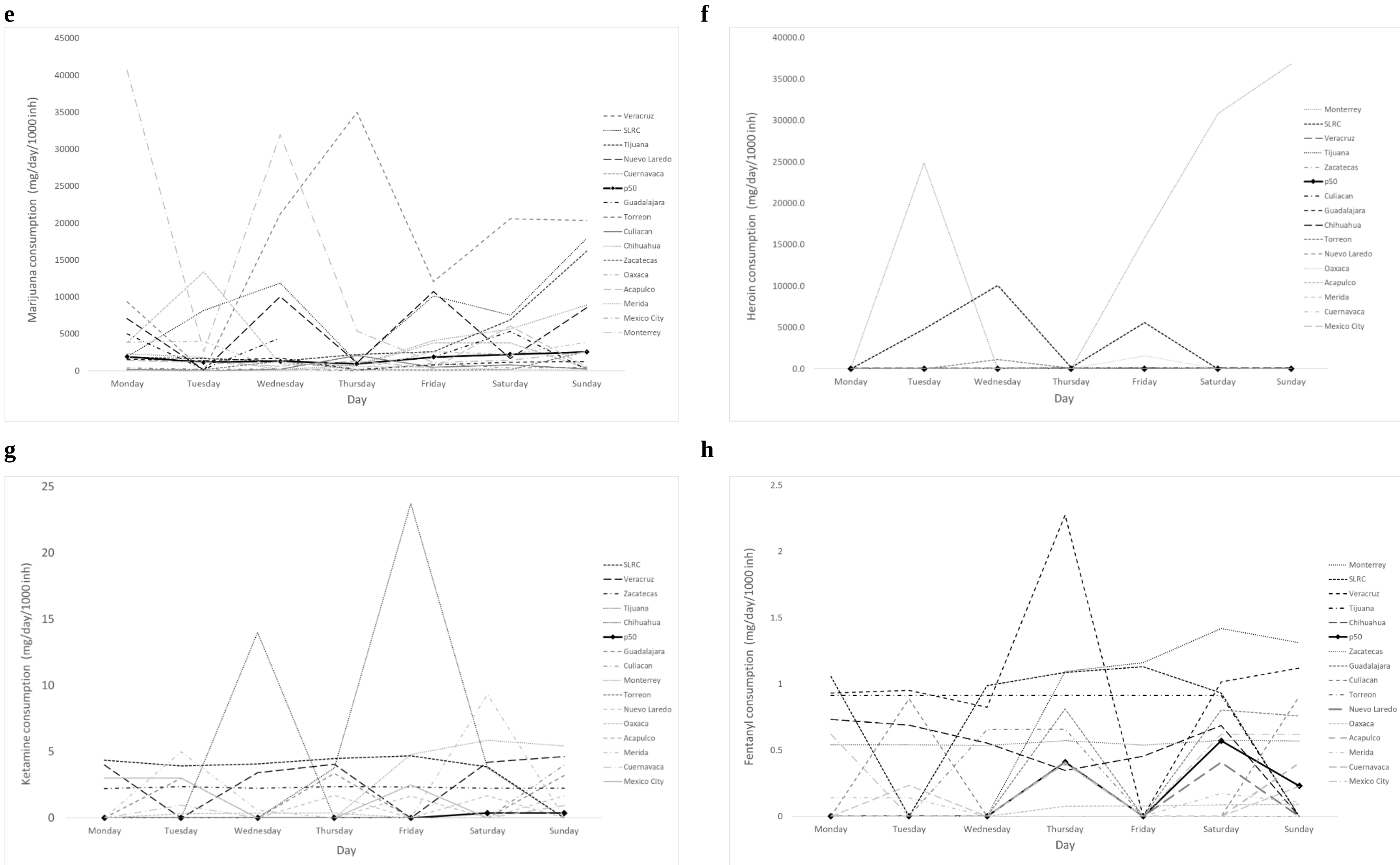

Figure S5 Weekly profile of drugs consumption in Mexican cities (mg/day/1000 inh). e) Marijuana consumption profile, f) Heroin consumption profile, g) Ketamine consumption profile, h) Fentanyl consumption profile. Black line represents the whole week median (continuous line); gray and discontinuous line represent consumption profile by city

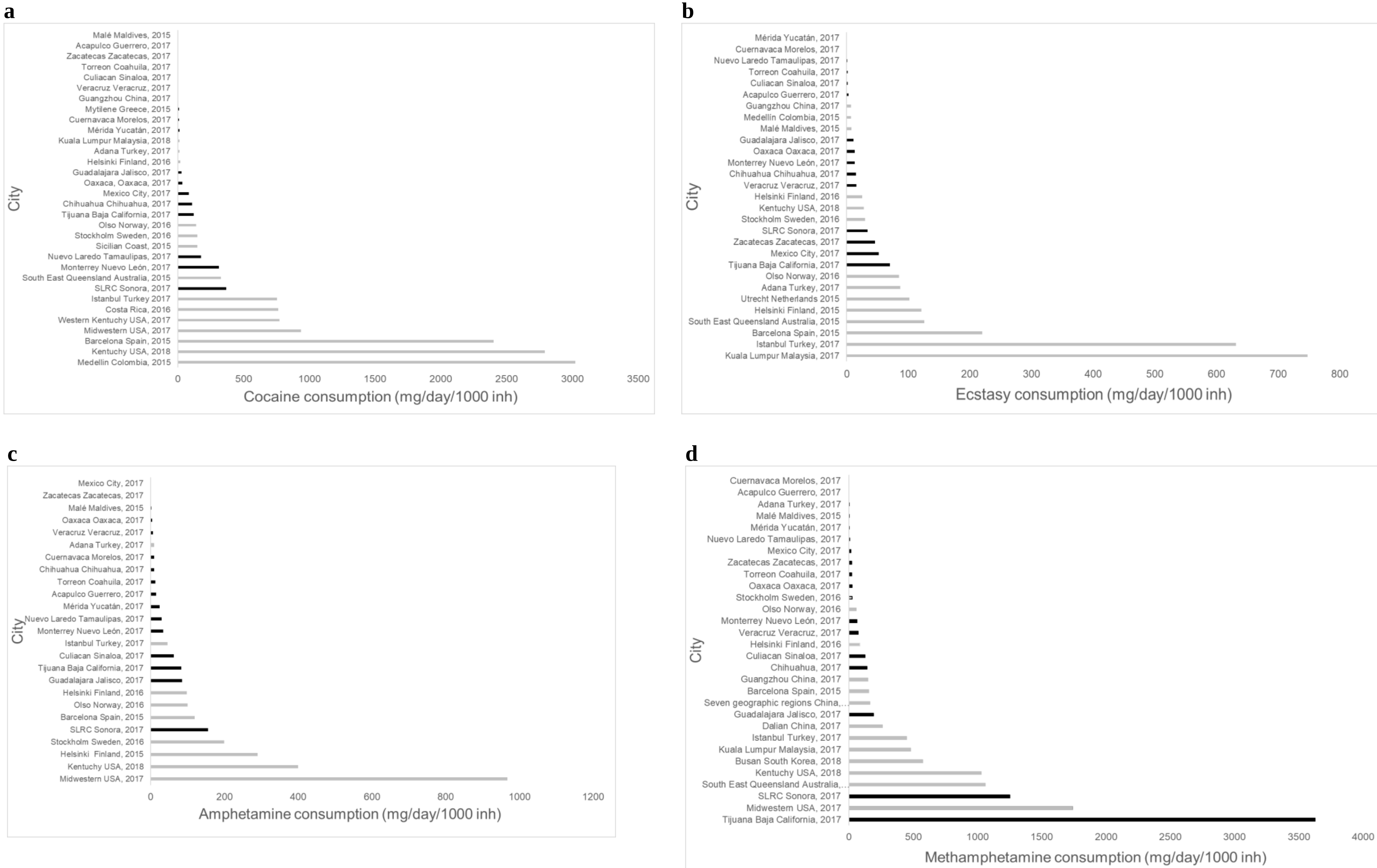

Figure S6 Comparison of drugs consumption between Mexican cities (mg/day/1000 inh) and other cities. Black bars represent Mexican cities median data; gray bars represent consumption data from other studies. The studies were obtained from a systematic review under the next inclusion criteria: studies with data collection between 2015 and 2019, studies with 24 h wastewater samples collected during seven or more consecutive days, and studies with drugs consumption calculation through back-calculated procedure or data with availability to perform back-calculation.

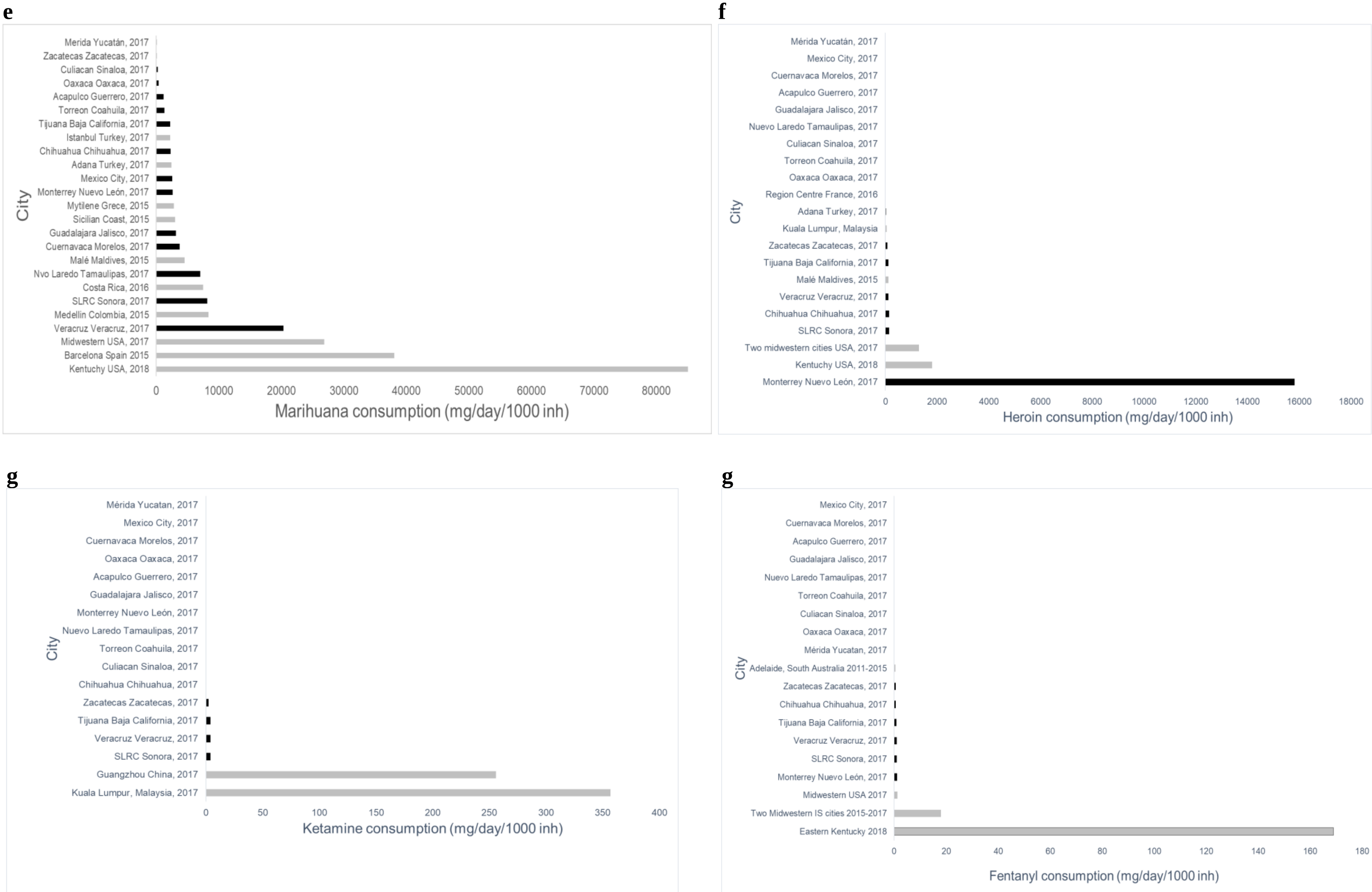

Figure S6 Comparison of drugs consumption between Mexican cities (mg/day/1000 inh) and other cities. Black bars represent Mexican cities median data; gray bars represent consumption data from other studies. The studies were obtained from a systematic review under the next inclusion criteria: studies with data collection between 2015 and 2019, studies with 24 h wastewater samples collected during seven or more consecutive days, and studies with drugs consumption calculation through back-calculated procedure or data with availability to perform back-calculation.