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Mass loading and removal of pharmaceuticals and personal care products including psychoactives, antihypertensives, and antibiotics in two sewage treatment plants in Southern India

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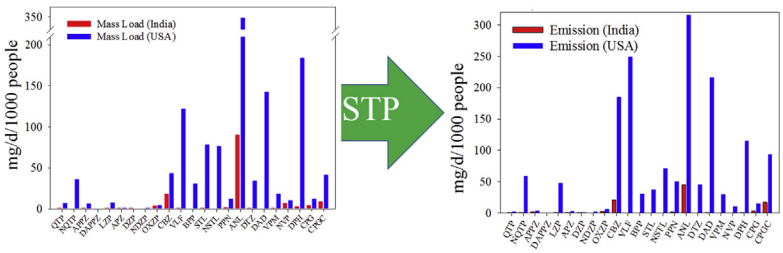
HIGHLIGHTS

- 29 PPCPs and 6 metabolites were detected in two STPs in India.
- Atorvastatin, mefenamic acid and paraxanthine found for the first time in Indian STPs.
- Metabolites were found 7 times higher than their parent drugs.
- Conventional STP did not remove carbamazepine, diazepam and clopidogrel.
- First study in India to report mass loading and emission of PPCPs into the environment.

GRAPHICAL ABSTRACT



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ABSTRACT

Environmental contamination by pharmaceuticals and personal care products (PPCPs) is barely studied in India despite being one of the largest global producers and consumers of pharmaceuticals. In this study, 29 pharmaceuticals and six metabolites were determined in sewage treatment plants (STPs) in Udupi (STP_U: population served ~150,000) and Mangalore (STP_M: population served ~450,000); the measured mean concentrations ranged from 12 to 61,000 ng/L and 5.0 to 31,000 ng/L, respectively. Atorvastatin (the most prescribed antihypercholesterolemic in India), mefenamic acid, and paraxanthine were found for the first time in wastewater in India at the mean concentrations of 395 ng/L, 1100 ng/L, and 13,000 ng/L, respectively. Select pharmaceutical metabolites (norverapamil and clopidogrel carboxylic acid) were found at concentrations of upto 7 times higher than their parent drugs in wastewater influent and effluent. This is the first study in India to report mass loading and emission of PPCPs and their select metabolites in STPs. The total mass load of all PPCPs analyzed in this study at STP_U (4.97 g/d/1000 inhabitants) was 3.6 times higher than calculated for STP_M. Select recalcitrant PPCPs (carbamazepine, diazepam, and clopidogrel) were found to have negative or no removal from STP_U while additional

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Removal efficiency
Wastewater
Sludge
Sewage treatment plant
India

treatment with upflow anaerobic sludge blanket reactor at STP_M removed (up to 95%) these PPCPs from STP_M. Overall, 5.1 kg of caffeine, 4.1 kg of atenolol, 2.7 kg of ibuprofen, and 1.9 kg of triclocarban were discharged annually from STP_U. The PPCP contamination profile in the Indian STP was compared with a similar study in the USA.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) enter the environment as parent drugs, their metabolites, and transformed products through industrial and/or domestic wastewater discharge as well as the farm application of sewage sludge from sewage treatment plants (STPs) (Halling-Sorensen et al., 1998). There is a concern over the ecological threats posed by "down-the-drain" chemicals including PPCPs in India; India is one of the top five emerging pharmaceutical manufacturing countries due to the recent relocation of global pharmaceutical industry from North America and EU to Asian countries (Rehman et al., 2015). India is one of the countries with largest drug consumption (Mathew and Unnikrishnan, 2012), and ranked 11th in the global consumption of over-the-counter drugs (Nagaraj et al., 2015).

In addition to the environmental discharge of significant amount of residual PPCPs through industrial effluent (Gunnarsson et al., 2009; Larsson et al., 2007), the treatment capacity of domestic sewage in India is far below the quantity of sewage generated from 1.3 billion people (Subedi et al., 2015). In 2008, only 31% of the total sewage produced (~38,254 million liters per day; MLD) in 908 cities were treated (CPCB, 2010). The shortage in demand and supply for the sewage treatment, inadequate maintenance of STPs, and sewage overflow every year for ~3–4 months of monsoon, consequently, can outweigh the estimated environmental emission of PPCPs. The majority of centralized wastewater treatment systems in developing countries consist of low-cost stabilization tank and septic ponds, while advanced secondary/tertiary treatments are common in developed countries (Kivaisi, 2001).

Over the past decade, research determining sources and overall fates of PPCPs through STPs, and their distribution in multiple environmental matrices including biota (Subedi et al., 2012), wastewater (Metcalfe et al., 2010; Radjenovic et al., 2009), surface water (Kolpin et al., 2002), drinking water (Benotti et al., 2009), and sludge (Subedi et al., 2013) were studied in developed countries. However, the frequency and concentration of PPCPs can vary depending on the consumption pattern of drugs and the effectiveness of wastewater treatment strategies (Kolpin et al., 2002). The fates of PPCPs in STPs in India are poorly reported (Anumol et al., 2016; Subedi et al., 2015). Moreover, the reported PPCPs fate studies focused only on parent drugs (Anumol et al., 2016; Prabhasankar et al., 2016; Singh et al., 2014). Environmental fate studies are particularly important because 30–90% of the administered dose of drugs is typically excreted through urine or feces (Halling-Sorensen et al., 1998), and metabolites of pharmaceuticals can be as much pharmacologically active as their parent drugs (Schwartz et al., 1985). A pilot study reported recently by our research group was the first to estimate mass loadings, removal efficiency, and environmental emission of wide-range of PPCPs and their select metabolites including illicit drugs and artificial sweetener in India (Subedi et al., 2015). We found ng/L to μg/L levels of PPCP residues including some of the highest reported concentrations in wastewater and sludge from Indian STPs.

PPCPs are capable of exerting toxic effects at relatively low concentrations through specific modes of action, and can affect non-target organisms at different trophic levels (Rehman et al., 2015). Antipsychotics, antidepressants, and antibacterials are the three most toxic classes of pharmaceuticals in the environment (Fent et al., 2006; Asimakopoulos and Kannan, 2016). For instance, at environmentally relevant concentrations, carbamazepine altered the ultrastructural cellular reactions in liver, kidney and gills of *Oncorhynchus mykiss* (rainbow trout) and *Cyprinus carpio* (common carp) (Triebskorn et al., 2007); diazepam inhibited polyp regeneration of *Hydra vulgaris* (Pascoe et al., 2003), and metoprolol and verapamil significantly changed the heartbeat of *Daphnia magna* (Villegas-Navarro et al., 2003). Therefore, studies on the environmental occurrence of fate of PPCPs are important. A detailed discussion on the toxicity of pharmaceutical residues in various test organisms has been compiled by Halling-Sorensen et al. (1998).

In this study, occurrence and removal of 29 PPCPs and nine metabolites including antischizophrenic, sedative-hypnotic-anxiolytic, antidepressant, antihypertension, antimicrobial, antibiotic, analgesic, antihistamine, antiplatelet, antihypercholesterolemic, UV-filter, and stimulant were determined in two STPs located at Udupi and Mangalore in southern India. Profiles of occurrence of PPCPs in wastewater influent in India were also compared with that in wastewater influents in the USA. This is the first study to determine mass loading of a wide-range of PPCPs in STPs from a week-long sampling event, removal efficiency, and environmental emission of these chemicals from Indian STPs.

2. Material and methods

2.1. Reagents and chemicals

Standard stock solutions (100 or 1000 μg/mL) of individual pharmaceuticals, metabolites, and their corresponding isotopically-labeled standards were purchased from commercial vendors, as described elsewhere (Subedi et al., 2015; Subedi and Kannan, 2015). Purity of all of the standards were ≥95%. Formic acid (98.2%) was from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure Deionized water was prepared with the Barnstead ultrapure system (Barnstead International, Dubuque, IA, USA). All standard stock solutions were stored at −20 °C.

2.2. Sample collection and preparation

Wastewater samples including raw wastewater (influent), treated wastewater (effluent), and sludge were collected over a seven-day period, from Sunday, May 19, 2013 to Saturday, May 25, 2013, consecutively from two STPs located in Udupi (STP_U) and Mangalore (STP_M) in the State of Karnataka, southern India. The wastewater influents were collected after the initial treatment that included screening of large-sized debris followed by grit removal. The wastewater effluents were collected at an outlet after the treatment processes prior to discharge into the Arabian Sea. STP_U involves a conventional aerobic biological treatment with activated sludge followed by final clarification and chlorine disinfection. STP_M involves upflow anaerobic sludge blanket reactor (UASBR),

secondary aerobic biological treatment with activated sludge, final clarification, and chlorine disinfection (Fig. S1). UASBR is expected to remove >85% of organic materials (measured as chemical oxygen demand) due to the simultaneous anaerobic bio-degradation and sedimentation process. The removal of PPCPs from STP_U was due to the aerobic bio-degradation while the removal of PPCPs from STP_M was due to combined anaerobic and aerobic bio-degradation (inside UASBR). The two plants STP_U (population served ~150,000) and STP_M (population served ~450,000) has an installed treatment capacity of 14.2 MLD (current flow rate of 7.5 MLD) and 81.7 MLD (current flow rate of 12 MLD), respectively. Both plants received wastewater predominantly (>75%) from household waste.

Sludge samples were collected for seven days in both STPs within a sampling week from final clarifier tank at STP_U and from sludge pit (after UASBR) at STP_M. The total sludge production (TSP) at final clarification tank of STP_U was 400 kg/d whereas TSP at sludge pit in STP_M was 133 kg/d. All samples were collected in pre-cleaned polypropylene bottles rinsed with site wastewater prior to sample collection, shipped frozen within 6 days to the Wadsworth Center, Albany, USA where it was stored at −20 °C until extraction and further analysis.

Wastewater samples were analyzed by following the methods described elsewhere with some modifications (Subedi et al., 2015; Subedi and Kannan, 2015). Briefly, wastewater samples were thawed at room temperature, 100 mL was spiked with a mixture of isotopically labeled internal standards of PPCPs (50 ng), mixed well, and allowed to equilibrate for ~30 min at room temperature. The aqueous samples were extracted by passage through Oasis® HLB 6 cm^3 (200 mg; Waters, Milford, MA) solid phase extraction (SPE) cartridges. Prior to use, the cartridges were conditioned with 5 mL of methanol and 5 mL of milli-Q water, and wastewater samples were loaded at ~1 mL/min. Then, cartridges were allowed to dry for ~30 min under vacuum and then eluted with 6 mL of methanol followed by 4 mL of a mixture of acetone, methanol, and ethyl acetate (2:2:1 v/v/v). Cartridges were also eluted with 4 mL of methanol containing 5% ammonia. The eluents were combined and concentrated to ~100 μL under a gentle stream of nitrogen at 35° C using a TurboVap® Evaporator (Zymark, Inc., Hopkinton, MA). The final volume of the extract was adjusted to 1 mL with methanol in an amber glass vial, and 10 μL of the extract was injected into HPLC-MS/MS.

Sludge samples were analyzed by following the method described elsewhere with some modifications (Subedi et al., 2013, 2014). Briefly, ~0.1 g of freeze-dried sludge was spiked with a mixture of isotopically labeled internal standards and allowed to equilibrate for ~30 min at room temperature. Spiked sludge samples were vortex-mixed for 1 min and extracted with 6 mL of methanol:water mixture (5:3 v/v) using an ultrasonic bath (Branson® Ultrasonics 3510R-DTH; Danbury, CT) for 30 min. Extracts were centrifuged at 4000 rpm for 5 min (Eppendorf Centrifuge 5804, Hamburg, Germany), the supernatant was collected in a polypropylene tube, and the extraction was repeated with 6 mL of methanol. The extracts were combined and concentrated to ~1 mL under a gentle stream of nitrogen. The concentrated extract was diluted with milli-Q water to ~10 mL and purified by passage through Oasis® HLB (6 cm^3, 200 mg) cartridges, as described above for wastewater samples. The final volume of the extract was adjusted to 1 mL with methanol in an amber glass vial, and 10 μL of the extract was injected into HPLC-MS/MS for analysis.

2.3. Instrumental analysis

PPCPs were analyzed using an API 2000 electrospray triple quadrupole mass spectrometer (ESI-MS/MS; Applied Biosystems, Foster City, CA), interfaced with an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA). The autosampler was maintained at 4 °C. The analytes were separated using an Ultra Biphenyl column (100 mm × 2.1 mm, 5 μm) (Restek® Corporation, Bellefonte, PA). Methanol and water (0.1% formic acid) as well as methanol and 0.5 mM ammonia (*aq*) were used as mobile phases; a description of the mobile phase gradient flow is presented in Table S1. Target analytes were determined by multiple-reaction monitoring (MRM) in positive mode of ionization while triclocarban, ibuprofen, and sulfamethoxazole were monitored in negative ionization mode. Detailed information on the MRM transitions, analyte peak identification, and quantification is provided in Table S2 (Subedi et al., 2014). Briefly, analyte peak identification was based on the retention time (±0.05 min) and the ratio of qualitative to quantitative transition-ion responses (±20%). The quantitation of pharmaceuticals was based on the isotope dilution method; however, bupropion, acetaminophen, mefenamic acid, clindamycin, lincomycin, miconazole, and tiabendazole were quantified based on the response of the corresponding internal standards. Metabolites were quantified using the internal standards of the corresponding parent compounds (due to the lack of labeled standards available for the metabolites). The calibration curves were prepared by plotting concentration-dependent response factor of each target analyte (peak area of analyte divided by peak area of internal standard) versus the response-dependent concentration factor (concentration of analyte divided by concentration of internal standard). The regression coefficients (r^2) for seven-to nine-point calibration standards calculated by equal weighting quadratic regression were ≥0.99 for all target analytes. The limits of quantitation (LOQs) were determined as a minimum concentration of analytes in sample extracts that provided a signal to noise ratio ≥10. LOQs of the target pharmaceuticals and their metabolites in wastewater and sludge samples ranged 0.1–50 ng/L and 0.5–50 ng/g, respectively. Continuing calibration verification standards were injected before and after every batch of samples, which showed recoveries at 100 ± 22%. A method blank was analyzed with every batch of samples. The concentrations of target pharmaceuticals and their metabolites in method blanks were below the corresponding LOQ. The concentrations of the target chemicals in sludge are reported on a dry-weight basis unless stated otherwise.

One sample was selected randomly for matrix spike (MS) and matrix spike duplicate (MSD) analyses with each batch of samples analyzed. Target pharmaceuticals (50 ng) and their corresponding internal standards (50 ng) were each spiked, and were passed through the entire analytical procedure. The average relative recoveries of target pharmaceuticals from wastewater and sludge ranged from 75 to 100%. The recoveries of all pharmaceuticals and their metabolites in spiked wastewater and sludge samples were reported elsewhere (Subedi et al., 2013, 2014, 2015; Subedi and Kannan, 2015).

2.4. Calculations

The mass loadings of pharmaceuticals and their select metabolites in STPs, removal efficiency through the treatment processes, and emission from STPs were calculated using the following equations (Eqns (1)–(3)), as reported elsewhere (Subedi et al., 2015; Subedi and Kannan, 2014, 2015).

$$\text{Mass load/1000 inhabitants} = C_i \times F \times \left(\frac{1}{10^6}\right) \cdot \left(\frac{1000}{\text{Population}}\right) \quad (1)$$

$$\text{Removal Efficiency } (\%) = \frac{[C_i \times F] - [(C_e \times F) + (C_s \times TSP)]}{[C_i \times F]} \times 100 \quad (2)$$

$$\text{Emission/1000 inhabitants} = [(C_e \times F) + (C_s \times TSP)] \times \left(\frac{1}{10^6}\right)\left(\frac{1000}{\text{Population}}\right) \quad (3)$$

where C_i is the mean concentration of analyte in wastewater influent (ng/L), C_e is the mean concentration of analyte in wastewater effluent (ng/L), F is the daily flow of wastewater influent (L/d), mass load/1000 inhabitants is the mean amount of individual pharmaceutical compound introduced into STP (mg/d/1000 inhabitants), C_s is the mean concentration of analyte in sludge (ng/g wet weight), TSP is the total sludge production (g/d wet weight), population is the number of inhabitants served by the STP, and emission/1000 inhabitants is the mean amount of individual pharmaceutical compound discharged through wastewater effluent and sludge (mg/d/1000 inhabitants).

The partition ratio for each PPCP at STP_U was calculated based on the assumption that PPCPs are in equilibrium between sludge and effluent after aerobic biological treatment. Thus, the calculated partition ratio was used to calculate the concentration of PPCPs in sludge after aerobic treatment in STP_M, and used to calculate the removal efficiency and environmental emission.

3. Results and discussion

3.1. PPCPs in influent

All 29 PPCPs including two antischizophrenics (quetiapine and aripiprazole), three sedatives-hypnotics-anxiolytics (lorazepam, alprazolam, and carbamazepine), three antidepressants (venlafaxine, bupropion, and sertraline), four antihypertensions (propranolol, atenolol, diltiazem, and verapamil), one antimicrobial (triclocarban), six antibiotics (trimethoprim, sulfamethoxazole, clindamycin, lincomycin, miconazole, and tiabendazole), four analgesics (ibuprofen, acetaminophen, codeine, and mefenamic acid), one antihistamine (diphenhydramine), one antiplatelet (clopidogrel), one antihypercholesterolemic (atorvastatin), one UV-filter (oxybenzone), and one stimulant (caffeine) were found in wastewater influents in the two STPs (Table 1). Six of the nine pharmaceutical metabolites analyzed were also found in wastewater influent (Table 1). Metabolites of antischizophrenics (norquetiapine and dehydro-aripiprazole) and an antidepressant (norsertraline) were not detected in influents from both STPs. The concentrations of most of the PPCPs in influents at STP_U were higher than the concentrations of PPCPs at STP_M. Antibiotics (trimethoprim and clindamycin) and antidepressants (venlafaxine and a metabolite of verapamil) were found at concentrations ≥6 times higher in STP_U than in STP_M influents. The sum of mean concentrations of total 29 pharmaceuticals and six metabolites entering STP_U and STP_M were 99,500 ng/L and 51,800 ng/L, respectively.

Aripiprazole is the most selling prescription drug in the USA in 2013 (http://www.drugs.com/stats), and aripiprazole and quetiapine are among the top six antischizophrenics prescribed in India. The mean concentrations of quetiapine and aripiprazole in influents were similar at STP_U (Table 1), despite the higher prescription frequency and mean dose of quetiapine than aripiprazole (Grover and Avasthi, 2010). Typically, psychotic patients in India are prescribed with two or more psychoactives (Grover and Avasthi, 2010). Diazepam was measured at a mean concentration of 25 ng/L in 100% of influent samples from STP_M. Similarly, carbamazepine is one of the most commonly prescribed and co-prescribed antiepileptic drugs with antischizophrenics and anxiolytics in India (Haroon et al., 2012). The mean concentration of carbamazepine in influent in both STPs (~500 ng/L) was similar as reported in wastewater from other parts of India (Anumol et al., 2016; Subedi and Kannan, 2015) and Korea (Choi et al., 2008), while but two times lower than in Canada (Lajeunesse et al., 2012).

Venlafaxine and bupropion were found in 100% of influent samples from STP_U at mean concentrations of 38 ng/L and 19 ng/L, respectively.

Hypertension is a significant contributor of cardiovascular disease which is estimated to increase by 111% from 1990 (2.3 million deaths in India) to 2020 in India (Gupta, 2004). All antihypertensions (atenolol, propranolol, diltiazem, and verapamil) were detected in 100% influent samples in both STPs. Atenolol is the most prescribed antihypertensive (19.4%) in India (Rachana et al., 2014). The mean concentration of atenolol in influent was 33–91 times higher than other antidepressants.

The mean concentration of triclocarban (an antimicrobial) was 3.3–138 times higher than the concentrations found for antibiotics (Table 1). Antibiotics and antimicrobials constitute 6% of all human prescription medicines and >70% of all veterinary medicines (Thiele-Bruhn, 2003). The extensive agricultural and veterinary facilities in Mangalore than in Udupi can explain two times higher concentration of tiabendazole (a fungicide) at STP_M than in STP_U. Analgesics are the largest group of over-the-counter drugs in India (Nagaraj et al., 2015). Acetaminophen was detected as the next highest compound after caffeine in 100% influent samples in both STPs. The mean concentration of ibuprofen (1300 ng/L) was similar to that reported previously in other five STPs in India (Subedi et al., 2015) while mefenamic acid (1100 ng/L) was found for the first time in wastewater in India. Atorvastatin is the second most commonly prescribed drug in the USA (http://www.rxlist.com) and the most prescribed antihypercholesterolemic in India, but has not been reported in wastewater prior to this study. The mean concentration of atorvastatin was 395 ng/L detected for the first time in wastewater from two STPs in India.

The higher concentration of metabolites of select drugs their parent drugs emphasizes the importance of monitoring metabolites in wastewater for the calculation of mass loads of drugs in STPs. For example, the concentration of diltiazem in STP_U was ~2 times lower than its parent drug while the mean concentration of a metabolite of verapamil was 7.2 times higher than that of its parent drug. Similarly, clopidogrel carboxylic acid, a metabolite of clopidogrel (an antiplatelet), was found more frequently and at higher concentrations than its parent drug.

3.2. Comparison of PPCP profiles in wastewater influent from India and the USA

The mass loadings of select PPCPs in wastewater influents and effluents from a STP in Udupi, India (STP_U) and a STP in New York, USA (Subedi and Kannan, 2015) serving similar size populations were compared (Fig. 1). Wastewater samples from both STPs were analyzed utilizing the same analytical method including the same set of analytical instrumentations. The mean concentrations of antipsychotics such as venlafaxine and bupropion were 8.8 and 7.7 times higher, respectively, in the USA than in India. However, the mean concentrations of antipsychotics such as aripiprazole, alprazolam, and diazepam and an antihypertension (atenolol) were ≥5 times higher in India than in the USA. Moreover, the mean concentrations of norverapamil (a metabolite of antihypertensive verapamil) and oxazepam were 29 and 21 times higher in India than in the USA. The ratio of the concentration of norverapamil to

Table 1
Mean (n = 7) concentrations of pharmaceuticals and their select metabolites in wastewater (ng/L) and sludge (ng/g dw) from two Indian sewage treatment plants (STPs).

Analytes	Udupi STP			Mangalore STP		
	Influent	Effluent	Sludge	Influent	Effluent	Sludge
Antischizophrenics						
Quetiapine	**38**	**20**	**35**	**15**	**5.2**	**23**
	17-76 (100)	16-24 (100)	21-59 (100)	4.0–61 (100)	3.0–8.0 (100)	12-45 (100)
Norquetiapine	<LOQ	<LOQ	**43.9**	<LOQ	<LOQ	**51**
	<LOQ	<LOQ	24-71 (100)	<LOQ	<LOQ	30-76 (100)
Aripiprazole	**44**	**71**	**20**	**29**	**0.4**	**16**
	ND-100 (71)	38-150 (100)	8-45 (100)	ND-46 (71)	ND-0.4/29	12-18 (100)
Dehydro-aripiprazole	ND	ND	**3.5**	ND	ND	**16**
	ND	ND	ND-6.0 (86)	ND	ND	ND-14 (71)
Sedatives-hypnotics-anxiolytics						
Lorazepam	**46**	**23**	ND	**26**	**12**	ND
	ND-95 (86)	ND-47 (57)	ND	ND-31 (71)	ND-22 (57)	ND
Alprazolam	**41**	**33**	**24**	<LOQ	25	**24**
	<LOQ-69 (29)	<LOQ-57 (29)	ND-25/29	<LOQ	ND-25.0/29	ND-24.0/29
Diazepam	**23**	**36**	**9.0**	**25**	**9.5**	**6.7**
	ND-85 (71)	6.0–100 (100)	ND-12 (86)	27-62 (100)	3.0–28 (43)	ND-8.0 (57)
Oxazepam	**140**	**85**	**48**	**50**	**50**	<LOQ
	<LOQ-210 (71)	<LOQ-210 (29)	ND-48/29	<LOQ-50.0/29	ND-50.0/29	<LOQ
Nordiazepam	**12**	**17**	**5.0**	**5.9**	**25**	**5.2**
	5.0–17 (71)	13-20 (100)	ND-5.0/29	<LOQ-11 (29)	ND-25.0/29	ND-8.0 (57)
Carbamazepine	**450**	**580**	**21.9**	**550**	**480**	**22**
	240-750 (100)	450-770 (100)	17-31 (100)	460-660 (100)	290-710 (100)	16-30 (100)
Antidepressants						
Venlafaxine	**38**	**14**	**6.7**	**5.0**	**5.0**	**7.1**
	11-76 (100)	12-18 (100)	5.0–13 (29)	ND-5.0/57	ND-5.0/29	<LOQ-13/29
Bupropion	**19**	**14**	**12**	**23**	**5.0**	**16**
	13-32 (100)	13-16 (100)	ND-16 (43)	ND-64 (71)	ND-66 (57)	5.0–44 (57)
Sertraline	**23**	**18**	**220**	**40.0**	1.7	**101**
	ND-38 (71)	8.0–21 (100)	81-310 (100)	ND-91 (57)	ND-3.0/57	77-210 (100)
Norsetraline	ND	ND	**78**	ND	ND	**67**
	ND	ND	46-150 (43)	ND	ND	<LOQ -136 (14)
Antihypertensions						
Propranolol	**51**	**43**	**46**	**43**	**28**	**54**
	42-62 (100)	36-52 (100)	27-60/100	30-54 (100)	21-33 (100)	41-73 (100)
Atenolol	**2900**	**1500**	**49.0**	**1400**	**590**	**13**
	2100-3800 (100)	<LOQ-500 (86)	ND-110 (29)	1200-1900 (100)	510-7100 (100)	<LOQ-21.4/57
Diltiazem	**55**	**5.0**	**19**	**16**	**1.8**	**8.9**
	23-120 (100)	3.0–7.0 (100)	2.0–35 (86)	6.0–19 (100)	1.0–2.0 (100)	ND-22 (29)
Desacetyl diltiazem	**32**	**44**	**15**	<LOQ	**10**	**8.4**
	20-64 (100)	28-65 (100)	9.0–20 (100)	<LOQ	<LOQ -18 (57)	<LOQ-18 (57)
Verapamil	**36**	**2.0**	**33**	**25**	ND	**24**
	16-103 (100)	ND-6.0 (43)	25-46 (100)	12-50 (100)	ND	20-32 (100)
Norverapamil	**260**	**4.0**	**169**	**47**	ND	**190**
	60-550 (100)	<LOQ-15/29	54-250 (100)	<LOQ-95 (86)	ND	57-420 (100)
Antimicrobial						
Triclocarban	**2400**	**540**	**21,000**	**4000**	**260**	**13,000**
	1300-4300 (100)	300-860 (100)	13,000-28000 (100)	1200-10000 (100)	215-358 (100)	10,000-23000 (100)
Antibiotics/fungicides						
Trimethoprim	**180**	<LOQ	**13**	**29**	**25**	**7.6**
	68-400 (100)	ND-<LOQ	ND-16 (86)	<LOQ-51 (29)	ND-25.0/29	<LOQ-10.4/57
Sulfamethoxazole	**220**	**260**	ND	**100**	**25**	<LOQ
	55-690 (100)	120-420 (100)	ND	<LOQ-170 (71)	ND-25.0/29	ND-<LOQ
Clindamycin	**210**	<LOQ	**2.4**	**31**	**25**	**2.6**
	25-790 (86)	ND-<LOQ	<LOQ-2.4/29	ND-31.0/29	ND-25.0/29	ND-3.63/57
Lincomycin	**730**	**430**	**4.6**	**230**	**130**	**3.8**
	ND-1800 (71)	280-510 (100)	3.0–7.0 (100)	ND-230/29	ND-310 (29)	2.0–5.0 (100)
Miconazole	**67**	**8.0**	**240**	**42**	**25**	**240**
	ND-180 (86)	<LOQ-21 (29)	210-300 (100)	21-99 (100)	ND-25.0/29	180-280 (100)
Tiabendazole	**64**	**79**	ND	**123**	**25**	**3.5**
	<LOQ-210 (29)	<LOQ-370 (29)	ND	25-440 (71)	ND-25.0/29	ND-5.8/57
Analgesics						
Ibuprofen	**1200**	**980**	**48**	**1400**	**630**	**48**
	<LOQ-2800 (71)	270-1940 (100)	ND-49 (57)	970-1900 (100)	290-710 (100)	ND-49 (29)
Acetaminophen	**9000**	**690**	**340**	**4500**	**340**	**480**
	5400-11000 (100)	330-1200 (100)	120-970 (57)	2900-7500 (100)	<LOQ -490 (86)	<LOQ -970 (86)
Codeine	**160**	**82**	ND	**79**	**25**	<LOQ
	ND-280 (86)	<LOQ-120 (86)	ND	ND-103/29	ND-52.0/57	ND-<LOQ
Mefenamic acid	**1100**	**570**	**110**	**1100**	**440**	**72**
	ND-2800 (86)	320-750 (100)	73-130 (100)	490-3800 (100)	250-750 (100)	<LOQ -100 (86)
Antihistamine						
Diphenhydramine	**97**	**32**	**100**	**44**	**15**	**100**
	49-180 (100)	23-35 (100)	50-160 (100)	13-98 (100)	10-19 (100)	60-200 (100)

(*continued on next page*)

Table 1 (*continued*)

Analytes	Udupi STP			Mangalore STP		
	Influent	Effluent	Sludge	Influent	Effluent	Sludge
Antiplatelet						
Clopidogrel	**130**	<LOQ	**120**	**130**	**54**	**130**
	ND-125/29	<LOQ	110-120 (43)	ND-125/29	ND-125/57	<LOQ-180 (57)
Clopidogrel carboxylic acid	**200**	**430**	**19**	**300**	**460**	**20**
	ND-420 (86)	130-740 (100)	15-23 (100)	260-420 (100)	310-570 (100)	14-28 (100)
Antihypercholesterolemic						
Atorvastatin	**410**	**280**	**75**	**380**	**340**	**96**
	110-690 (100)	<LOQ-500 (100)	ND-110 (29)	310-460 (100)	280-510 (100)	<LOQ-140 (57)
UV-filter						
Oxybenzone	**52**	**7.0**	**39**	**39**	**1.1**	**26**
	32-85 (100)	ND-29 (43)	ND-29 (43)	25-48 (100)	ND-3.0/57	<LOQ-36 (57)
Stimulant						
Caffeine	**61,000**	**1100**	**34**	**30,000**	**3400**	**32**
	40,000-120,000 (100)	810-1700 (100)	10-120 (71)	16,000-45000 (100)	2400-4400 (100)	12-70 (100)
Paraxanthine	**19,000**	**760**	ND	**7400**	**1500**	ND
	15,000-24000 (100)	610-950 (100)	ND	5500-9300 (100)	1400-2300 (100)	ND

ND: non-detects; LOQ: limit of quantitation; pharmaceutical metabolites are italicized; bold values are the mean concentrations; values in parenthesis are the detection frequency. The concentrations that were <LOQ were substituted with ½ LOQ values for statistical analysis.

verapamil in influent from STP_U in India was six times higher than in the USA (1.2), which can either result from the microbial transformation in STP_U and/or the potential direct discharge of verapamil into the wastewater in the USA. The mean concentrations of antipsychotics such as aripiprazole, diazepam, and oxazepam in effluent from India were 42, 14, and 11 times, respectively, higher than in the USA, whereas the mean concentrations of venlafaxine, diltiazem, and verapamil in effluent from the USA were 24, 39, and 13 times, respectively, higher than in India. The different profiles of pharmaceuticals and their metabolites in wastewater from STPs in India and the USA indicate the different consumption patterns of drugs as well as the effectiveness of wastewater treatment strategies and/or different extent of dilution. It may be important to note that the samples were collected in Indian WWTPs prior to monsoon season (therefore, dilution can be significantly lower than in monsoon season). Moreover, the different contamination profiles of PPCPs in India and in the USA highlight the importance of region-specific monitoring (Anumol et al., 2016).

3.3. Mass loading of PPCPs into STPs

The mass of PPCPs enters into the STPs via wastewater influent was calculated as mg/d/1000 inhabitants, using the concentration of PPCPs in influent, population served, and the volume of wastewater inflow (Eqn (1)). The total mass load of all PPCPs analyzed in this study at STP_U (4.97 g/d/1000 inhabitants) was 3.6 times higher than that calculated for STP_M (Fig. 1 and Table S3). The sum of the mass loads of caffeine and its metabolite, paraxanthine, (~4.0 g/d/1000 inhabitants) were ~4 times higher than the total mass loads of all other pharmaceuticals in STP_U. In both STPs, the mass loading of caffeine (a stimulant) was followed by analgesics (acetaminophen, ibuprofen, and mefenamic acid), an antihypertensive (atenolol), and an antimicrobial (triclocarban) (Fig. 1 and Table S3). Considering that the sum mass loads of all analytes in a STP as 100%, percentage loads of each class of PPCPs were calculated. Average percentage mass loading of the stimulant in STP_U and STP_M was 75.5% followed by analgesics (12.5%), antimicrobial (5.1%), antihypertensions (3.2%), antipsychotics (1.3%), and antibiotics (1.3%) (Fig. 2A and B). Moreover, the mass loads of norverapamil (metabolite of verapamil) and clopidogrel carboxylic acid (metabolite of clopidogrel) were 7.2 and 1.6 times higher than their parent drugs, respectively. Verapamil and norverapamil excrete in the concentration ratio of 0.47 through human urine (Schwartz et al., 1985), which suggests that approximately two times higher concentration of norverapamil than verapamil in wastewater influent. However, the microbial or chemical ambience in

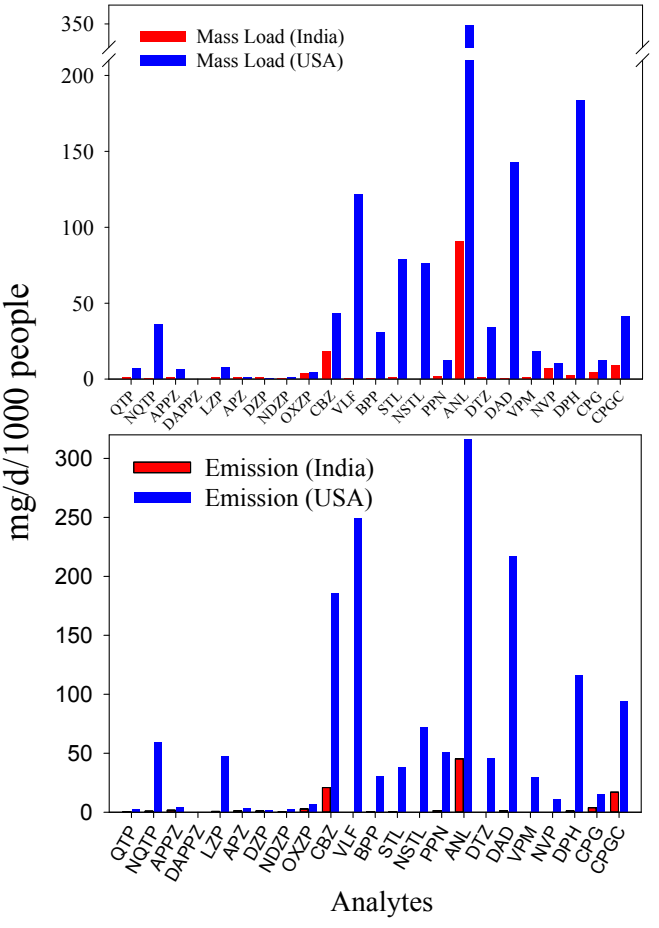

Fig. 1. The average mass loading and environmental emission of select PPCPs from two STPs in India and two STPs in New York, USA (Subedi and Kannan, 2015). APPZ: aripiprazole; DAPPZ: dehydro-aripiprazole; QTP: quetiapine; NQTP: norquetiapine; LZP: lorazepam; APZ: alprazolam; DZP: diazepam; OxZP: oxazepam; NDZP: nordiazepam; CBZ: carbamazepine; VLF: venlafaxine; BPP: bupropion, STL: sertraline; NSTL: norsetraline; PPN: propranolol; ANL: atenolol; DTZ: diltiazem; DAD: desacetyl diltiazem; VPM: verapamil; NVP: norverapamil; DPH: diphenhydramine; CPG: clopidogrel; CPGC: clopidogrel carboxylic acid.

wastewater can enhance degradation/transformation of verapamil including *O*-demethylation that can lower verapamil levels in influents.

Most studies that report mass loadings of PPCPs into the STPs and/or discharge from STPs based only on the concentrations of parent drugs measured. However, this study demonstrates that the estimated mass loadings can be underestimated by two fold by excluding the concentrations of metabolites in wastewater. Based on the average per capita usage of water in an Indian urban community (250 L), water usage by the population in Udupi (38 MLD) was calculated as ~3 times more than the existing capacity of STP_U. Similarly, water usage by the population in Mangalore (113 MLD) is ~1.5 times more than the existing capacity of STP_M. Owing to these imbalances in the estimated demand and supply, there is a potential growth in water replenishment strategies including water reuse schemes in Indian communities (Anumol et al., 2016). This study shows the need for further studies to understand the mass loads and fates of PPCPs in water.

3.4. Removal of PPCPs

STP_U employs a conventional aerobic bio-degradation with activated sludge while STP_M uses UASBR (anaerobic bio-degradation) as well as secondary aerobic biological treatment with activated sludge followed by final clarification and chlorine disinfection. Efficiency (%) of STP_U and STP_M to remove PPCPs was calculated using Eqn (2). Average removal efficiency of triclocarban, miconazole, acetaminophen, diltiazem, verapamil, norverapamil, oxybenzone, caffeine, and paraxanthine were 82–99% while trimethoprim, clindamycin, mefenamic acid, quetiapine, lorazepam, diphenhydramine, and atenolol were removed at ≥50% (Fig. 3). The removal efficiency of psychoactive drugs was found similar to that from STPs in the USA (Subedi et al., 2015). Recalcitrant PPCPs such as carbamazepine, diazepam, and clopidogrel were found to have negative or no removal from STP_U while UASBR treatment (anaerobic bio-degradation) at STP_M provided significant (up to 95%) removal of these PPCPs from STP_M. In addition to the limited treatment, high mass loading in the STP_M and low contact time for biological treatment contribute to a lower PPCP removal efficiency in comparison with that of STP_U. PPCP metabolites such as desacetyl diltiazem (metabolite of diltiazem) and clopidogrel carboxylic acid (metabolite of clopidogrel) were found to have negative removal efficiency. Microbial transformation of parent or conjugated forms of these drugs during wastewater treatment process can increase the levels of metabolites in waste streams (Calisto and Esteves, 2009; Subedi et al., 2015).

3.5. PPCPs emission into the environment

Although select PPCPs were significantly removed during wastewater treatment processes, significant levels of other PPCPs were found in effluents and sewage sludge (used as biosolids). The total mass of PPCPs and their select metabolites (mg/d/1000 inhabitants) discharged into the environment through effluent discharge and sludge disposal was calculated based on the concentration of PPCPs in effluent, wastewater outflow rate,

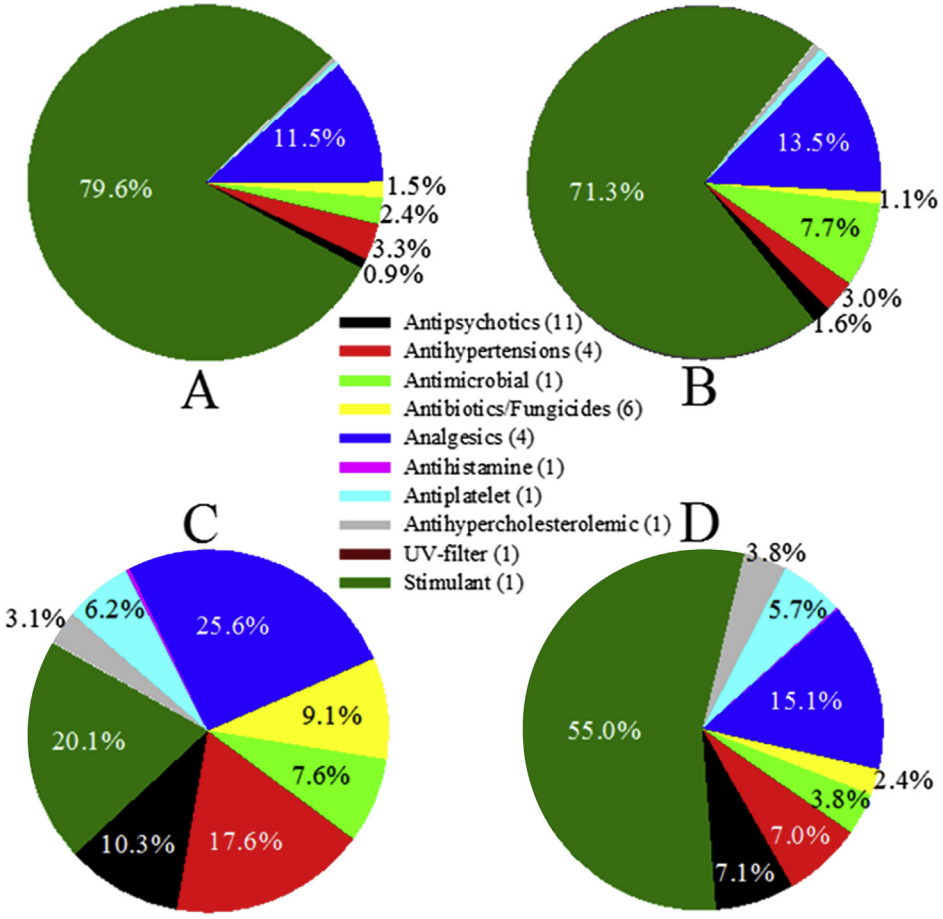

Fig. 2. Percentage composition profiles of selected PPCPs in wastewater. **A**: STP_U influent; **B**: STP_M influent; **C**: STP_U effluent; and **D**: STP_M effluent.

concentration of PPCPs in sewage sludge, total amount of sewage sludge produced, and the population served by the STP (Eqn (3)).

The total emission/discharge of PPCPs from STP_U was significantly higher than from STP_M (Mann-Whitney Rank Sum Test: $p = 0.003$). Caffeine, atenolol, ibuprofen, acetaminophen, mefenamic acid, triclocarban, carbamazepine, clopidogrel, and atorvastatin were the major PPCPs discharged from both STPs (Fig. 1 and Table S3). The total emission of caffeine and its metabolite (stimulant, 91.4 mg/d/1000 inhabitants) from STP_U was followed by atenolol (a hypertensive, 74.9 mg/d/1000 inhabitants), ibuprofen (an analgesic, 49.0 mg/d/1000 inhabitants, and triclocarban (an antimicrobial, 34.6 mg/d/1000 inhabitants). The emission of atenolol from STP_U (serving 150,000 people) was two times lower than that from a STP in Spain (serving 277,000 people: 149.5 mg/d/1000 inhabitants) (Radjenovic et al., 2009) and 4.7 times lower than that reported for a STP in the USA (serving ~100,000 people: 355 mg/d/1000 inhabitants) (Subedi and Kannan, 2015). Similarly, the emission of carbamazepine from STP_U was similar to that from a STP in Spain (36.8 mg/d/1000 inhabitants) (Radjenovic et al., 2009) but approximately an order of magnitude lower than that of a STP in the USA (226 mg/d/1000 inhabitants) (Subedi and Kannan, 2015). Overall, 5.1 kg of caffeine, 4.1 kg of atenolol, 2.7 kg of ibuprofen, and 1.9 kg of triclocarban were discharged annually from STP_U that employed a conventional aerobic biological treatment with activated sludge and serves an average population of 150,000 in India. Similarly, 39.5 kg of PPCPs (analytes in this study) were discharged annually from STP_M that employed UASBR and secondary aerobic biological treatment with activated sludge and serves an average population of 450,000 in India. Carbamazepine (an antipsychotic), clopidogrel (antiplatelet and its metabolite clopidogrel carboxylic acid), and atorvastatin (an antihypercholesterolemic) were discharged at 9–29 mg/d/1000 inhabitants in both STPs.

India currently regulates the chemical discharge from municipal wastewater effluent and sewage sludge through Hazardous Waste (Management, Handling, and Transboundary Movement) Wastes Rules (GIMEF, 2008) as the regulations of organic contaminants in wastewater including PPCPs in the USA (USEPA) and the regulation of land application of sewage sludge in Europe (CEC, 1986). Insufficient number of STPs and lack of enforcement of regulations can result in higher environmental emission of PPCPs than we estimated in this study. This study has provided important information on the fate of different classes of PPCPs in Indian STPs which can be useful for regulatory authorities to evaluate the environmental discharge of PPCPs and implement strategic guidelines to alleviate ecological risk and to improve environmental sustainability.

4. Conclusions

Twenty-nine pharmaceuticals and six of their metabolites were determined in two STPs in southern India. The contamination profiles of selected pharmaceuticals and their metabolites in wastewater were found different in comparison with that in the USA, which indicates different consumption patterns of drugs, effectiveness of wastewater treatment strategies, and/or different extent of dilution. Average percentage mass loading of the selected 29 PPCPs in STP_U and STP_M was found in the order of stimulant (75.5%) > analgesics (12.5%) > antimicrobial (5.1%) > antihypertensions (3.2%) > antipsychotics (1.3%) > antibiotics (1.3%). Average removal efficiency of most of the PPCPs were ≥50% in both STPs, while selected PPCPs such as carbamazepine, diazepam, and clopidogrel were removed significantly (up to 95%) at STP_M (which employs UASBR treatment) but negative or no removal at STP_U. It was found that 5.1 kg of caffeine, 4.1 kg of atenolol, 2.7 g of ibuprofen, and 1.9 kg of triclocarban were discharged annually from STP_U that employed a conventional aerobic biological treatment with activated sludge.

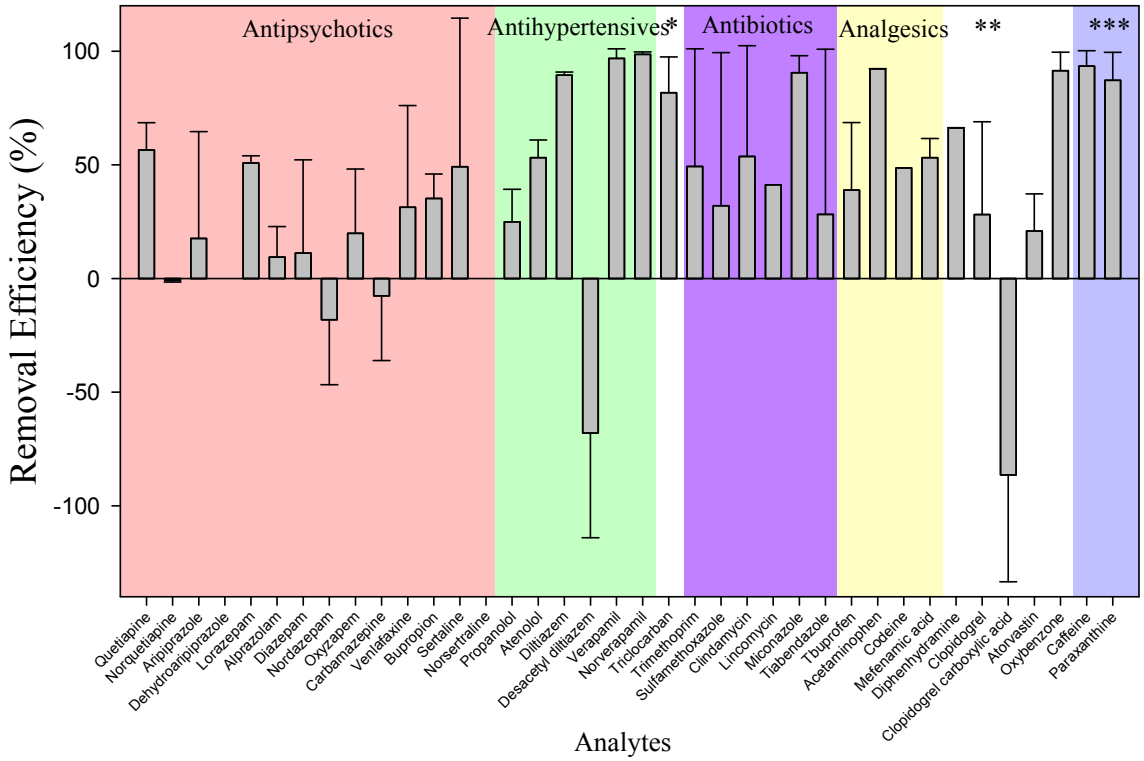

Fig. 3. Average removal efficiency (% ± StDev) of PPCPs from STP_U and STP_M. *antimicrobial; **antihistamine (diphenhydramine), antiplatelet (clopidogrel and its metabolite clopidogrel carboxylic acid), hypercholesteremic (Atorvastin), and UV-filter (oxybenzone); ***stimulant (caffeine and its metabolite paraxanthine).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.10.026.

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