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Contamination profiles, mass loadings, and sewage epidemiology of neuropsychiatric and illicit drugs in wastewater and river waters from a community in the Midwestern United States

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Abstract

In this study, residues of the neuropsychiatric and illicit drugs including stimulants, opioids, hallucinogens, anti-schizophrenics, sedatives, and antidepressants were determined in influent and effluent samples from a small wastewater treatment plant, a receiving creek, and river waters in the Four Rivers region of the Midwestern United States. Nineteen neuropsychiatric drugs, eight illicit drugs, and three metabolites of illicit drugs were detected and quantitated in the water samples using HPLC–MS/MS. Residual concentrations of the drugs varied from below the detection limit to sub-μg/L levels. The source of residual cocaine and benzoylecgonine in wastewater is primarily from human consumption of cocaine rather than direct disposal. Wastewater based epidemiology is utilized to estimate the community usage of drugs based on the concentration of drug residues in wastewater, wastewater inflow, and the population served by the centralized wastewater treatment plant. The per-capita consumption rate of methamphetamine (1740 mg/d/1000 people) and amphetamine (970 mg/d/1000 people) found in this study were the highest reported per-capita consumption rates in the USA. Antidepressant venlafaxine found to have the highest environmental emission from the WWTP(333 ± 160 mg/d/1000 people) followed by citalopram (132 ± 60.2 mg/d/1000 people), methamphetamine (111 ± 43.6 mg/d/1000 people), and hydrocodone (108 ± 90.1 mg/d/1000 people). Bee Creek, an immediate receiving water body, is found to be a source of several neuropsychiatric and illicit drugs including methamphetamine, methadone, alprazolam, oxazepam, temazepam, carbamazepine, venlafaxine, citalopram, sertraline, oxycodone, and hydrocodone ($p < 0.036$) in the Clarks River.

Graphical Abstract

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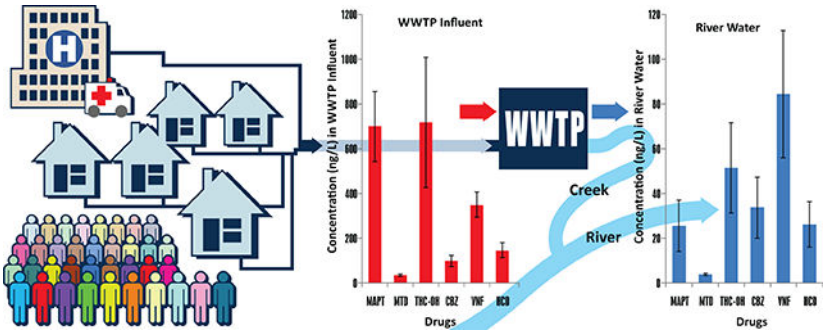
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.03.060.



Keywords

Illicit drugs; Sewage epidemiology; Wastewater; Wastewater treatment plant; River water; Community consumption

1. Introduction

Neuropsychiatric and illicit drug abuse have been an increasing socioeconomic issue on a global scale (Subedi and Kannan, 2014, 2015; Subedi et al., 2013). The abuse and addiction of opioids including fentanyl, heroin, hydrocodone, and oxycodone is a national crisis affecting public health (63.1% of drug overdose deaths) as well as social and economic welfare (economic burden of $78.5 billion in 2013) in the USA (Florence et al., 2016; NIDA, 2017). Approximately 15% of the U.S. population ≥ 12 years of age used illicit drugs including cocaine, heroin, opiates, MDA, and MDMA in 2016 (UNODC, 2016). Recently, abuse and addiction of opioids in the Midwestern USA are considered as major public health issues owing particularly to an elevated rate of heroin seizures (increased by 428% from 2010 to 2013 in KY) (USDEA, 2014). The Midwestern USA is considered a transshipment and distribution hub for Mexican drug trafficking organizations, and are facing epidemic levels of abuse and diversion of drugs including cocaine, heroin, marijuana, and methamphetamine to several other states in the Midwest and Southeast (USDJ, 2011). In the Commonwealth of Kentucky, the Justice and Public Safety Cabinet, deaths due to drug overdose rose by 31% between the years of 2014–2016 and of those deaths in 2016, morphine (a metabolite of heroin) was present in approximately 45% of all cases (KODCP, 2015). In addition, clandestine meth-labs and indoor/outdoor cannabis cultivation have consistently posed a threat of drug abuse in the Midwestern and Southeastern United States (KODCP, 2015).

Community usage of neuropsychiatric and illicit drugs has been estimated conventionally by survey questionnaires or crime statistics that typically underestimate the actual use of drugs in the community. Sewage epidemiology is a rapidly expanding method that utilizes the concentration of target drugs (and/or metabolites) in wastewater influent from centralized WWTPs to back-calculate the community use of drugs. Sewage epidemiology can provide a more comprehensive, real-time, and cost-effective measure of drug abuse in a community as a complement to other conventional methods (Subedi and Kannan, 2014). Many European countries including Italy, Spain, Switzerland, and the UK have successfully utilized sewage

epidemiology to provide an early warning system of new drugs of abuse (Gonzalez-Marino et al., 2016), to identify the effectiveness of new drug treatment and prevention, and identify susceptible areas/populations for policy development (Been et al., 2015; Castiglioni et al., 2014; McCall et al., 2016). Despite the fact that the fundamental concept of using wastewater testing to estimate the community usage of illicit drugs was originated at the U.S. Environmental Protection Agency (USEPA) (Daughton, 2002), it is underused in the USA. Nevertheless, there are several challenges associated with sewage epidemiology approach including significant degradation of drugs in wastewater prior/during analysis, the dynamic population in a community, lack of representativeness sample, and flooding of the WWTP due to excessive rain (Subedi, 2018; van Nuijs et al., 2011).

Following ingestion, pharmaceuticals including controlled neuropsychiatric and illicit drugs are routinely flushed down the drain as residuals, bioconjugates, and their metabolites and reach a centralized wastewater treatment plant (WWTP) (Loganathan et al., 2009; Subedi et al., 2012; Subedi and Kannan, 2014). After treatment, the discharged effluent from a WWTP in this study opens into the Bee Creek and finally discharged into the Clarks River. It has been reported that the treated wastewater effluent can be a major source of residual drugs in the aquatic ecosystem. Contamination of streams, rivers, lakes and other aquatic ecosystems with pharmaceuticals and illicit drugs are of great concern in the recent years due to their harmful biological effects in wildlife as well as in humans (Asimakopoulos and Kannan, 2016; Daughton, 2002; Jones-Lepp et al., 2011; Subedi and Loganathan, 2016).

Chronic exposure of fish to a mixture of psychoactive and illicit drugs reduced the reproductive output, increased DNA fragmentation, and induced gene expression that mimics human expression profiles of an individual diagnosed with the idiopathic autism spectrum disorder (Thomas et al., 2012; Thomas and Klaper, 2012). Not only is the ecological impact of residual drugs in local water sources an alarming matter, but the Clarks River is used for recreational purposes as well as a source of drinking water for the local community. The research on contamination levels of neuropsychiatric and illicit drugs, long-term exposure of the residual drugs in drinking water is limited. Further research in the occurrence of drugs in water sources is vital in understanding the impact of exposure on aquatic life as well as in humans.

In this study, eight illicit drugs (cocaine, amphetamine, methamphetamine, morphine, methadone, MDMA, MDA, and THC), 19 prescribed neuropsychiatric drugs (aripiprazole, quetiapine, lorazepam, alprazolam, diazepam, oxazepam, temazepam, carbamazepine, sertraline, fluoxetine, venlafaxine, citalopram, methylphenidate, codeine, fentanyl, oxycodone, hydrocodone, hydromorphone, buprenorphine), and their five metabolites (benzoylecgonine, norcocaine, cocaethylene, THC-COOH, THC-OH) were determined in wastewater influent, effluent, and surface water samples from the Bee Creek (immediate receiving water body), the upstream Clarks River, and the Clarks River in the Midwestern United States. The per-capita consumption rate of drugs was determined using WBE. Per-capita environmental emission of drugs were determined based on residual levels of drugs in wastewater effluent while a potential source of contamination of drugs in the Clarks River was determined based on the residual levels of drugs in surface water.

2. Materials and methods

2.1. Reagents and chemicals

Standard stock solutions (100 or 1000 ppm) of individual illicit drugs, neuropsychiatric drugs, metabolites, and their corresponding deuterated internal standards were purchased from Cerilliant (Round Rock, TX). Illicit drugs and their metabolites include stimulants [cocaine (CCN), benzoylecgonine (BEG), norcocaine (NCCN), cocaethylene (CCE), amphetamine (AMP), and methamphetamine (MAPT)], opioids [morphine (MPH), methadone (MTD), and 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)], hallucinogens [3,4 – methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), 3,4 – methylenedioxyamphetamine (MDA), (−)-Δ^9-tetrahydrocannabinol (THC), (±)-11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCA), (±)-11-hydroxy-Δ^9-tetrahydrocannabinol (THCOH)]. Neuropsychiatric drugs and their metabolites include antischizophrenics [aripiprazole (APPZ), quetiapine (QTP)], sedatives-hypnotics-anxiolytics [lorazepam (LZP), alprazolam (APZ), diazepam (DZP), oxazepam (OXZ), temazepam (TMZ), carbamazepine (CBZ)], antidepressants [sertraline (SRT), fluoxetine (FLX), venlafaxine (VNF), citalopram (CTP), methylphenidate (MPD)], and opioids [codeine (CDN), fentanyl (FNT), oxycodone (OCD), hydrocodone (HCD), hydromorphone (HMP), buprenorphine (BPN)]. Internal standards include CCN-D_3, BEG-D_8, NCCN-D_3, CCE-D_8, AMP-D_8, MAPT-D_8, MPH-D_6, MTD-D_9, EDDP-D_3, MDMA-D_5, MDEA-D_5, MDA-D_5, THC-D_3, THCA-D_3, THCOH-D_3, APPZ-D_8, QTP-D_8, LZP-D_4, APZ-D_5, DZP-D_5, OXZ-D_5, TMZ-D_5, CBZ-D_{10}, SRT-D_3, FLX-D_6, VNF-D_6, CTP-D_6, MPD-D_9, CDN-D_6, FNT-D_5, OCD-D_6, HCD-D_6, HMP-D_3, BPN-D_4. HPLC grade methanol and formic acid (99.5% purity) were purchased from Fisher Scientific (Hampton, NH). Ultrapure water was prepared using a Barnstead Ultrapure System. All standard solutions were stored at −20 °C.

2.2. Sample collection

Wastewater influent and effluent samples (composited an aliquot every 15 min for 24 h using a time proportional autosampler) were collected from a WWTP. Ort et al. (2010) compare and contrast the limitations of different sampling techniques for the determination of drugs in wastewater (Ort et al., 2010). WWTP treats ~5 million gallons per day (MGD) of wastewater serving ~20,000 people (predominantly a university students' population) in the Southeastern United States. The WWTP receives predominantly (>95%) the domestic wastewater and <5% industrial waste. After the screening of large-size debris and grit removal, wastewater feeds through the vertical loop reactor, the oxidation ditches for an aerobic treatment, clarifiers, UV disinfection, flow down the reaeration ladder, and discharges into the Bee Creek. The Bee Creek primarily carries the wastewater effluents from the WWTP and subsequently opens into the Clarks River (Fig. 1). The influent samples were collected after the screening of large-size debris and grit removal and effluent samples were collected after the treated wastewater flowing down the reaeration ladder. The treated wastewater discharges into the Bee Creek and opens into the Clarks River which subsequently joins the Ohio River (Fig. 1). Surface grab water samples were collected from the Bee Creek (36°37′50″ N, 88°17′35″ W), upstream Clarks River (36°36′46″ N, 88°17′16″ W), and downstream Clarks River (36°39′14″ N, 88°16′46″ W). All samples

were collected for seven consecutive days from October 2 to 8, 2017. All of the samples were collected in 250 mL certified precleaned amber glass bottles and transported to the laboratory at Murray State University on ice. The limitations of sampling collection, storage, and preparation of wastewater samples for pharmaceuticals and illicit drugs are described elsewhere (Baker and Kasprzyk-Hordern, 2011). Upon arrival to the lab, the samples were stored at −20 °C until analysis.

2.3. Sample preparation

All of the samples were allowed to equilibrate to the room temperature, mixed well, centrifuged 100 mL of wastewater and 200 mL for surface water at 4500 rpm (1924 ×*g*) for 5 min, filtered the supernatant through Glass Fiber Filter (1.2 μm pore size) using vacuum filtration. Filtered water samples were spiked with a mixture of internal standards (50–150 ng), mixed well, and extracted using Oasis® HLB solid phase extraction (SPE) cartridges. The cartridges were conditioned with 3 mL of methanol followed by 3 mL ultrapure water (Barnstead™ EASYpure® II UF Ultrapure Water System) prior to extraction. The samples were extracted through the SPE cartridges at a rate of ~1 mL/min. The cartridges were allowed to dry under vacuum and eluted with 4 mL methanol followed by 3 mL of 5% ammonia in methanol. The combined eluate was concentrated to ~250 μL under a gentle stream of nitrogen at room temperature using the Reacti-Vap™ Evaporator. The concentrate was quantitatively transferred to an amber LC vial and the final volume was adjusted to 1 mL with methanol. One and half microliters of prepared samples were injected for HPLC–MS/MS analysis.

2.4. Instrumental analysis

Target analytes were analyzed using Agilent 1290 Infinity II LC System coupled to Agilent 6460 Triple Quadrupole mass spectrometer (Santa Clara, CA). Analytes were separated using methanol and 0.1% aqueous solution of formic acid (Table S1) through a Force Biphenyl column (100 mm × 2.1 mm × 1.8 μm) (Thermo Fisher Scientific, Waltham, MA). Identification of analytes was based on retention time (±0.05 min), quantitative and qualitative *m/z* ion transitions (Table S2) in positive ionization mode, and a relative abundance of qualitative to quantitative ions (±20%). Analytes were quantified based on an isotopic dilution method of quantification. The calibrations curves were prepared by plotting concentration-dependent response factor of each analyte (peak area of analyte divided by peak area of internal standard) versus the response-dependent concentration factor (concentrations of analyte divided by the concentration of internal standard). The regression coefficients (r^2) for five- to ten-point calibration standards calculated by linear or quadratic regression were ≥0.99 for all analytes.

2.5. Quality assurance and quality control

A randomly selected wastewater sample was spiked with target analytes (75–200 ng) and their corresponding internal standards (50–150 ng) for matrix spike (MS) and matrix spike duplicate (MSD) analyses and processed as described in Section 2.3. The average spiking recoveries and RSDs ranged from 85 ± 1.3% (LZP) to 133 ± 4.3% (CBZ); however, average spiking recoveries of CDN, HCD, and THC-OH were 142 ± 1.4%, 145 ± 0.2%, and 164 ± 7.0%, respectively. The reported spiking recoveries are absolute recoveries (i.e. internal

standards were added right before the injection to LC–MS/MS); however, a mixture of internal standards containing isotopic analogs of each drugs and metabolites was spiked in wastewater samples prior extraction. Spiking internal standards prior extraction corrects for any loss of target analytes during sample preparations and instrumental analysis. A continuous calibration verification standard (CCV) solution containing target analytes at approximately the mid-calibration level was analyzed prior and after every ten samples. The average recoveries of analytes were 122%. A method blank was spiked with a mixture of internal standards, prepared accordingly, and analyzed. All analytical data presented here are blank corrected. The limits of detection (LODs) and limits of quantitation (LOQs) were determined as a minimum concentration of analytes that provide a signal to noise ratio ≥ 3 and ≥10, respectively. LODs for analytes ranged from 0.13 to 0.20 ng/mL whereas LOQs ranged from 0.50 to 16 ng/mL of an extract.

2.6. Method validation

An entire analytical method was validated with a triplicate spiking and recovery study on wastewater samples and river surface water samples. Wastewater (100 mL, n = 3) and river water (200 mL, n = 3) were spiked with a mixture of target analytes (75–200 ng) and extracted by the same method described in Section 2.3. A non-spike matrix blank sample of wastewater and river water was also prepared and analyzed. After the eluates were concentrated, triplicate samples were spiked with a mixture of internal standards (50–150 ng) and the final volume was adjusted to 1 mL with methanol. Triplicate recoveries and RSDs in wastewater ranged from 53 ± 6.1% (EDDP) to 114 ± 7.4% (MPD); however, the recoveries of MPH, THC, THCA, and THC-OH were 159 ± 17.2%, 39 ± 74%, 171 ± 9.0%, and 155 ± 9.5%, respectively (Fig. 2). Similarly, triplicate recoveries and RSDs in river water ranged from 50 ± 11.5% (EDDP) to 117 ± 9.2% (FNT); however, the recoveries of FLX, APPZ, THCA, and THC were 47 ± 9.6%, 27 ± 10%, 150 ± 4.8%, and 11 ± 19%, respectively (Fig. 2).

3. Results and discussion

3.1. Occurrence of drugs in wastewater influent

Five of eight illicit drugs including cocaine (110 ± 34.8 ng/L), amphetamine (337 ± 30.7 ng/L), methamphetamine (700 ± 157 ng/L), morphine (91.5 ± 13.2 ng/L), and methadone (36.4 ± 5.7 ng/L) were detected in all wastewater influent samples (Table 1). Benzoylecgonine (424 ± 120 ng/L, a metabolite of cocaine) and THC-OH (718 ± 290 ng/L, a metabolite of THC) were also found in all wastewater influent samples. Similarly, prescribed neuropsychiatric drugs including one of two antischizophrenics (quetiapine), four among six sedatives-hypnoticsanxiolytics (alprazolam, oxazepam, temazepam, and carbamazepine), all of five antidepressants (sertraline, fluoxetine, venlafaxine, citalopram, and methylphenidate), and five of six opioids (codeine, oxycodone, hydrocodone, hydromorphone, and buprenorphine) were also found in all wastewater influent samples (Table 1).

3.2. Community usage of drugs

Mass loading (mg/d) of drugs into the WWTP was determined using Eq. (1) as described in (Subedi and Kannan, 2014) and (Foppe and Subedi, 2018). Briefly, C is the concentration of drugs in wastewater influent (ng/L), F is the daily average flow rate of wastewater influent (L), stability is a factor for the correction of 12-h stability as reported elsewhere (Baker and Kasprzyk-Hordern, 2011). It is important to note that the mass loading of only a few drugs was able to be corrected for their stability in wastewater due to limited literature.

$$Mass\ Load = C \times F \times \left(\frac{100}{100 + Stability}\right) \times \frac{1}{1.0 \times 10^6} \quad (1)$$

The consumption rate of drugs (mg/d/1000 people) in a community was calculated using Eq. (2) as described in (Subedi and Kannan, 2014). The consumption rate of drugs was corrected with the percentage excretion rate from the human body (Postigo et al., 2008). The molar ratio of parent drug (MW_{par}) and the corresponding metabolite (MW_{met}) was used when metabolite was used to determine the consumption rate of parent drugs such as cocaine and THC. The population is the number of inhabitants served by the WWTP.

$$Consumption\ Rate = \text{Mass Load} \times \left(\frac{100}{\text{Excretion}}\right) \times \left(\frac{MW_{par}}{MW_{met}}\right) \times \left(\frac{1000}{\text{Population}}\right) \quad (2)$$

The mass loading of methamphetamine into the WWTP (14,900 mg/d) in this study was ~2 fold higher than that of benzoylecgonine (Table 2). However, the mass loading of benzoylecgonine (6980 mg/d) in a similar size community in Albany, NY was ~250 fold higher than the mass loading of methamphetamine (Subedi and Kannan, 2014). It shows that two similar size communities can also have different use patterns of illicit drugs, which can potentially depends on the demographic location, per-capita income, population dynamicity, and age groups. The Midwestern USA has been considered for the clandestine production of methamphetamine and a transshipment and distribution hub for Mexican drug trafficking organizations. Relatively higher production and use volume of methamphetamine in the Midwestern region than several other parts of the country can have resulted in the higher mass loading of methamphetamine (USDJ, 2011).

The ratio of mass loading of cocaine and benzoylecgonine ranged from 0.22 to 0.38, which suggested that that cocaine and benzoylecgonine measured in wastewater influent is primarily from human consumption of cocaine rather than direct disposal (Table 2). A higher ratio of cocaine and benzoylecgonine (0.85) in wastewater influent from Schiphol Airport, Netherland was described as potential disposal of unused cocaine in sewer system (Bijlsma et al., 2012). The mass loadings of codeine and hydrocodone (~2600 mg/d) were ~2 fold higher than morphine and oxycodone and ~4 fold higher than methadone and hydromorphone. Similarly, mass loading of venlafaxine and citalopram (two major antidepressants) were 2.9–3.8 fold higher than the mass loading of temazepam and carbamazepine (two major sedatives-hypnotics-anxiolytics). The mass loading of prescribed

neuropsychiatric drugs in this study was ~2 to 8 fold higher than the similar size community in Albany, NY (Subedi and Kannan, 2014) except for sertraline (2 fold lower).

The per-capita consumption rate of methamphetamine (1740 mg/d/100 people) and amphetamine (967 mg/d/100 people) found in this study were the highest reported per-capita consumption rates in the USA. Considering 30 mg as a typical dose of methamphetamine (Postigo et al., 2008), the average consumption rate of methamphetamine (1740 mg/d/1000 people) estimated in this study corresponds to 58 doses of methamphetamine per 1000 people in this community. This means that the estimated 5.8% of population in this community consumed methamphetamine in a study week, which is two-fold higher than the United Nation Office on Drugs and Crime's estimation (UNODC, 2017). The consumption rate of methamphetamine was 2–3 fold higher than in the Western and Southern USA (Chiaia et al., 2008) while ~300 fold higher than in a similar size community in Albany, NY (Subedi and Kannan, 2014). It is important to note that the estimated per-capita consumption rates in this study were determined based on the average residual levels of drugs in wastewater influent for only one week period. Several other studies reported the daily and seasonal variations in community consumption of drugs (Huerta-Fontela et al., 2008; Bijlsma et al., 2012). Also, the average per-capita consumption rate of methamphetamine was ~2 fold higher than the consumption rate of cocaine (determined based on the mass loading of a metabolite: benzoylecgonine). Similarly, the consumption rate of cocaine was ~2 fold lower than in a community in the Southern USA (Chiaia et al., 2008) and in Albany, NY (Subedi and Kannan, 2014). Utilizing sewage epidemiology, the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) reported the consumption rate of cocaine (10–1000 mg/d/100 people), amphetamine (5–250 mg/d/1000 people), methamphetamine (10–750 mg/d/1000 people), and MDMA (5–150 mg/d/1000 people) among over 60 European cities in 2016 (EMCDDA, 2017). To our knowledge, this is the first study to estimate the per-capita consumption of THC using sewage epidemiology in the USA. Per-capita consumption of THC was 26,900 mg/d/1000 people using the concentration of its metabolite (THC-OH). Morphine is the most commonly used analgesic in clinical practices including postoperative pain control in the USA. Moreover, Kentucky was one of the eight most opioid prescribing States (>107 prescriptions per 100 people in 2014) in the USA (CDC, 2015). Therefore, the higher consumption rate of morphine (1780 mg/d/1000 people) may suggest the multiple sources of morphine (Table 2). Several prescribed drugs including codeine, ethylmorphine, pholcodine, and nicomorphine transform into morphine.

Per-capita consumption rates of methadone, codeine, and hydrocodone ranged from ~129 to 140 mg/d/1000 people which were 1.3 to 4.6 fold higher than that reported in seven different communities across the USA (Chiaia et al., 2008). Higher per-capita consumption of methadone, hydrocodone, and codeine in this study is consistent with the higher consumption of these opioids in Midwest USA (Subedi and Loganathan, 2016; USDJ, 2011).

3.3. Per-capita environmental emission of drugs via wastewater effluent

The environmental emission (mg/d/1000 people) of neuropsychiatric and illicit drugs through wastewater effluent was determined using Eq. (3) as reported elsewhere (Subedi and Kannan, 2015).

$$Emission\ (mg/d/1000\ people) = C_e \times F \times \left(\frac{1000}{population}\right) \times \frac{1}{10^6} \quad (3)$$

where C_e is the concentration of drugs in ng/L, F is the daily average flow rate of wastewater influent (L).

Four illicit drugs (benzoylecgonine, amphetamine, methamphetamine, and methadone); five sedatives-hypnotics-anxiolytics (lorazepam, alprazolam, oxazepam, temazepam, and carbamazepine); four antidepressants (sertraline, fluoxetine, venlafaxine, and citalopram); and three prescribed opioids (oxycodone, codeine, and hydrocodone) were detected in all wastewater effluent samples (Table 1). Antidepressant venlafaxine was found to have the highest environmental emission from the WWTP (333 ± 160 mg/d/1000 people) followed by citalopram (132 ± 60.2 mg/d/1000 people), methamphetamine (111 ± 43.6 mg/d/1000 people), and hydrocodone (108 ± 90.1 mg/d/1000 people) (Table 2). Per-capita environmental emissions of venlafaxine and citalopram were similar as that reported in similar size community in Albany, NY (Subedi and Kannan, 2015); however, per-capita environmental emission of methamphetamine in this study was six-fold higher.

3.4. Occurrence of drugs in surface water

Bee Creek receives the treated wastewater effluent from the WWTP and opens into the Clarks River. Three illicit drugs (benzoylecgonine, amphetamine, and methamphetamine); five sedatives-hypnotics-anxiolytics (lorazepam, alprazolam, oxazepam, temazepam, and carbamazepine); four antidepressants (sertraline, fluoxetine, venlafaxine, and citalopram); and four prescribed opioids (oxycodone, codeine, hydrocodone, and fentanyl) were detected (1.40 to 243 ng/L) in all samples from the Bee Creek (Table 1). The concentration of select illicit drugs such as methamphetamine ($p = 0.036$) and methadone ($p < 0.001$) as well as the concentration of select prescribed drugs such as alprazolam ($p = 0.031$), oxazepam ($p < 0.001$), temazepam ($p = 0.016$), carbamazepine ($p = 0.002$), venlafaxine ($p = 0.016$), citalopram ($p = 0.031$), sertraline ($p = 0.016$), oxycodone ($p = 0.006$), and hydrocodone ($p = 0.002$) are significantly higher than their concentrations in the downstream Clarks River (Paired *t*-test using SigmaPlot 12.0). Therefore, the Bee Creek as an immediate receiver of treated wastewater is found to be a source of several neuropsychiatric and illicit drugs in the Clarks River (Table 1).

4. Conclusions

Neuropsychiatric pharmaceuticals and illicit drugs are emerging environmental contaminants of concern. Very limited studies have been conducted dealing with their characterization, sources, levels, biological effects and epidemiological issues. In this study, an analytical method capable of simultaneous analysis of eight illicit drugs, nineteen prescribed drugs, and their five metabolites was developed and validated. Twenty-six

neuropsychiatric drugs, illicit drugs, and their three metabolites were detected in wastewater. The source of residual cocaine and benzoylecgonine in wastewater is found primarily from human consumption of cocaine rather than direct disposal. WBE is utilized to estimate the community usage of drugs based on the concentration of drug residues in wastewater, wastewater inflow, and the population served by the centralized wastewater treatment plant. The per-capita consumption rate of methamphetamine (1740 mg/d/100 people) and amphetamine (967 mg/d/100 people) found in this study were the highest reported per-capita consumption rates in the USA. Antidepressant venlafaxine was found to have the highest environmental emission from the WWTP (333 ± 160 mg/d/1000 people) followed by citalopram (132 ± 60.2 mg/d/1000 people), methamphetamine (111 ± 43.6 mg/d/1000 people), and hydrocodone (108 ± 90.1 mg/d/1000 people). Bee Creek, an immediate receiving water body, is found to be a source of several neuropsychiatric and illicit drugs including methamphetamine, methadone, alprazolam, oxazepam, temazepam, carbamazepine, venlafaxine, citalopram, sertraline, oxycodone, and hydrocodone ($p < 0.036$) in the Clarks River. Moreover, the mass discharge of illicit and neuropsychiatric drugs into the environment (as demonstrated in this study) may warrant design or operational considerations for wastewater treatment plants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Sewage epidemiology was utilized to determine community use rate of drugs.
- Per-capita consumption of methamphetamine and amphetamine was the highest ever reported in the USA.
- Codeine and hydrocodone were the most consumed prescription opioids.
- Venlafaxine and citalopram were discharged at the highest rate from the WWTP to the adjacent creek.
- Wastewater effluent found to be a source of drugs in the receiving creek and river

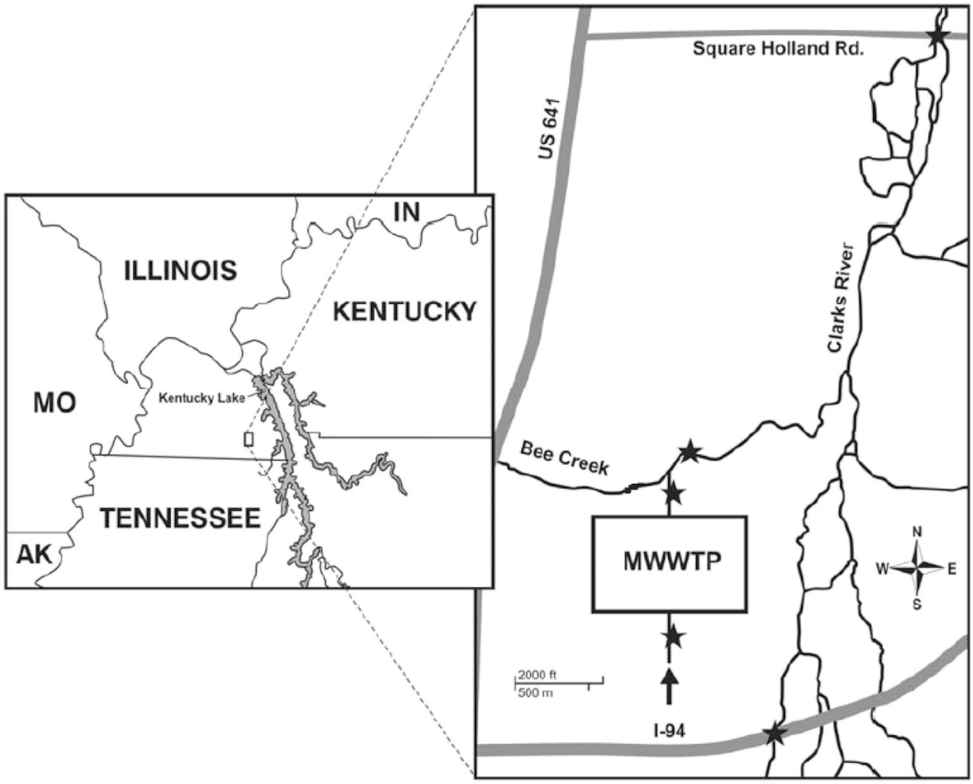

Fig. 1.

Sampling sites (★) at wastewater treatment plant, the Bee Creek, and the Clarks River. (Figure is modified from Loganathan et al. (2009).)

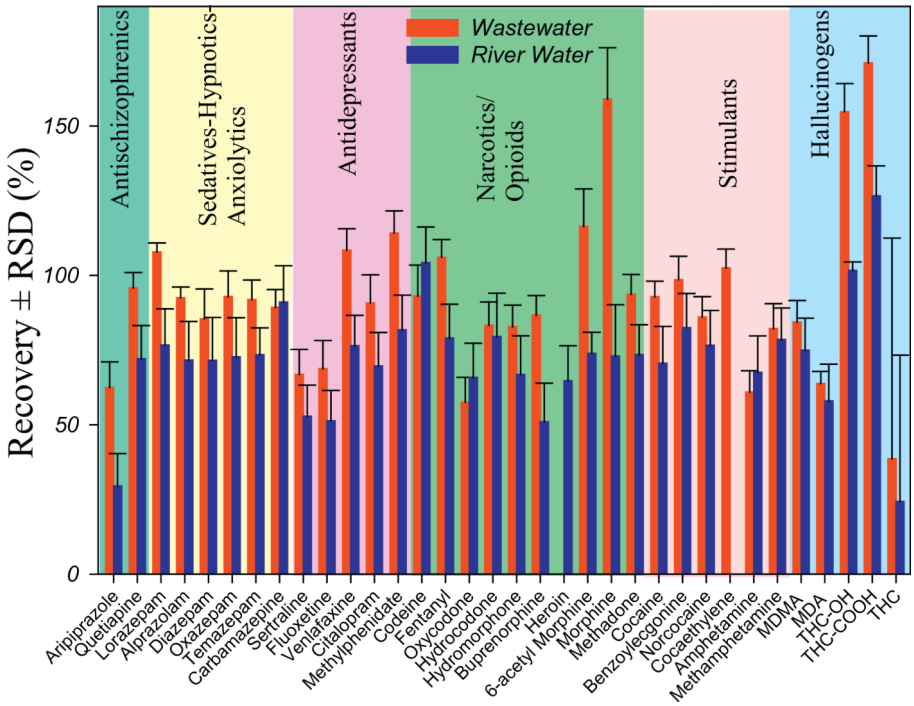

Fig. 2.

Percentage recoveries (n=3) of spiked analytes in wastewater and river water.

Table 1

Concentration of illicit drugs, neuropsychiatric drugs, and their metabolites (ng/L) in a centralized wastewater treatment plant, immediate connecting creek, and a receiving river in a community in the Midwestern United States.

Analytes	Mean concentration ± StDev in WWTP		Mean concentration ± StDev in surface water		
	Influent	Effluent	Bee Creek	Clarks River upstream	Clarks River downstream
Stimulants					
Cocaine (CCN)	110 ± 34.8	19.4 ± 12.6	3.40 ± 0.8	2.50 ± 0.2	<LOQ
Benzoylecgonine (BEG)	424 ± 120	31.5 ± 14.5	14.2 ± 10.0	2.40 ± 0.1	6.30 ± 1.4
Norcocaine (NCCN)	<LOQ	12.7 ± 16.3	3.70 ± 1.0	4.40 ± 0.9	3.90 ± 0.7
Amphetamine (APT)	337 ± 30.7	21.8 ± 18.1	5.10 ± 2.6	2.50 ± 0.2	<LOQ
Methamphetamine (MAPT)	700 ± 157	125 ± 32.8	86.4 ± 64.3	2.70 ± 0.3	25.7 ± 11.5
Opioids/narcotics					
Morphine (MPH)	91.5 ± 13.2	43.0 ± 39.3	6.2 ± 1.1	<LOQ	<LOQ
Methadone (MTD)	36.4 ± 5.7	25.2 ± 12.6	17.8 ± 2.3	2.4 ± 0.2	3.9 ± 0.6
Hallucinogens					
MDMA	<LOQ	<LOQ	6.1 ± 0.3	<LOQ	<LOQ
MDA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
THC	ND	ND	ND	ND	ND
THCA	ND	ND	ND	ND	ND
THC-OH	718 ± 290	<LOQ	339 ± 403	97.8 ± 54.8	51.6 ± 20.1
Antischizophrenics					
Aripiprazole (APPZ)	ND	<LOQ	5.1 ± 1.3	8.3 ± 4.3	7.0 ± 2.9
Quetiapine (QTP)	41.9 ± 7.4	13.3 ± 15.6	4.4 ± 1.8	<LOQ	<LOQ
Sedatives-hypnotics-anxiolytics					
Lorazepam (LZP)	30.4 ± 6.0	28.1 ± 11.5	15.8 ± 3.9	ND	ND
Alprazolam (APZ)	8.30 ± 2.4	11.6 ± 9.9	6.10 ± 2.4	<LOQ	2.40 ± 0.8
Diazepam (DZP)	4.80 ± 0.3	15.0 ± 15.2	6.10 ± 1.9	2.60 ± 0.2	3.00 ± 0.2
Oxazepam (OXZ)	54.1 ± 6.3	59.7 ± 12.5	34.5 ± 8.4	<LOQ	22.5 ± 4.5
Temazepam (TMZ)	89.4 ± 10.4	87.2 ± 11.9	60.9 ± 22.2	3.40 ± 1.0	28.7 ± 9.4
Carbamazepine (CBZ)	99.1 ± 26.0	99.7 ± 15.5	63.1 ± 24.6	3.80 ± 0.5	33.8 ± 13.6
Antidepressants					

Analytes	Mean concentration ± StDev in WWTP		Mean concentration ± StDev in surface water		
	Influent	Effluent	Bee Creek	Clarks River upstream	Clarks River downstream
Sertraline (SRT)	54.9 ± 19.7	24.5 ± 14.7	24.2 ± 10.2	6.10 ± 1.1	3.80 ± 1.5
Fluoxetine (FLX)	31.5 ± 10.5	13.4 ± 11.8	9.60 ± 2.9	3.50 ± 0.9	<LOQ
Venlafaxine (VNF)	350 ± 56.3	362 ± 21.6	243 ± 97.3	2.60 ± 0.6	84.5 ± 28.5
Citalopram (CTP)	278 ± 50.7	144 ± 16.1	95.1 ± 43.5	3.80 ± 0.0	4.90 ± 1.2
Methylphenidate (MPD)	10.7 ± 2.7	<LOQ	3.90 ± 1.2	2.70 ± 0.4	<LOQ
Prescribed opioids					
Codeine (CDN)	148 ± 53.4	27.9 ± 26.8	34.4 ± 11.5	<LOQ	4.20 ± 0.3
Fentanyl (FNT)	1.60 ± 0.1	19.9 ± 26.1	1.40 ± 0.6	1.50 ± 0.5	<LOQ
Oxycodone (OCD)	72.2 ± 22.6	38.1 ± 16.8	27.0 ± 8.6	2.90 ± 2.1	10.3 ± 2.0
Hydrocodone (HCD)	147 ± 34.2	107 ± 27.0	126 ± 57.5	5.00 ± 0.6	26.2 ± 10.1
Hydromorphone (HMP)	42.4 ± 7.6	21.8 ± 22.7	9.10 ± 3.1	<LOQ	<LOQ
Buprenorphine (BPN)	13.9 ± 2.5	<LOQ	<LOQ	<LOQ	<LOQ

ND: non-detects; <LOQ: less than limit of quantitation.

Table 2

Mass loading of illicit drugs, neuropsychiatric drugs, and their metabolites (mg/d) into the WWTP, their community usage, and environmental emission in the Midwestern United States.

Analytes	Mass loading (mg/d)	Per-capita consumption rate (mg/d/1000 people)	Per-capita emission via effluent (mg/d/1000 people)
Stimulants			
Cocaine (CCN)	2250 ± 1630	938 ± 706[a]	25.9 ± 28.2
Benzoylecgonine (*BEG*)	8040 ± 6050	N/A	30.4 ± 23.1
Norcocaine (*NCCN*)	244	N/A	18.9 ± 32.8
Amphetamine (APT)	5800 ± 2730	967 ± 455	20.7 ± 27.1
Methamphetamine (MAPT)	14,900 ± 10,200	1740 ± 1190	111 ± 43.6
Opioids/narcotics			
Morphine (MPH)	1170 ± 667	1780 ± 1020	71.5 ± 85.0
Methadone (MTD)	733 ± 459	131 ± 81.9	27.7 ± 31.7
Hallucinogens			
THC-OH	11,300 ± 35,000	26,900 ± 8310[b]	100
Antischizophrenics			
Quetiapine (QTP)	789 ± 481	39.4 ± 24.1	20.1 ± 32.1
Sedatives-hypnotics-anxiolytics			
Lorazepam (LZP)	437 ± 73.3	21.9 ± 3.67	29.7 ± 30.9
Alprazolam (APZ)	158 ± 109	7.92 ± 5.47	14.2 ± 21.5
Diazepam (DZP)	122 ± 62	6.09 ± 3.08	21.6 ± 32.4
Oxazepam (OXZ)	1070 ± 579	53.7 ± 29.0	59.4 ± 46.2
Temazepam (TMZ)	1800 ± 1060	89.8 ± 53.1	83.9 ± 54.6
Carbamazepine (CBZ)	1940 ± 1470	97.0 ± 73.4	97.0 ± 67.1
Antidepressants			
Sertraline (SRT)	1120 ± 1040	56.2 ± 51.9	27.6 ± 34.5
Fluoxetine (FLX)	670 ± 583	33.5 ± 29.2	16.5 ± 25.3
Venlafaxine (VNF)	7010 ± 4260	351 ± 213	333 ± 160
Citalopram (CTP)	5260 ± 3300	263 ± 165	132 ± 60.2
methylphenidate (MPD)	194 ± 93	9.70 ± 4.63	72.1

Analytes	Mass loading (mg/d)	Per-capita consumption rate (mg/d/1000 people)	Per-capita emission via effluent (mg/d/1000 people)
Prescribed opioids			
Codeine (CDN)	2800 ± 2550	140 ± 127	35.1 ± 56.6
Fentanyl (FNT)	25.0 ± 2.0	1.27 ± 0.10	36.1 ± 49.7
Oxycodone (OCD)	1250 ± 767	62.7 ± 38.4	40.7 ± 43.2
Hydrocodone (HCD)	2580 ± 1730	129 ± 86.3	108 ± 90.1
Hydromorphone (HMP)	656 ± 411	32.8 ± 20.5	31.7 ± 48.0
Buprenorphine (BPN)	257 ± 178	12.8 ± 8.92	91.0

[a]Concentration of benzoylecgonine was used to determine the consumption rate of cocaine.

[b]Is the consumption rate of THC that was determined based on the concentration of THC-OH. The correction factors for the percentage excretion rates from human body were taken from Postigo et al. (2008) as benzoylecgonine (45%), amphetamine (30%), methamphetamine (43%), morphine (4.2%), methadone (28%), and THCOH (2%).