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Normalized Diurnal and Between-Day Trends in Illicit and Legal Drug Loads that Account for Changes in Population

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Abstract

Drug concentrations in composite municipal wastewater samples and census-based estimates of population are used to derive daily loads of illicit substances that are indexed to population. However, such estimates do not provide information on the diurnal trends of substance excretion nor can they account for changes in population. To address these limitations, a series of 1 h composites created by sampling wastewater influent at 6 min intervals was collected over four consecutive days at a single wastewater treatment plant. Creatinine (a urinary indicator), caffeine, methamphetamine, benzoylecgonine (BZE), and cocaine were analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS). Diurnal trends and between-day trends were substance specific and related to the number of estimated doses and excretory half-life. Normalization to creatinine yielded trends in substances that differed significantly from non-normalized trends by accounting for changes in population within the municipality studied. Increases in normalized substance excretion observed during early morning hours originate from individuals among the resident population of the municipality due to the absence of commuters.

INTRODUCTION

Illicit substance use in the United States is a social and economic problem. There is an ever present need for information regarding the use, distribution and presence of illicit drugs of concern at small (e.g., individual municipalities) and larger (e.g., regional and national) geographic scales. Conventional methods for assessing drug use utilize indirect indicator data sources such as self-report surveys, drug treatment admissions, and fatal overdose data that are limited by low numbers of observations that are lagged in time and subject to known bias.[1] Consequently, there is a need for data that directly measures drug consumption, which is obtained at smaller time intervals in order to determine temporal changes in usage patterns and impacts of interventions.

Municipal wastewater influent is used as a means for examining the loadings and source characteristics of chemicals used by humans including those contained in drugs of abuse,[2,3] personal care products,[4] household chemicals,[5,6] and pharmaceuticals.[6] Recent work indicates that illicit drug loads in wastewater influent and associated dose estimates are potentially complementary data sources to traditional indicators on substance abuse.[7–10] The

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current method that uses wastewater influent concentrations to calculate per capita loads relies upon two measured values (concentration and wastewater flow) and one estimated value (population).[2,7] Population is suggested as one of the greatest uncertainty when estimating substance use from per capita loads obtained from wastewater data.[2,8,11–14] Annual population estimates obtained from census data are lagged in time. For example, the US census is conducted every 10 years with the last occurring on April 1st, 2010.[15] Moreover, current census-based population estimates cannot reflect within-day or between-day changes in population. Indirect population indicators such as nitrogen, chemical oxygen demand, biological oxygen demand, phosphorus[2] in wastewater are proposed as strategies to account for changes in population in addition to non-traditional indicators such as prescription pharmaceuticals[16] and electricity use within a municipality.[17] However, there currently are no widely-accepted techniques to account for intra- and inter-day variation in population on the municipality scale.

Creatinine is an endogenous urine indicator that has potential for use as a surrogate population indicator.[8,14] Creatinine is produced at steady state in the body and is routinely used to verify urine authenticity and to account for dilution when testing human urine for illicit substances.[18] Water soluble, urinary-derived creatinine is excreted with every void and it remains in the dissolved phase along with water-soluble substances including illicit drugs.

Creatinine concentrations and the corresponding uncertainty about the concentrations in human urine are well documented for large (tens of thousands) numbers of individuals.[18,19] A large, detailed dataset published by Barr et al.[18] provides information on the creatinine variation with ethnicity/age and the uncertainty about those values for 22,245 subjects. Activities such as ingesting dietary (creatine) supplements[20,21] and the consumption of meat[14,22] potentially affect creatinine concentrations in urine. For specific activities such as meat consumption to impact the interpretation of diurnal trends in substances ratioed to creatinine requires that subpopulations of the municipality (e.g., illicit drug consumers) disproportionately engage in activities that alter creatinine concentrations in urine. Variability in the excretion of creatinine and other substances, including illicit substances, may be equally affected by differences in metabolism between individuals. However, measures of creatinine and other substances obtained from influent wastewater aggregate the metabolic variability among individuals at the whole-municipal scale. For this reason, we hypothesize that creatinine can be used to account for relative changes in population. Further, we posit that normalization to creatinine offers a different interpretation of trends in illicit drug use that that offered by the current method of reporting per capita values (mass/person*day), which are obtained by dividing mass flows by a static, census-based population. Normalization of illicit substances to creatinine potentially addresses a current limitation in wastewater epidemiology and provides insight into diurnal and between day trends in loads of illicit substances.

The uncertainty about computed loads for illicit and other substances is usually not assessed,[23] with few exceptions.[24] Representatively capturing packets (e.g., “pulses”) of wastewater influent containing urine when creating composite samples is critical for more accurate data and in turn more accurate interpretations of temporal and spatial trends for a wide range of environmentally-relevant contaminants and illicit substances. With some exceptions,[25] wastewater is typically collected as 24 h composites in a flow- or time-proportional manner. However, diurnal trends are masked by the current practice of collecting a single 24 h composite sample for each day. Alternatively, hourly (diurnal) trends for substances in wastewater influent can provide information on the daily patterns of substances used and consumed by humans. For example, a more detailed understanding of human consumption of illicit substance potentially aids in interpreting substance abuse

within municipalities. Others have employed diurnal sampling to detect changes in concentrations of personal care products, steroids, and alkylphenols in wastewater effluent.[4] Hourly data can then be compared to human urinary excretion of substances that are reported in the literature on the time scale of minutes to hours.[26–28] Sampling to create single daily composites typically occurs at hourly time intervals;[3,8,29,30] however, there is an increasing trend toward more frequent sampling[31–34] because frequencies of > 20 min are likely to miss significant pulses of urine.[16]

The first objective of this study was to determine diurnal trends in selected substances with high sampling frequency on a 6 min interval to create sets of hourly composite samples over a period of 4 days at a single wastewater treatment plant (WWTP). Four days was selected only to demonstrate diurnal variability in the selected substances and the methodology to assess such trends. The time period used for this study was selected because a significant change in population was expected to occur due to university and public school students and families leaving for spring break. The second objective was to compare trends in selected substances with and without normalizing to creatinine in order to account for relative changes in population within the municipality. Substances selected for study include caffeine, which is a legal and widely-used substance that is considered a marker for wastewater contamination.[8,35–37] Methamphetamine is a stimulant that is largely used as an illegal substance[38] with only minor legitimate use in the US.[39] Cocaine is a widely-used illicit stimulant[40] and its major metabolite, benzoylecgonine (BZE), has been used for back-calculating numbers of cocaine doses from wastewater data.[2,3,7,41]

EXPERIMENTAL METHODS

Location, Wastewater System, and Sampling

The municipality selected for this study is located in the Pacific Northwest of the United States with a 2010 census-based population of 54,462 (April 1st 2010) and is characterized by the following age distribution: 0–17 yrs (13%), 18–64 yrs (72%), and +61 yrs (13%) (Table S1 in the Supporting Information; SI).[42]

The WWTP collects wastewater from a mainly gravity-fed sewer system (two short sections are pressurized) consisting of 350 km of sewer piping and seven lift stations that are located throughout the catchment. Two main lift stations pump ~25% of the total daily wastewater volume (Figure 1a). Although pump activity at lift stations could significantly impact the temporal loads of illicit substances, discerning the contributions of individual pumps was beyond the scope of this study. Ongoing work is focused on understanding the potential influences of lift station pumps and the information will be used to inform sampling practices for systems in which pumps move a significant fraction of the total wastewater flow.

At the influent works of the WWTP, sewer flows are collected in a wet well that is continuously pumped at variable flow rates to maintain a constant level in the wet well. All raw wastewater samples were taken after the wet well and after the water had passed through a series of screens. The flow provided by the WWTP was recorded at 1 min intervals and used to compute hourly flows. Intra-hour variation in flow within the four days was characterized by a relative standard deviation (RSD) of less than 34% with only 15 of 96 hrs exceeding a RSD of 20% (see Table S2). The wastewater temperature was 14–15°C over the four sampling days and there was no precipitation.

Sampling was carried out from March 17th 2010 at 8 am through 8 am on March 21st. The sampling device (ISCO 3700 auto-sampler; Teledyne Isco Inc., Lincoln NE) was filled with ice to maintain a temperature of 4 °C and configured to acquire 1 h composite samples. The

ISCO sampler collected a sample every 6 min, which resulted in ten 25 mL samples to form each hourly composite. Following each day of sample collection, the 24 × 1 h composite samples were retrieved from the autosampler at 8 am and transported on ice (4 °C) to the laboratory. A 40 mL subsample of each composite was transferred to a HDPE centrifuge tube and stored at −20 °C without chemical preservation.

Chemical Analyses

Samples were analyzed by large volume injection liquid chromatography/tandem mass spectrometry as described by Chiaia et al.[8] with minor modifications. Briefly, wastewater was centrifuged and the supernatant was spiked with a mixture of deuterated internal standards. Calibration curves were constructed by using five standards. For more information see the SI. Sequences contained blanks (buffer), quality control standards, and a third party, certified-reference standard (UTAK, INC Valencia, CA). Details on transitions, instrument parameters, preparation of calibration standards and curves as well as the preparation of the certified-reference standard are given in Table S2.

Analytical precision of the whole method, as indicated by the RSD, was determined from four replicate analyses of a single wastewater sample within each analytical sequence and typically were below < 20% (Table S4). Accuracy, as indicated by the % agreement with the certified third party reference standard, ranged from 68–91% (Table S5).

Uncertainty Analysis

Total uncertainties about the measured total hourly loads for each substance were calculated as the square root of the sum of the squares of the individual RSDs associated with the chemical analyses, the selected subsampling frequency, and flow measurements made by the WWTP (Equation S1, Table S6).[5,16,23] The uncertainties about the ratios of substances normalized to creatinine were calculated as the square root of the sum of the squares of the individual RSDs associated with the analysis and the sampling uncertainty (Equation S2). Sampling uncertainties were assigned considering the nature of the diurnal trend for each substance and the short 6 min sampling frequency. A sampling uncertainty of 5% was applied to caffeine and creatinine given their non-episodic (e.g., cyclic with one ‘wave’ or peak per day) diurnal trends and for the remaining substances a value of 15% was estimated.[5,23] Flow measurement uncertainty was ± 5%, according to WWTP calibration protocols.

Stability of Analytes During Storage

The stability of analytes in wastewater samples stored for up to 24 h during collection at 4 °C was investigated by collecting single 500 mL grab samples of influent in a high density polyethylene bottle on each of three different days. The 500 mL samples were stored at 4 °C and aliquots were subsampled at 1–7, 12, and 24 h after collection and immediately frozen until analysis. For further details on the stability study see the SI.

First-order disappearance rate constants for each analyte were calculated by plotting the natural log of concentration versus time (Figures S1–S5). The mass lost during storage was computed by dividing the measured analyte concentration for each hourly composite sample by the term e^{-kt} where k is the average first-order rate constant (Table S7) and reaction times (t) that ranged from 24 h for the first composite sample collected to 1 h for the last hourly composite collected. Average rate constants for caffeine (Figure S2), methamphetamine (Figure S3), and cocaine (Figure S5) were not significantly different from zero but variation about the measured rate constants resulted in computed losses ranging from 5–16% (Table S7). The estimated mass lost for each substance for each hourly composite sample was added to the measured mass to compute daily total loads for each

substance. Error bars indicating the uncertainty about the estimated load of each analyte lost during storage were constructed using the upper and lower rate constants as indicated by the 95% CI about the average rate constant.

RESULTS AND DISSCUSSION

Hydraulics

The total wastewater flow decreased 17% from 3.82×10^7 L/d on Wednesday to 3.18×10^7 L/d on Saturday (Table 1) for the WWTP investigated. Hourly flows reflect a regular diurnal pattern with a factor of two change in flow from a minimum at night to a morning maximum (Figure 1A). A mean wastewater in-sewer residence time (R_t) of ≤1.5 hrs was estimated from the location of the population's highest density and an average wastewater velocity of 0.6 m/s in the gravity fed system. For convenience, a R_t of 1 h can be used to back estimate the time of excretion within the municipality. For example, substances captured in an hourly composite collected from 8 to 9 am are attributed to excretion events within the municipality occurring from 7 to 8 am. However, no information can be inferred regarding the time of substance ingestion.

Creatinine

Creatinine is the only endogenous substance investigated in this study. Creatinine is excreted with every void; therefore, it does not have a half-life (the time required for the urine concentration of a substance to decrease by 50%) per se. For creatinine and all other substances investigated in this study, the estimated mass lost during storage is added to the measured load to represent the total daily load excreted by the entire municipality.

The temporal trends exhibited by creatinine loads (concentrations multiplied by flow; Figure 1B) and concentrations (Figure S6) are similar to the diurnal pattern of wastewater flow (Figure 1A). The cyclic diurnal trends in creatinine are consistent with a large population excreting a correspondingly large number of creatinine-containing urine 'pulses'.[5]

The diurnal trends in creatinine observed at the WWTP are characterized by peak loads from 8 am until 12–1 pm that then drop to a plateau that lasts until 12 pm–1am (Figure 1B). The observed diurnal patterns for creatinine are similar to those observed for ammonia, a more conventional urine indicator.[43,44,45] Ammonia was not measured in this study because, many other sources of ammonia exist within municipalities and because the WWTP investigated receives landfill leachate that is rich in ammonia.[46] Therefore, the observed trends in creatinine loads reflect known patterns of human urine output that are governed by the circadian rhythm of urination.[47,48]

The rapid increase in early morning creatinine is likely due to people waking up followed by their first void of the day. First voids are characterized by only 5% higher creatinine concentrations[18] and cannot fully account for the ~30% larger loads of creatinine in the morning. The decline after 12 pm (midnight) is due to a large fraction of the resident population going to sleep. Peak creatinine loads shift later (i.e., 10 am) on Saturday, presumably due to later waking times (Figure 1B).

Commuters also potentially contribute to the greater loads of creatinine in the morning with a net weekday influx of 9,000 commuters into this municipality, which is 17% of the census-based population of 55,000.[49] No demographic data was available for the commuters. However, age impact creatinine excretion more than ethnicity[18] and commuters are likely to be of working age (e.g., 18–60 year olds), which is the age range that describes 72% of the municipality's base population (Table S1).[42] For this reason, the diurnal trends and changes

in daily creatinine loads are attributed to changes in total population and not to shifts in age, race, sex, or diet.

The measured daily loads of creatinine (Table 1), which is the sum of the hourly loads (g) from 8 am to 8 am, decrease from Wednesday (36 kg) to Saturday (19 kg). As indicated, this time period was deliberately selected because the population is known to decline due to the departure of students for spring break. The movements of the university's student population, which may include a fraction of the commuters, and that represent 40% of the municipality's census-based population along with public school students and their families are likely to significantly affect measured creatinine loads. Given the lack of independent population measures, creatinine is used to account for relative changes in population so that the trends in substances ratioed to creatinine offer apparent changes in per capita loads of illicit and other substances.

Because there was no independent information on the actual population served by this WWTP, two approaches were taken to estimate the population utilizing the WWTP during the study period. The first approach consisted of back calculating the population by first assembling a weighted estimate of the municipality's creatinine urine concentration based on the municipality's ethnic/age demographics and the corresponding median, 10^{th} percentile, and 90^{th} percentile creatinine concentrations for each ethnic/age group reported by Barr et al.[18] (see SI for details). The weighted median creatinine in urine value was 1,300 mg/L while the 10^{th} and 90^{th} percentile values were 410 and 2,400 mg/L, respectively. Using the median value together with the measured creatinine mass load for each day, the volume of urine for each day was estimated from Equation S3. The population of the municipality was then further estimated from Equation S3 by incorporating the reported average ± one standard deviation of the volumes of urine produced per person per day (1.1±0.5 L/day) as reported by Murakami et al. for n=654 subjects.[50] The estimated population ranged from a high of 17,000 to 31,000 on Wednesday, which is 26–49% of the total maximum population (weekday with residents + commuters) down to a low of 9,000 to 16,000 on Saturday, which is 14–30% of the resident-only (weekend) population (Table S8). As indicated earlier, the accuracy of the back calculated population is unknown and was not within the scope of the current study.

The second approach was to compare the wastewater flow and drinking water consumption. However, no data is collected by the municipality on the actual water consumption for a single day. For this reason, the annual estimate of the daily average drinking water consumed (1.73×10^7 L/d) was compared to the wastewater flows recorded during this dry weather study period (Table 1). Assuming no loss of drinking water in the distribution system and if it all entered the wastewater system, 1.45×10^7 L/d to 2.09×10^7 L/d (45–54%) of the total wastewater flow must originate from other sources. The municipality's sewer system can be characterized as having a substantial infiltration and inflow impact.[46] Detailed investigations in over 200 European sewer catchments reveal normal infiltration and inflow impacts between 35–65% of total wastewater flows during dry weather flow conditions,[51–54] even in relatively well-maintained systems.[55] For this reason, it was not possible to correlate drinking water consumption and total wastewater flow as an independent estimate of the municipality's population during the study period.

Because 'open' municipalities are subject to changes in population within and between-day, more research is needed to refine our understanding of the correlation between creatinine and actual numbers of individuals. On-going research in this laboratory is focused on testing hypotheses for creatinine in prisons, which are closed systems with very well-defined hourly and daily populations. In addition, creatinine biodegradation during transit in sewers may decrease measured loads. Biodegradation is likely affected by factors related to the

infrastructure of sewer systems including wastewater residence time, low and variable oxygen concentrations, lower temperatures (e.g., 14–15 °C), and the presence of an active biofilm. Although the biodegradation of creatinine and the other substances may be occurring during transit, the rates of biodegradation may be effectively constant for a municipality and its fixed infrastructure. Thus, we assume that hourly and daily trends in creatinine loads are proportional to population. For this reason, the mass loads of all substances investigated in this study were ratioed (normalized) to those of creatinine in order to interpret trends in apparent per capita loads that potentially different from those obtained using static (census-based) population estimates.

Fecal-associated substances such as coprostanol, were suggested as potential biomarkers to estimate population size.[8,14] However, in addition to the recognized challenges related to sampling for suspended and settleable solids, additional confounding factors exist when considering fecal-associated biomarkers for normalizing the mass flows of water-soluble substances. For example, sedimentation and non-continuous movement of feces cause storage of feces in sewer systems (house connections) and consequently leads to unknown lag times. In contrast to urination events that occur throughout the day, on average, there is only one fecal-related toilet flush per person per day.[44,45] Therefore, most commuters may not contribute to the total feces load but proportionally to the urine load. For these reasons, fecal-associated biomarkers were not considered for this study.

Caffeine

Caffeine was selected to benchmark illicit substances for which consumption patterns are less well known. As expected, the wide consumption of caffeine is reflected in its higher loads (Figure 1C) and concentrations (Figure S6) when compared to those of the illegal substances. The non-normalized hourly loads (Figure 1C) depict cyclic diurnal trends that repeat on each of the four sampling days.

The first peak in non-normalized caffeine hourly loads observed at the WWTP occurs from 8–9 am and lasts until 12 am–1pm (Figure 1C). The largest loads in caffeine occur slightly later than those of creatinine and they shift to later times due to the population waking later in the morning on Saturday. The trends in loads for the two substances may also differ because caffeine ingestion typically begins after the first morning void. Furthermore, caffeine has a 6–9 h excretory half-life (the time required for the urine concentration of a substance to decrease by 50%),[56–58] whereas creatinine is excreted with every void. The hourly loads of caffeine normalized to creatinine (Figure 1D) offer a significantly different picture of caffeine excretion than that by non-normalized hourly loads (Figure 1C) with greater apparent per capita loads of caffeine in the middle of the day rather than the morning.

Non-normalized daily total loads of caffeine decrease from 1,600 g on Wednesday to 1,400 g on Saturday (Table 1) and indicate decreased consumption over the four-day period. Non-normalized loads (mass/day) offer the same information as the more commonly reported per capita loads (mass/person*day) because the population estimate used in the denominator is a constant. In contrast, apparent per-capita caffeine consumption increases from Wednesday to Saturday when loads are normalized to creatinine (Table 1). Such an increase requires that students and commuters leaving the community for spring break/weekend consume less caffeine than those who remain during this time period. The example of caffeine illustrates that normalizing substances to creatinine changes the interpretation of trends in diurnal and between-day trends in loads.

The number of caffeine doses was estimated assuming that 1%[59,60] of a caffeine dose containing 150 mg[57] is excreted (Equation S4). A large number of caffeine doses (9.6×10^5

to 1.1×10^6; Table 2) indicates a large number of caffeine-containing urine 'pulses.' A large number of pulses combined with a urinary half-life of 6 to 9 hrs[57,58,60–62] is consistent with the observed cyclic diurnal trends in caffeine. However, the number of estimated caffeine doses (17–20 doses per person) is much larger than expected based on consumption alone, which may indicate that other sources such as the disposal of caffeine-containing beverages are entering the wastewater system. In addition, the assumed dose may be low given the presence of caffeine supplements and drinks in the marketplace that contain very high levels of caffeine. For this reason, caffeine is not an unambiguous human urine indicator.

Methamphetamine

Methamphetamine was quantified in each hourly composite sample over the four sampling days and is consistent with the characterization of this municipality as a high-methamphetamine use municipality as described in Chiaia et al.[8] Diurnal trends in non-normalized methamphetamine hourly loads were relatively uniform (Figure 2A) with maximum to minimum loads exhibiting the least variability among the substances studied. In contrast, normalized-methamphetamine displays a distinct peak from 3–7 am (Figure 2B). Methamphetamine is a potent, relatively long-acting stimulant so activity among methamphetamine users late at night/early morning is not unexpected. Increased apparent per-capita loads during this time frame, when commuters are largely absent, is assumed to result from individuals among the resident population of the municipality.

The total daily methamphetamine loads, both non-normalized and normalized, increase Wednesday through Saturday (Table 1). However, normalized loads show a greater increase in methamphetamine compared to non-normalized loads (Table 1). The increase in apparent per-capita excretion of methamphetamine on Friday and Saturday is due to the lower measured creatinine loads at the beginning of spring break and the onset of the weekend. This increase is assumed to result from the resident population of the municipality.

The total number of estimated methamphetamine doses ranged from 270 to 330 (Table 2) and were computed assuming a dose of 100 mg[63] and that 40% of the dose is excreted[27,63] (Equation S5).[34] In the region where this municipality is located, legal use of methamphetamine as a component of prescription medications is estimated to account for only 3–8% of the observed methamphetamine loads.[39] Because the estimated number of methamphetamine doses is lower (270–330; Table 2) than that of caffeine, the number of methamphetamine-containing urine pulses is lower. While one might expect episodic (e.g., multiple peaks of shorter duration) trends in methamphetamine loads, methamphetamine has a longer half–life (9–24 h) than caffeine,[28,64] which may explain why the diurnal trends in methamphetamine are cyclic and not episodic.

Benzoylecgonine and Cocaine

Benzoylecgonine, the major human metabolite of cocaine, was observed in each hourly composite sample (Figure 3A), which was not the case for the parent substance, cocaine (Figure 3C). The non-normalized hourly loads of BZE and cocaine are episodic with no discernible diurnal or between-day trends, which is in contrast to methamphetamine. Normalized loads of BZE and cocaine have weak but discernible peaks during the very early morning hours from 2–7 am (Figure 3B and 3D) when commuters are largely absent and, as such, are attributed to cocaine users within the resident population.

The non-normalized total daily loads of BZE and cocaine indicate no apparent trend between days (Table 1). In contrast, normalization to creatinine provides evidence that the apparent per capita BZE and cocaine loads actually increase from Wednesday to Saturday (Table 1). Given the decrease in students and commuters as the weekend approaches,

increased excretion of cocaine and BZE is interpreted as resulting from individuals among the municipality's resident population due to the absence of the commuters during this time period. Increased cocaine usage on the weekend is consistent with other report indicating increases on weekends,[3,32] holidays,[65] and sporting events[31] that rely on non-normalized cocaine/BZE data and that assume a constant population. However, such 'weekend effects' may actually be larger or smaller than reported if population changes are taken into account by normalizing to creatinine loads.

The estimated number of doses per day of cocaine calculated from the total daily load of BZE (Table 1; Equation S6)[2] ranged from 35 to 45 (Table 2), which is low compared to other municipalities.[32,34] Alternatively the number of doses if cocaine itself[66] is used for the estimate ranged from 160–330 (Table 2; Equation S7). When considering the more accepted approach of estimating doses from BZE, the number of cocaine doses is the lowest among the substances investigated. The relatively low number of doses combined with the short excretory half-life of cocaine (1–4 hrs)[26] is consistent with episodic cocaine loads. Even though BZE is excreted over long time periods (e.g., 3 days)[26] that could then result in cyclic diurnal patterns, the low number of cocaine doses may explain the observed episodic nature of BZE loads.

The ratio of BZE mass load (Figure 3A) to cocaine mass load (Figure 3C) ranged from 2–32 (data not shown), which is consistent with the reported ratios for 24 h wastewater composites that range from 2 up to 10.[41,67] Ratios of the BZE/cocaine mass loads for wastewater indicate that BZE is only ten times greater than cocaine, which is much lower than those reported in detailed datasets describing the mass balance and continuous monitoring of cocaine and its metabolites in human subjects over the time scale of minutes to hours.[32] Others speculate that impacts of alcohol co-consumption and biodegradation in transit to WWTPs as well as deliberate release (e.g., dumping) of cocaine result in low BZE/cocaine ratios.[2,32] While 'dumping' could result in low BZE/cocaine ratios, one would also expect such events to occur as rather large discrete peaks in the diurnal trends in cocaine loads. However, no large peaks are apparent among the hourly composites (Figure 3D). For this reason, 'dumping' does not appear to account for the low ratios in this system. Alternatively, "wash off", may occur over the course of the day when washing cocaine-contaminated hands, surfaces, and implements involved in cocaine administration. In addition, wash off may occur after packaging or re-formulating powder cocaine to "crack", which is often done at the local level following importation. If cocaine is washed off then 100% of the cocaine mass enters the wastewater stream instead of 1% had it been metabolized. Therefore, even small amounts of unmetabolized cocaine entering the waste stream due to wash off could significantly shift BZE/cocaine ratios to the low observed values.

Implications

The overarching goal of this study was to develop an approach that can account for relative changes in population when estimating illicit substance loads for municipalities. The methodology was applied to discern diurnal trends of illicit substances that, by their very nature of their use, are likely substance-, time-, and location-specific. The generalized methodology can be applied to determine the diurnal trends for these and other substances to any other series of days, seasons or locations.

Diurnal sampling offers detailed insight into substance loads within a day on the scale of a whole municipality that single 24 h composites simply cannot provide. Diurnal trends in creatinine were attributed to apparent per-capita changes in population and used to account for within and between day changes in substance loads. Normalization to creatinine changed the interpretation of within and between-day trends. Thus, normalization to creatinine is a

tool that shows potential for adjusting loads of substances obtained from single daily 24 h composites in order to account for changes in population. Normalization to creatinine revealed the late night and early morning excretion of illicit substances that was interpreted as resulting from individuals among the municipality's resident population. Interventions to address substance use can be better targeted with more precise information about the population (resident and/or nonresident) consuming substances that is gained from within-day patterns of excretion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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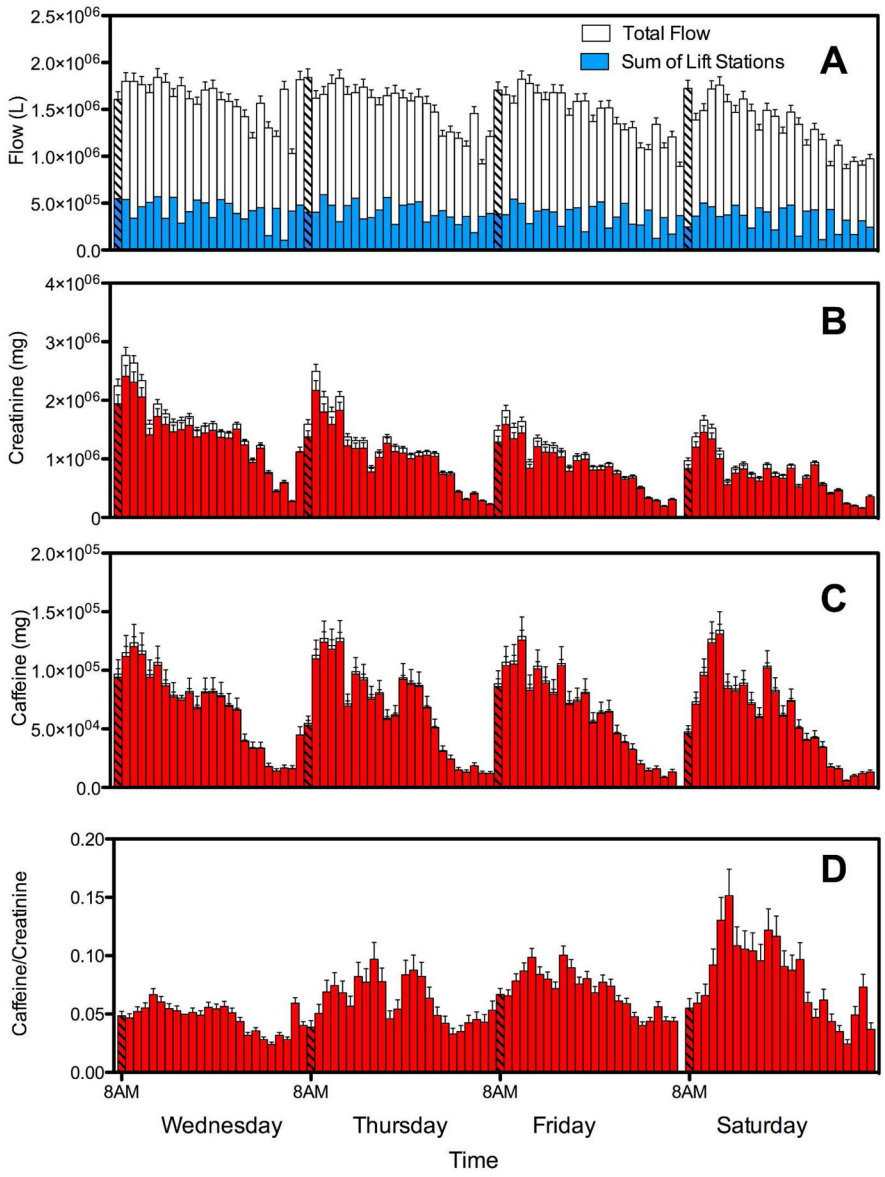

Figure 1.

Total hourly wastewater flows (gravity and pumped) from March 17th – March 21st (A), hourly loads (mg) of creatinine (B), caffeine (C), and caffeine/creatinine (D). Blue bars indicate sum of lift station wastewater flow. Red bars indicate measured load and the corresponding error (See SI for error calculations). Clear bars indicate the estimated load loss during collection and storage at 4 °C and the corresponding error calculated from the 95% CI of the rate constant measured during the stability study. Black hatched bars indicate 8 am as a time reference for each day.[1]

[1]The bottle collecting the 7–8 am sample on Saturday broke during collection.

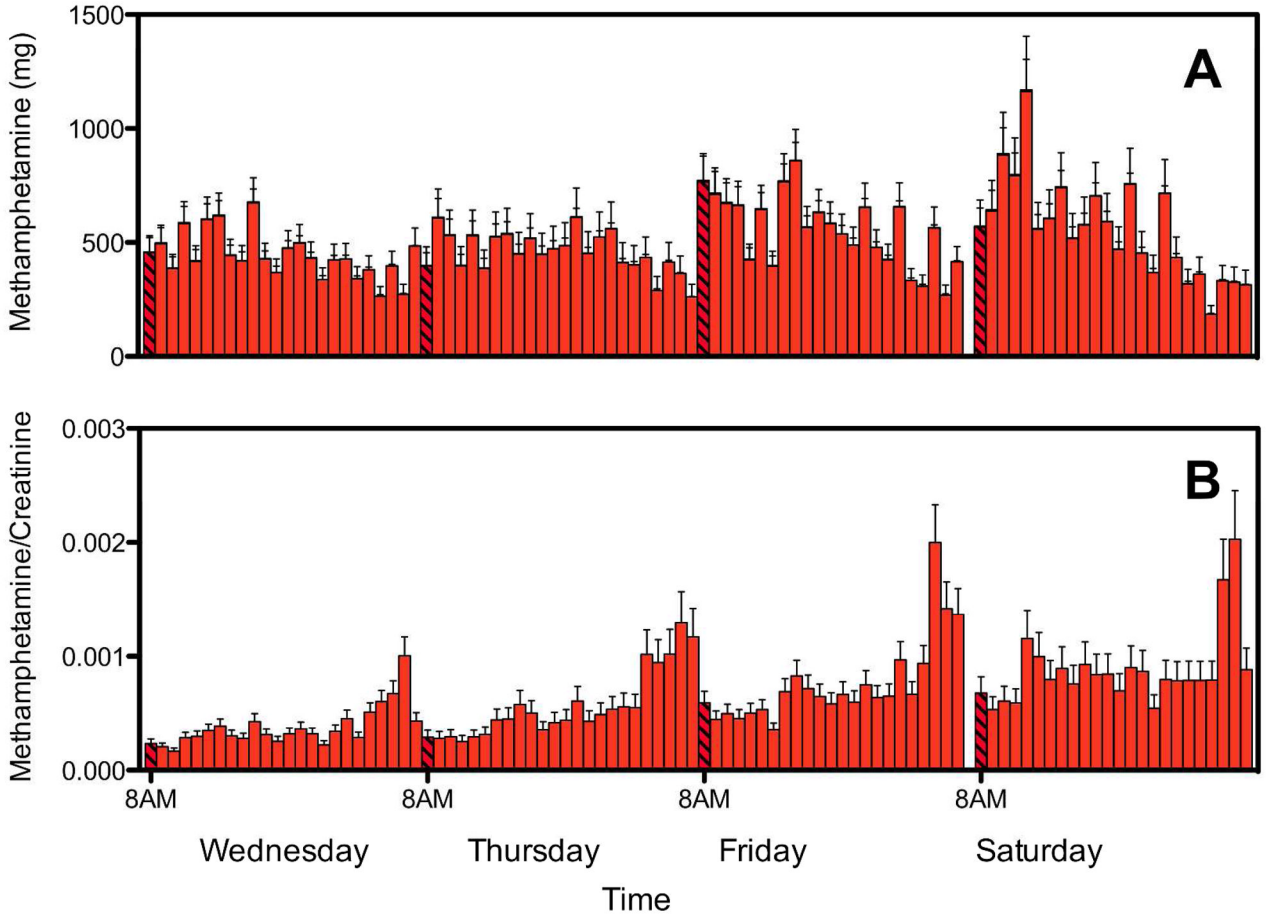

Figure 2.

Hourly load (mg) of methamphetamine (A) and methamphetamine/creatinine (B) for Wednesday March 17th – Saturday March 21st. Colored bars indicate measured load and the corresponding error (See SI for error calculations). Clear (but very small) bars indicate the estimated load loss during collection and storage at 4 °C and the corresponding error calculated from the 95% CI of the rate constant measured during the stability study. Black hatched bars indicate 8 am as a time reference for each day.[1]

[1]The bottle collecting the 7–8 am sample on Saturday broke during collection.

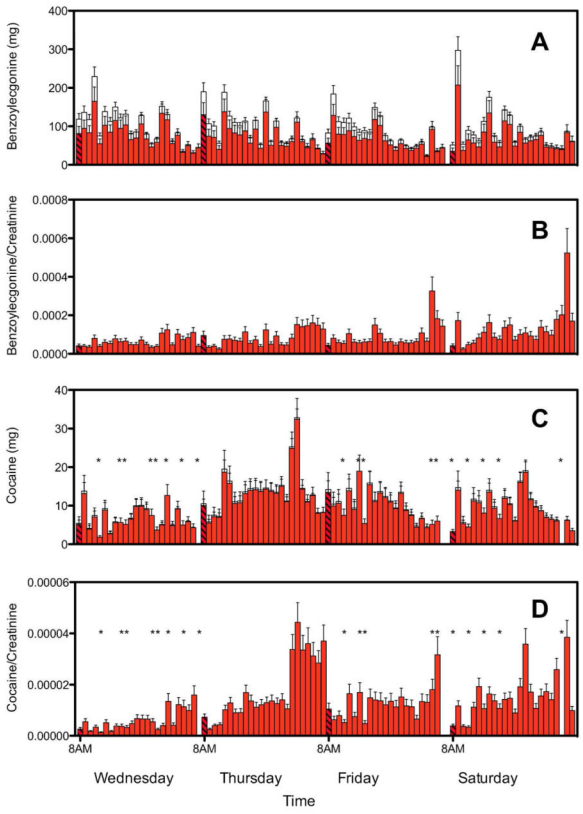

Figure 3.

Hourly load (mg) of benzoylecgonine (A), benzoylecgonine/creatinine (B), cocaine (C), and cocaine/creatinine (D) for Wednesday March 17th – Saturday March 21st. Colored bars indicate measured load and the corresponding error (See SI for error calculations). Clear bars indicate the estimated load loss during collection and storage at 4 °C and the corresponding error calculated from the 95% CI of the rate constant measured during the stability study. Black hatched bars indicate 8 am as a time reference for each day.[1]

[1]The bottle collecting the 7–8 am sample on Saturday broke during collection.

*Samples with signal-to-noise ratios <10 determined from calibration curves are included for qualitative purpose.

Table 1

Total wastewater flow entering the WWTP and total loads of measured plus average estimated loss during storage for creatinine, caffeine, methamphetamine, benzoylecgonine (BZE), and cocaine, and normalized load (load substance/load creatinine) for each sampling day starting at 8 am.

Substance	Wednesday	Thursday	Friday	Saturday
Total wastewater flow (L)	3.82×10^{7}	3.65×10^{7}	3.48×10^{7}	3.18×10^{7}
Creatinine (kg)	36	27	22	19
Caffeine				
(g)	1600	1600	1500	1400
normalized	0.05	0.06	0.07	0.08
Methamphetamine				
(g)	11	11	13	13
normalized	3.1×10^{-4}	4.1×10^{-4}	5.9×10^{-4}	6.8×10^{-4}
BZE				
(g)	2.4	2.2	1.9	2.1
normalized	6.7×10^{-5}	8.1×10^{-5}	8.6×10^{-5}	1.1×10^{-4}
Cocaine				
(g)	0.15	0.33	0.23	0.21
normalized	4.2×10^{-6}	1.2×10^{-5}	1.0×10^{-5}	1.1×10^{-5}

Table 2

Estimated number of doses of caffeine, methamphetamine, cocaine (computed from BZE), and cocaine (from cocaine). For assumptions and calculations see Equations S4–S7.

Substance	Wednesday	Thursday	Friday	Saturday
Caffeine	1.1×10^6	1.0×10^6	1.0×10^6	0.96×10^6
Methamphetamine	270	280	320	330
Cocaine (computed from BZE)	60	50	40	50
Cocaine (computed from cocaine)	160	330	220	210