Contaminants of Emerging Concern in Wastewaters in Barbados, West Indies

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ABSTRACT

Contaminants of emerging concern (CECs), including pharmaceuticals and drugs of abuse have been detected in wastewater worldwide, but there have been few reports in the peer-reviewed literature on the levels of CECs in municipal wastewater from the Caribbean region. In this study of wastewater collected from two wastewater treatment plants in Barbados, caffeine and ibuprofen were detected at µg/L concentrations, whereas two steroid hormones (i.e. androstenedione, estrone) and several prescription pharmaceuticals were detected at ng/L concentrations. Among drugs of abuse, benzoylecgonine (i.e. metabolite of cocaine), MDMA (i.e. Ecstasy) and MDA (i.e. 3,4-methylenedioxyamphetamine) were present at the highest concentrations in untreated wastewater. Overall, these data show that there is potential impact in the marine environment in Barbados as a result of the discharge of CECs into the coastal zone.

Keywords: *Caffeine; pharmaceuticals; drugs of abuse; wastewater; Barbados; West Indies*

Numerous studies conducted throughout the world have shown that contaminants of emerging concern (CECs), including pharmaceuticals, drugs of abuse and steroid hormones are present in domestic wastewater and are released into surface waters (Luo et al, 2014), including discharges into the marine environment (Arpin-Pont et al., 2016). In the Caribbean region, most domestic wastewater is treated by on-site septic systems or discharged into open pits, and less than 15% of populations are connected to wastewater treatment plants (UNEP, 2004). This is also the case on the Caribbean island of Barbados, where approximately 14% of the sewage produced by the population of about 300,000 people is treated in wastewater treatment plants (Nurse et al., 2012).

There are two wastewater treatment plants (WWTPs) operating in Barbados. The Bridgetown Sewerage System services about 12% of the town of Bridgetown (population 120,000), and the sewage is collected and treated in a secondary treatment plant and then discharged into the marine environment through a short outfall. The South Coast Sewerage System has approximately 3,000 connections and sewage is treated in a primary WWTP and again discharged into the marine environment. A number of hotels use "package" sewage treatment plants for on-site sewage treatment before discharge into the marine environment. Discharges of domestic wastewater into the marine environment are a threat to marine organisms, including coral reefs (Mora, 2008). Pharmaceuticals and other contaminants of emerging concern (CECs) present in wastewater may contribute to impacts on marine ecosystems (Prichard and Granek, 2016).

The objective of the present study was to determine if domestic wastewater in Barbados, West Indies is contaminated with CECs. We monitored wastewater for several classes of CECs, including caffeine, pharmaceuticals, drugs of abuse and steroid hormones. Grab samples of untreated and treated wastewater were collected from the two WWTPs operating on the island

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during two sampling periods in 2013, in February during the high tourism season, and in June during the tourism off-season. These samples were extracted using solid phase extraction (SPE) cartridges and analyzed for caffeine, pharmaceuticals and estrogen hormones by liquid chromatography with tandem mass spectrometry (LC-MS/MS) and for drugs of abuse by liquid chromatography with high resolution mass spectrometry (LC-HRMS).

METHODS AND MATERIALS

Wastewater samples were collected from the WWTP located in the city of Bridgetown (i.e. WWTP 1) and the WWTP on the South Coast (i.e. WWTP 2) of Barbados. WWTP 1 services about 1,500 sewer connections, which are mainly from commercial establishments and WWTP 2 services about 3,000 connections, which are mainly from private residences and hotels. The sewage flows in WWTP 1 are typically between 9,500 and 11,300 m^3/d and the treatment technology is an activated sludge system. However, this plant is >30 years old and is poorly maintained (Nurse et al., 2012). Sewage flows in WWTP 2 are typically between 2,600 and 3,800 m^3/d and this is a primary treatment plant that serves an area along the south coast of the island where there are many hotels and night entertainment establishments. For both WWTPs, treated wastewater is discharged through short outfalls into the marine environment. The high tourist season in Barbados extends from the month of December to April, which contributes to the maximum use of water resources during this period of the year (Cashman and Moore, 2012).

For analysis of pharmaceuticals in treated and untreated wastewater, sampling at WWTP 1 and WWTP 2 took place in February and June of 2013. For analysis of drugs of abuse, only samples of untreated wastewater were collected, and these samples were collected only in June of 2013. During sampling campaigns, grab samples (1 L) of treated wastewater (i.e. effluent) and untreated wastewater (i.e. influent) were collected in amber glass bottles at both WWTPs and immediately transported to the University of the West Indies, Cave Hill Campus and frozen. Two days after collection, the frozen samples were transported by air freight in a cooler with ice packs to Trent University in Peterborough, ON, Canada. On arrival at Trent University, 3 days after shipment from Barbados, the thawed samples were stored overnight in the dark at 4°C and they were extracted the following morning.

The compounds listed in Table 1 were selected as analytes. Isotopically labeled surrogates were added as internal standards (IS) to aid in quantification by compensating for matrix effects (Table 1). The pharmaceuticals, as well as androstenedione and estrone were all purchased from Sigma Aldrich (Oakville, ON, Canada). The stable isotope surrogates for the analytes were purchased from either CDN Isotopes (Pointe-Claire, QC, Canada) or Cambridge Isotopes (Tewksbury, MA, USA). HPLC grade solvents, as well as high purity sodium hydroxide, hydrochloric acid, formic acid and ammonium hydroxide were purchased from Fisher Scientific (Ottawa, ON, Canada). Oasis MAX (6cc. 200 mg) and Oasis MCX (6cc. 200 mg) solid phase extraction cartridges were purchased from Waters Corporation (Milford, MA, USA).

Aliquots (100 mL) of wastewater were extracted by concentrating the analytes onto SPE cartridges. The field samples were thawed and filtered through a glass-fiber filter (mesh size 1.0 μm). Caffeine, pharmaceuticals and the steroid hormones were extracted using Oasis MAX anion exchange cartridges, as described previously by Li et al. (2010). The drugs of abuse were extracted using Oasis MCX cation exchange cartridges, as described previously for illicit drugs (Metcalfe et al., 2010) and modified to include opioid drugs (Yargeau et al., 2014). The extracts were evaporated to near dryness and reconstituted in 0.4 mL methanol for analysis. All samples were extracted and analyzed in triplicate (n =3). Procedural blanks with deionized (MilliQ) water were extracted simultaneously with field samples.

Table 1. Target compounds analyzed in wastewater samples from Barbados and their internal standards. Notations include abbreviation, molecular weight and uses of each target compound.

Compound	**MW (g/mol)**	**Internal Standard**	**Uses**
Pharmaceuticals			
Caffeine	194.2	Caffeine-$^{13}C_3$	Stimulant
Gemfibrozil	151.2	Gemfibrozil-d_6	Hypolipidemic
Ibuprofen	206.3	Ibuprofen-$^{13}C_3$	Analgesic
Carbamazepine	236.3	Carbamazepine-d_{10}	Anticonvulsant
Trimethoprim	290.3	Trimethoprim-$^{13}C_3$	Antibiotic
Sulfamethoxazole	253.3	Sulfamethoxazole-$^{13}C_3$	Antibiotic
Steroid hormones			
Androstenedione	286.4	Androstenedione-d_3	Natural androgen
Estrone	270.4	Estrone-$^{13}C_2$	Natural estrogen
Drugs of Abuse			
Amphetamine	135.2	Amphetamine-d_5	Illicit drug
Methamphetamine	149.2	Methamphetamine-d_5	Illicit drug
Methylene dioxyamphetamine (MDA)	179.2	MDA-d_5	Illicit drug
Ecstasy (MDMA)	193.3	MDMA-d_5	Illicit drug
Ephedrine	165.2	Ephedrine-d_3	Decongestant
Benzoylecgonine	289.3	Benzoylecgonine -d_3	Cocaine metabolite
Cocaine	303.4	Cocaine-d_3	Illicit drug
Morphine	285.3	Morphine-d_3	Prescription opioid
Dihydrocodeine	301.4	Dihydrocodeine-d_6	Prescription opioid
Codeine	299.4	Codeine-d_3	Prescription opioid
Oxycodone	315.4	Oxycodone-d_3	Prescription opioid
Ketamine	237.7	Ketamine-d_4	Veterinary anesthetic
Tramadol	263.4	Tramadol-$^{13}C_1$	Prescription opioid
Methadone	309.4	Methadone-d_9	Prescription opioid
EDDP	277.4	EDDP-d_3	Methadone metabolite
Fentanyl	336.5	Fentanyl-d_5	Prescription opioid

Caffeine, pharmaceuticals and steroid hormones were analyzed by high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) operated with an electrospray ionization source, essentially as described by Metcalfe et al. (2014). All analyses were conducted using an Agilent 1100 HPLC coupled to a 5500 QTrap mass spectrometer purchased from Applied Biosystems Sciex (Concord, ON, Canada) operated in either positive or negative ion mode, depending on the analyte. Detection was by Multiple Reaction Monitoring (MRM) of the precursor and product ion transitions. An external standard method with an eight-point calibration curve was used for quantification, and the data were adjusted according to response for the internal standards. The analysis of drugs of abuse was conducted as described by Rodayan et al. (2016) by ultra-high performance liquid chromatography with high resolution mass spectrometry (LC-HRMS) operated with electrospray ionization. The LC-HRMS was an Accela LC system coupled to LTQ Orbitrap XL instrument purchased from Thermo Fisher Scientific (Waltham, MA, USA). Quantification was performed using an internal standard method with an eight-point calibration curve spanning the range of the anticipated analyte concentrations in field samples. An area ratio (Area of compound/Area of internal standard) linear regression was performed. The stable isotopes surrogate internal standards were used to compensate for matrix-induced signal suppression and recoveries.

RESULTS AND DISCUSSION

The concentrations of caffeine, pharmaceuticals and steroid hormones detected in treated and untreated wastewater in February and June of 2013 are listed in Table 2 for WWTP 1 (i.e. Bridgetown) and WWTP 2 (i.e. South Coast). None of the target analytes were detected in procedural blank samples. The concentrations of caffeine, pharmaceuticals and steroid hormones in untreated wastewater (i.e. influent) were higher at the South Coast plant (WWTP 2) relative to the Bridgetown plant (WWTP 1) during both the February and June sampling campaigns. WWTP 1 serves about one-eighth of the capital city of Bridgetown, but the majority of the wastewater collected is from commercial enterprises. In contrast, WWTP 2 collects wastewater from many residences and hotels, so higher concentrations in wastewater probably reflect higher domestic inputs of the CECs in this area. Rainfall may also have affected the monitoring results, as the untreated wastewater at WWTP 1 flows through a primary clarifier that is open to dilution from rainfall, whereas the primary clarifier for WWTP 2 is located within an enclosed space.

Table 2. Mean ± SD (n=3) concentrations (ng/L) of CECs in wastewater collected from WWTP 1 and WWTP 2 in Barbados during February and June, 2013. < LOQ = less than limit of quantitation.

Compound	**WWTP 1**		**WWTP 2**	
	Mean (±SD) concentration in influent (ng/L)	Mean (±SD) concentration in effluent (ng/L)	Mean (±SD) concentration in influent (ng/L)	Mean (±SD) concentration in effluent (ng/L)
February				
Androstenedione	< LOQ	< LOQ	< LOQ	< LOQ
Estrone	< LOQ	< LOQ	< LOQ	< LOQ
Caffeine	112 ± 9	167 ± 11	430 ± 15	351 ± 10
Carbamazepine	0.4 ± 0.01	1.6 ± 0.03	0.5 ± 0.02	0.5 ± 0.01
Sulfamethoxazole	< LOQ	< LOQ	< LOQ	< LOQ
Trimethoprim	9.8 ± 0.6	8.5 ± 0.1	16 ± 1	16 ± 1
Gemfibrozil	0.3 ± 0.01	0.3 ± 0.02	0.1 ± 0.01	0.1 ± 0.01
Ibuprofen	2.0 ± 0.2	1.4 ± 0.1	9 ± 1	12 ± 1
June				
Androstenedione	27 ± 4	< LOQ	82 ± 4	< LOQ
Estrone	13 ± 1	< LOQ	21 ± 1	21 ± 1
Caffeine	1,281 ± 16	1,313 ± 20	18,967 ± 575	2,359 ± 109
Carbamazepine	15 ± 1	0.9 ± 0.01	364 ± 18	26 ± 12
Sulfamethoxazole	3.1 ± 0.3	1.9 ± 0.2	8.6 ± 0.5	1.2 ± 0.4
Trimethoprim	6.1 ± 0.4	2.8 ± 0.7	59 ± 1	1.4 ± 0.2
Gemfibrozil	20 ± 2	20 ± 2	53 ± 1	56 ± 1
Ibuprofen	675 ± 21	84 ± 8	1,765 ± 57	1,479 ± 18

At both WWTPs, the concentrations of the target analytes in influent and effluent were higher in the June sampling campaign than the February sampling campaign (Table 2). The tourist season in Barbados generally extends from mid-December to mid-April, so it was expected that concentrations of all target compounds would be higher during the February sampling period when tourism increases the population on the island. However, maximum water usage in

Barbados also occurs during the high tourist season (Cashman and Moore, 2012), so it may be that higher sewage flows during February were compensating the increased use/excretion of CECs in wastewater. Grab sampling is not an optimal method for monitoring CECs in wastewater because of the high temporal variability in the concentrations of these compounds in wastewater. Sampling of treated and untreated wastewater should also take into account the fact that the daily CEC load in the influent is distributed over multiple days in the effluent (Baalbaki et al., 2017). Therefore, no attempt was made to estimate removals of the target compounds from the data on the relative concentrations in influent and effluent.

Caffeine was present at elevated concentrations at both WWTPs, with a maximum mean concentration of about 19 μg/L in June in the influent at WWTP 2 (Table 2). This stimulant is consumed daily in many foods and beverages, and we previously detected this compound at high concentrations in untreated wastewater from Barbados (Edwards et al., 2015). Several studies have shown that secondary and tertiary treatment plants are very effective at removing caffeine from wastewater (Yang et al. 2011), but caffeine was still present at elevated concentrations in the effluents of both WWTPs (Table 2). The over-the-counter analgesic/anti-inflammatory drug, ibuprofen was also present at elevated concentrations in wastewater collected in June at both WWTPs (Table 2). The other target analytes were typically present at low ng/L concentrations in influent and lower concentrations in the effluent (Table 2). The concentrations of caffeine and pharmaceuticals detected in wastewater in Barbados were consistent with concentrations that have been reported in other studies (Martin et al., 2012; Luo et al., 2014). The steroid androgen, androstenedione and the steroid estrogen, estrone are intermediate compounds in steroidogenesis that terminates in synthesis of testosterone and estradiol. These compounds were detected in samples of influent collected in February, but at concentrations < LOQ. Both steroids were quantified in June samples of influent (Table 2). The concentrations detected in influent samples in June are consistent with the concentrations below limits of quantitation to 50 ng/L that have been previously reported for these steroids in wastewater (Velicu, 2009). Wise et al. (2011) suggested that activated sludge treatment is very effective in eliminating steroids, primarily because the intermediate log K_{ow} values of 2 - 3.4 for these compounds favour sorption onto sludge. This may explain why these compounds were not detected in the effluent of WWTP 1 (Table 2).

The release of antibiotics in wastewater may be the most serious concern because of the potential for the development of antimicrobial resistance in the environment. In the present study, the target analytes included the antibiotics, sulfamethoxazole and trimethoprim, which are often prescribed in combination (Yang et al., 2011). In WWTPs in Europe and North America, these compounds have been detected in untreated wastewater at concentrations as high as 1.3 μg/L (Lindberg et al. 2005; Martin et al., 2014), but relatively low concentrations (i.e. < 60 ng/L) of these two antimicrobial medications were detected in untreated wastewater in Barbados (Table 2).

The mean concentrations of the selected drugs of abuse measured in untreated wastewater collected in June from WWTP 1 and 2 are summarized in Table 3. All 16 of the targeted analytes were detected in wastewater in Barbados. For most drugs, similar concentrations of the targeted analytes were measured in wastewater from the WWTPs servicing both Bridgetown (i.e. WWTP 1) and the South Coast (i.e. WWTP 2). Note that it is possible to use data on the levels of drugs of abuse in untreated wastewater to estimate community drug consumption (Zuccato et al., 2008). However, because grab sampling is considered an inadequate approach for making these estimates, we did not attempt to estimate drug consumption for the Barbados communities serviced by the two WWTPs. In addition, we did not have accurate estimates of the sizes of the populations served by the WWTPs.

The data generated for cocaine should be interpreted with caution, as cocaine is relatively unstable in aqueous matrices and degradation of this compound may have occurred during shipment of wastewater from Barbados to Canada. However, the primary metabolite of cocaine, benzoylecgonine is more stable. Humans receiving a dose of cocaine excrete approximately 45% as this metabolite and about 5% as the parent drug (Zuccato et al. 2008). The relative concentrations of cocaine to benzoylecgonine in untreated wastewater from WWTP 1 approximate this ratio, but the concentrations of these two compounds were approximately equal in untreated wastewater from WWTP 2 (Table 3). Equal concentrations of cocaine and benzoylecgonine observed in wastewater have been interpreted as evidence of direct inputs of cocaine into the sewer system, rather than excretion from individuals dosed with cocaine (Yargeau et al., 2014). However, the concentrations of cocaine and benzoylecgonine in wastewater from Barbados were orders of magnitude lower than the µg/L concentrations detected in untreated wastewater in Martinique, Caribbean; a known transit area for cocaine from South America through the Caribbean to North America and Europe (Devault et al., 2014).

Table 3. Mean (± SD) concentrations (ng/L) of drugs of abuse measured in untreated wastewater collected at two WWTPs in Barbados in June, 2013. < LOQ = below limit of quantification; EDDP = Methadone metabolite, MDMA = Ecstasy, MDA = 3,4-methylenedioxyamphetamine.

Drugs of Abuse	**Mean concentration (ng/L)** ± SD	
	WWTP 1	**WWTP 2**
Amphetamine	< LOQ	< LOQ
Methamphetamine	< LOQ	< LOQ
Cocaine	4 ± 0.2	15 ± 1.1
Benzoylecgonine	49 ± 5	18 ± 0.5
MDMA (Ecstasy)	42 ± 5	18 ± 1.3
MDA	29 ± 0.1	29 ± 0.1
Morphine	3 ± 0.01	4 ± 0.2
Methadone	5 ± 0.6	7 ± 0.7
EDDP	70 ± 11	31 ± 1
Codeine	2 ± 0.1	9 ± 0.5
Dihydrocodeine	6 ± 0.4	9 ± 0.3
Ephedrine	7 ± 1.8	23 ± 3
Tramadol	34 ± 1.2	< LOQ
Oxycodone	5 ± 1.1	4 ± 0.1
Ketamine	4 ± 0.1	5 ± 0.7
Fentanyl	22 ± 2	55 ± 4

The amphetamine compound, 3,4-methylenedioxyamphetamine (MDA) was detected at mean concentrations of 29 ng/L in untreated wastewater from both WWTPs in Barbados (Table 3). In addition, MDMA (Ecstasy) was detected in untreated wastewater from both WWTPs. MDA is rarely used as a recreational drug compared to other amphetamines, but it is a primary metabolite of MDMA and is often a contaminant of illicit MDMA products, so this could be the primary source of MDA in the wastewater in Barbados. Amphetamine and methamphetamine were detected at both WWTPs, but at concentrations below the quantification limit, which signifies low usage in Barbados.

Several opioid drugs were detected in wastewater from Barbados. The abuse of prescription opioid painkillers, including fentanyl, oxycodone and tramadol is a growing problem in Europe and North America (Thomas et al. 2012), and these narcotics may also be a problem of abuse in Barbados. However, it cannot be ruled out that these opioids originate from legitimate use as prescription painkillers. Several opioid drugs, such as tramadol, were present at higher concentrations at WWTP 1. This trend may be due to the fact that this plant receives wastewater from the major hospital on the island, which is likely to discharge opioid drugs into the sewage system because of medicinal use. The mean concentrations of fentanyl in untreated wastewater in Barbados of 22 and 55 ng/L were lower than the estimated concentration of 155 ng/L of fentanyl detected in untreated wastewater in an WWTP sampled in Ontario, Canada in 2013 (Yargeau et al., 2014). However, this opioid was detected at very low concentrations (i.e. < LOQ to 1 ng/L) in untreated wastewater collected in 2015 from WWTPs in Adelaide, Australia (Tscharke et al., 2016). The world is experiencing a crisis in the illicit use of fentanyl, and analysis of wastewater can be a useful tool in monitoring temporal and geographic trends for this dangerous opioid drug.

The two WWTPs in Barbados discharge treated wastewater through relatively short submarine outfalls into the nearshore coastal zone, so pharmaceuticals and other CECs of wastewater origin are released into the marine environment. There is evidence that pharmaceuticals, personal care products and steroid hormones can be present at elevated concentrations in the marine environment (Arpin-Pont et al., 2016), and particularly in embayments where water circulation is limited (Singh et al., 2010). Therefore, it would be beneficial to monitor the marine environment in Barbados to evaluate the levels of CECs in coastal waters.

Overall, a range of pharmaceuticals, drugs of abuse, steroid hormones and caffeine were detected in wastewater collected from the two WWTPs that treat municipal sewage in Barbados, WI. The concentrations of these CECs were generally in the ng/L range, but concentrations of caffeine and ibuprofen were in the μg/L range in some samples of treated and untreated wastewater. In general, the concentrations of the target compounds were higher during the June sampling campaign, which may reflect greater dilution of wastewater as a result of higher water use during the high tourist season (i.e. December to April). Among the drugs of abuse analyzed in untreated wastewater, cocaine and its primary metabolite, benzoylecgonine, as well as MDA and MDMA (Ecstasy) were present at the highest concentrations. It is notable that the powerful opioid, fentanyl was detected in untreated wastewater, and the data reported in the present study may be a valuable baseline for future studies of community use of this and other drugs of abuse in Barbados. In order to provide accurate estimates of community drug use in Barbados and other small island developing states in the region, information is required on the number of people served by each WWTP and the daily wastewater flows.

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References

Arpin-Pont L, Martinez Bueno MJ, Gomez E, Fenet, H (2016). Occurrence of PPCPs in the marine environment: A review. Environ Sci Pollut Res 23:4978-4991.

Baalbaki Z, Sultana T, Maere T, Vanrolleghem P, Metcalfe CD, Yargeau V (2016) Fate and mass balance of contaminants of emerging concern during wastewater treatment determined using the fractionated approach. Sci Total Environ 573:1147-1158.

Cashman A, Moore W (2012) A market-based approach for encouraging water use efficiency in a tourism based economy. Int J Hospit Manag 31: 286-294.

Devault AD, Néfau T, Pascaline H, Karolak S, Levi Y (2014) First evaluation of illicit and licit drug consumption based on wastewater analysis in Fort de France urban area (Martinique, Caribbean), a transit area for drug smuggling. Sci Total Environ 490:970-978.

Edwards QA, Kulikov KM, Garner-O'Neale LD (2015) Caffeine in surface and wastewaters in Barbados, West Indies. SpringerPlus 4:1-12.

Edwards QA, Kulikov KM, Garner-O'Neale LD, Sultana T, Metcalfe CD (2017) Contaminants of emerging concern in surface waters in Barbados, West Indies. Environ Monit Assess 189: 636. https://doi.org/10.1007/s10661-017-6341-4.

Li H, Helm P, Metcalfe CD (2010) Sampling in the Great Lakes for pharmaceuticals, personal care products, and endocrine disrupting substances using the passive polar organic chemical integrative sampler. Environ Toxicol Chem 29:751-762.

Lindberg RH, Wennberg P, Johansson MI, Tysklind M, Andersson BAV (2005) Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. Environ Sci Technol 39:3421-3429.

Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J, Liang S, Wang XC (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci Total Environ 473:619-641.

Martín J, Camacho-Muñoz D, Santos JL, Aparicio I, Alonso E (2012) Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: removal and ecotoxicological impact of wastewater discharges and sludge disposal. J Hazard Mater 239: 40-47.

Metcalfe CD, Hoque ME, Sultana T, Murray C, Helm PA, Kleywegt S (2014) Monitoring for contaminants of emerging concern in drinking water using POCIS passive samplers. Environ Sci Processes & Impacts 16:473-481.

Metcalfe CD, Tindale K, Li H, Rodayan A, Yargeau V (2010) Illicit drugs in Canadian municipal wastewater and estimates of community drug use. Environ Poll 158:3179-3185.

Mora, C (2008) A clear human footprint in the coral reefs of the Caribbean. Proc Royal Soc B: Biological Sciences 275:767–73.

Nurse L, Cashman A, Mwansa J (2012) Confronting the challenges of sewerage management in the Caribbean: A case study from the island of Barbados. Environment 54:30-43.

Prichard E, Granek EF (2016) Effects of pharmaceuticals and personal care products on marine organisms: from single-species studies to an ecosystem-based approach. Environ Sci Pollut Res 23:22365-22384.

Rodayan A, Afana S, Segura PA, Sultana T, Metcalfe CD, Yargeau V (2016) Linking drugs of abuse in wastewater to contamination of surface and drinking water. Environ Toxicol Chem 35:843-849.

Singh SP, Azua A, Chaudhary A, Khan S, Willett KL, Gardinali PR (2010) Occurrence and distribution of steroids, hormones and selected pharmaceuticals in South Florida coastal environments. Ecotoxicol. 19:338-350.

Thomas KV, Bijlsma L, Castiglioni S, Covaci A, Emke E, Grabic R, Hernández F, Karolak S, Kasprzyk-Hordern B, Lindberg RH, Lopez de Alda M, Meierjohann A, Ort C, Pico Y, Quintana JB, Reid M, Rieckermann J, Terzic S, van Nuijs ALN, de Voogt P (2012) Comparing illicit drug use in 19 European Cities through sewage analysis. Sci Total Environ 432:432-439.

Tscharke BJ, Chen C, Gerber JP (2016). Temporal trends in drug use in Adelaide, South Australia by wastewater analysis. Sci Total Environ 565:384-391.

Velicu M, Suri R (2009). Presence of steroid hormones and antibiotics in surface water of agricultural, suburban and mixed-use areas. Environ Sci Technol 154:349-359.

Wise A, O'Brien K, Woodruff T (2011) Are oral contraceptives a significant contributor to the estrogenicity of drinking water? Environ Sci Technol 45:51-60.

Yang X, Flowers RC, Weinberg HS, Singer PC (2011) Occurrence and removal of pharmaceuticals and personal care products (PPCPs) in an advanced wastewater reclamation plant. Water Res 45: 2518-2528.

Yargeau V, Taylor B, Li H, Rodayan A, Metcalfe CD (2014) Analysis of drugs of abuse in wastewater from two Canadian cities. Sci Total Environ 487:722-730.

Zuccato E, Chiabrando C, Castiglioni S, Bagnati R, Fanelli R (2008) Estimating community drug abuse by wastewater analysis. Environ Health Perspect. 116:1027-1032.