

# Occurrences and fate of selected human antibiotics in influents and effluents of sewage treatment plant and effluent-receiving river Yamuna in Delhi (India)

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**Abstract** Antibiotics consumption has increased worldwide, and their residues are frequently reported in aquatic environments. It is believed that antibiotics reach aquatic water bodies through sewage. Medicine consumed for healthcare practices are often released into sewage, and after sewage treatment plant, it reaches the receiving water bodies of lakes or rivers. In the present study, we determined the fate of some commonly used antibiotics in a sewage treatment plant (STP) located in Delhi and the environmental concentration of these antibiotics in the Yamuna River, which receives the sewage and industrial effluent of Delhi. There are many reports on antibiotics occurrences in STP and river water worldwide, but monitoring data from the Indian subcontinent is sparse. Samples were taken from a STP and from six sampling sites on the Yamuna River. Several antibiotics were tested for using offline solid-phase extraction followed by high-performance liquid chromatography equipped with photodiode array analysis. Recoveries varied from 25.5–108.8 %. Ampicillin had the maximum concentration in wastewater influents ( $104.2 \pm 98.11 \mu\text{g l}^{-1}$ ) and effluents ( $12.68 \pm 8.38 \mu\text{g l}^{-1}$ ). The fluoroquinolones and cephalosporins had the lower concentrations. Treatment efficiencies varied between 55 and 99 %. Significant amounts of antibiotics were discharged in effluents and were detected in the receiving water body. The concentration of antibiotics in the Yamuna River varied from

not detected to  $13.75 \mu\text{g l}^{-1}$  (ampicillin) for the compounds investigated.

**Keywords** Antibiotics · Fluoroquinolones · Cephalosporins · Wastewater treatment plant · Yamuna River

## Introduction

Sewage treatment effluent quality is important because often, water is used, treated, and released for reuse by other communities. At every use–release cycle, water may be drawn for use with little or no treatment. In the last few years, a new category of pollutants (known as emerging contaminants, ECs) have been detected in effluents and receiving waters at trace levels. Antibiotics are among ECs detected in aquatic matrices. Antibiotics have important uses in both human and veterinary medicine for their antibacterial properties and as growth promoters. As their consumption increases, they are being detected in all the sectors of the environment. The total production of major antibiotics in India was more than 2,332 Mt in 2006–2007, with a growth rate of 10 %. Nearly 85 % of production is consumed in domestic markets (IDMA 2009). After administration to humans and animals, up to 90 % of the antibiotics can be excreted unchanged via urine and/or feces (Hirsch et al. 1998). These substances are only partially eliminated during sewage treatment (Ternes et al. 2003; Berset et al. 2004). Antibiotic residues entering in sewage are directly proportional to the amount of antibiotics used for human and other uses in particular area. In India, self-

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prescription rates are very high (more than 64 % of patients buy medicines over the counter without a prescription) (Greenhalgh 1987), and so residues may be higher than where human use is controlled by doctors.

Sewage treatment plants (STPs) are not designed to remove antibiotics. Antibiotic residues have been detected in different water matrices, including hospital wastewaters (Kummerer 2001; Lindberg et al. 2004), STP effluents (Batt et al. 2006; Watkinson et al. 2007; Mutiyar and Mittal 2013), STP biosolids (Kinney et al. 2006a), soil (Kinney et al. 2006b), surface waters (Kolpin et al. 2002, 2004; Batt et al. 2006), groundwater (Hirsch et al. 1999; Lindsey et al. 2001), sediments (Kerry et al. 1996; Kim and Carlson 2006), and drinking water (Zuccato et al. 2000, 2005). The first reported case of surface water contamination by antibiotics was in England in 1980, when Watts et al. (1982) detected at least one compound from the macrolide, sulfonamide, and tetracycline group of antibiotics in river water at concentrations of  $1 \mu\text{g l}^{-1}$ . Table 1 summarizes the literature reported antibiotic residue levels in different water and wastewater matrices. The occurrence of antibacterial agents in the aquatic environment has led to increasing concern for potential environmental risks. Hernando et al. (2006) showed adverse effects of pharmaceutical residues on bacteria, invertebrates, and algal populations, although Carlsson et al. (2006) did not support those findings. Kim et al. (2008) examined the acute aquatic toxicity on a marine bacterium (*Vibrio fischeri*), a freshwater invertebrate (*Daphnia magna*), and the Japanese Medaka fish (*Oryzias latipes*), and reported the  $\text{LC}_{50}$  for these organisms at two orders of magnitude higher than environmental concentrations. An overview of effect data for antibiotics residue on living beings is presented in Table 2. The effect data presented here are for acute toxicity, but chronic effects are possible from low-dose long-term exposures. In addition, the cocktail of antibiotics present in wastewater could result in synergistic or antagonistic effects. Discharge of antibiotics to the environment from the wastewater has been linked to the development of various resistant bacterial strains (Kummerer 2004). Therefore, the fate of pharmaceutical compounds in wastewater and other water systems should be properly investigated, including monitoring ambient concentrations in STPs and receiving water bodies. Previously, wastewater treatment plant (WWTP) effluents of Patancheru, Hyderabad, India reported to have the highest levels of ciprofloxacin (CIP) antibiotics residues (up to  $31,000 \mu\text{g l}^{-1}$ ) (Larsson et al.

2007). Thus, STPs are potentially important point source for these substances and present opportunities for applying centralized removal processes. Water bodies receiving WWTP effluents often have high pharmaceutical residues as peak high concentration of antibiotics residues (up to  $14,000 \mu\text{g l}^{-1}$ ) from surface, groundwater, and drinking water of Hyderabad area (Fick et al. 2009). Similarly, Tamiraparani River, Kaveri River, and Vellar River in southern part of India receiving treated sewage and industrial effluents detected high concentration of pharmaceuticals residues (Ramaswamy et al. 2011). Pharmaceutical substances in India are classified as a point of great concern. Wide range of pharmaceutical formulation is manufactured and used in India, which could lead to the release of more pharmaceuticals substances in the environment (Kurunthachalam 2012). Thus, the present study was carried out to investigate the antibiotics residues levels in Delhi's sewage and its receiving water body, Yamuna River. The objectives of this research were to establish contamination profiles of water matrices in Delhi and to determine environmental loadings of selected antibiotics from a STP. The selected antibiotics were ampicillin (AMP), ciprofloxacin (CIP), gatifloxacin (GAT), sparfloxacin (SPA), and cefuroxime (CEF) (Table 3). The antibiotics were selected to cover different groups of antibiotics ( $\beta$ -lactum, fluoroquinolones, and cephalosporins) and sales volume (IDMA 2009).

## Material and methods

### Site description

The National Capital Territory (NCT) is a part of the Indo-Gangetic Alluvial Plains and covers  $1,483 \text{ km}^2$ ; more than 60 % is urbanized with a population of 16.8 million (Census 2011). Yamuna River, a perennial river, originate from the Himalayan glaciers and passes through the NCT. The river is a major source of potable water to Delhi and is also the receiving water body for untreated and partially treated sewage from the city. Two percent of its total length of the river is in the city, but it receives 79 % of its total pollutant loading there, primarily through sewage and industrial discharges. Water quality in the Delhi, reaches of the Yamuna River, is characterized by high biological oxygen demand (BOD), chemical oxygen demand (COD), nutrients, total coliforms and fecal coliforms, and low dissolved oxygen (CPCB Report 2006). The water quality category of the river between

**Table 1** Some of the recent reported levels of antibiotics in water and wastewater matrices in literature

Sampling source	Conc. ( $\mu\text{g l}^{-1}$ for wastewater and $\mu\text{g kg}^{-1}$ for the sludge)										Reference	
	AMX	CFLO	CFX	CIP	NOR	OFX	PEN G	AMP	ERY			
<b>A. Sewage</b>												
STP (in), Spain				13.63	5.29							(Rosal et al. 2010)
WWTP (in), Hong Kong			0.54	0.72	0.06	0.34		0.40	0.22			(Li et al. 2009)
WWTP (eff), Hong Kong			0.38	0.07	0.01	0.56		0.13	0.10			(Li et al. 2009)
WWTP (in), Australia	6.94	6.15	64.00	1.11								(Watkinson et al. 2009)
WWTP (eff), Australia	0.05	1.80	2.5	ND								(Watkinson et al. 2009)
STP (eff), Wan Chai West Hongkong	1.0–1.67		2.93–3.05		0.85–1.69	5.81–6.83			1.59–3.94			(Minh et al. 2009)
STP (eff), North Point Hong Kong			4.98–5.02		2.07–2.51	4.15–4.65			1.89–2.05			(Minh et al. 2009)
STP (eff), Wan Chai East Hong Kong	0.67–1.66		4.70–5.07		1.31–3.70	5.90–7.78			2.81–3.15			(Minh et al. 2009)
STP (eff), Central Hong Kong	0.2–1.23		2.90–4.30		1.39–1.60	3.53–7.87			1.31–4.33			(Minh et al. 2009)
WWTP, Shijiazhuang China										Up to 389,000		(Li et al. 2008)
WWTP (eff) Brisbane, Australia	0.19–0.28	0.50–0.98	4.60–5.60	3.80–6.90								(Watkinson et al. 2007)
STP (eff), Italy				0.25		0.60						Zuccato et al. 2005
STP (eff), Michigan USA						0.20						Nakata et al. 2005
WWTP (eff), Atlanta			0.10–0.40			0.26–0.35						Renew and Huang 2004
Raw sewage, Switzerland			0.35–0.57		0.31–0.52							Golet et al. 2003
STP (eff), Germany									2.5–6.0			Termes and Hirsch 2000
STP Sludge, Switzerland			2500–3500		1700–3300							Golet et al. 2003
<b>B Surface water</b>												
Danube river, Serbia									0.06			Grujic et al. 2009
Tamis river, Serbia									0.04			Grujic et al. 2009
Rivers Po and Lambro, Italy			0.02–0.03			0.31			0.02–0.16			Calamari et al. 2003; Castiglioni et al. 2004; Zuccato et al. 2005
Lake Ocaga, Serbia									0.08			Grujic et al. 2009
<b>C Ground water</b>												
Ground water, Serbia									0.03–0.14			Grujic et al. 2009

Table 1 (continued)

Sampling source	Conc. ( $\mu\text{g l}^{-1}$ for wastewater and $\mu\text{g kg}^{-1}$ for the sludge)											Reference	
	AMX	CFLO	CFX	CIP	NOR	OFX	PEN G	AMP	ERY				
D Hospital effluents													
HWW (eff), Brisbane	0.90	10.00		15.00									Watkinson et al. 2009
HWW (eff), Brazil	35.12	801.02	300.1				434.46	389.13					Berto et al. 2009
Hospital of Santa Maria (eff), Brazil				140									Vasconcelos et al. 2009
HWW (eff), Hanoi USA				25800	15200								Duong et al. 2008
Sweden county hospital (eff) Kalmar, Sweden				3600–101000									Lindberg et al. 2004
University hospital (eff), Wurzburg Germany				2–51	44								Ohlsen et al. 2003
Different hospitals (eff), Germany				Up to 124,500									Hartmann et al. 1988
Indian conditions													
UCT hospital Ujjain (India)				7.6–31	5.7		66–73.2						Diwan et al. 2010
CRG hospital (Ujaain)				64.8–236.6	20.6–22.8		1.5–7.5						
CETP Hyderabad (India)				28000–31000	390–420		150–160						Larsson et al. 2007
CETP Hyderabad (India)				14000	25		55						Fick et al. 2009
Kazipeli lake Hyderabad				2500–6500	60–520		2–11						Fick et al. 2009
Isakavagu-Nakkavagu rivers, India				10–2500	ND-4.7		0.18–10						Fick et al. 2009
Ground water (wells)				0.04–14	ND-0.31		ND-0.48						Fick et al. 2009

*In* influents, *eff* effluents, *STP* sewage treatment plant, *WWTP* wastewater treatment plant, *HWW* hospital wastewater treatment plant effluent, *AMX* amoxicillin, *CFLO* Cefaclor, *CFX* cephalexin, *CIP* ciprofloxacin, *OFX* ofloxacin, *PEN G* penicillin G and its degradation products, *AMP* ampicillin, and *ERY* erythromycin

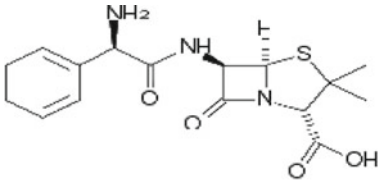
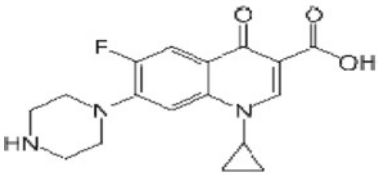
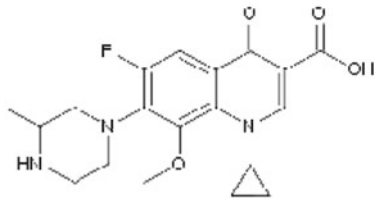
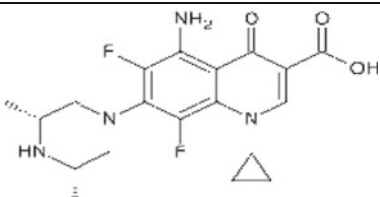
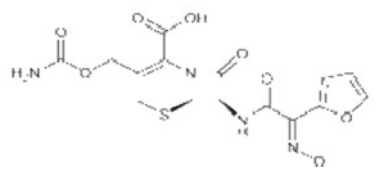
**Table 2** Overview of effect data for antibiotics residue on living beings

Test organisms	Compound	Antibiotic class	Measured unit	Concentration ( $\mu\text{g l}^{-1}$ )	Reference
Algae					
<i>M. aeruginosa</i>	Ciprofloxacin	Quinolones	24 h EC <sub>50</sub>	5.0	Halling-Sorensen et al. 2000; Boxall 2002
			Growth inhibition	<5.0	Halling-Sorensen et al. 2000
<i>C. meneghiniana</i>	Amoxicillin	$\beta$ lactum	EC <sub>50</sub>	3.7	Lutzhof et al. 1999
<i>P. subcapitata</i>	Sulfamethoxazole	Sulphonamide antibiotic	96 h EC <sub>50</sub>	2,400	Ferrari et al. 2003
<i>L. gibba</i>	Sulfamethoxazole	Sulphonamide antibiotic	96 h EC <sub>50</sub>	90	Ferrari et al. 2003
<i>S. leopoliensis</i>	Sulfamethoxazole	Sulphonamide antibiotic	7 day EC <sub>50</sub>	81	Brain et al. 2004
	Sulfamethoxazole	Sulphonamide antibiotic	96 h EC <sub>50</sub>	30	Nunes et al. 2005
	Sulfamethoxazole	Sulphonamide antibiotic	3 day EC <sub>50</sub>	520	Isidori et al. 2005
	Flumequine	Quinolones	EC <sub>50</sub>	159	Lutzhof et al. 1999
	Oxolinic acid	Quinolones	EC <sub>50</sub>	180	Lutzhof et al. 1999
	Sulfadiazine	Sulfonamides	EC <sub>50</sub>	135	Lutzhof et al. 1999
	Oxytetracycline	Tetracyclines	EC <sub>50</sub>	207	Lutzhof et al. 1999
<i>C. reinhardtii</i>	Streptomycin	Aminoglycoside	EC <sub>50</sub>	280	Harrass et al. 1985
<i>S. capricornum</i>	Streptomycin	Aminoglycoside	EC <sub>50</sub>	660	Harrass et al. 1985
	Erythromycin	Quinolones	3 day EC <sub>50</sub>	20	Isidori et al. 2005
	Ciprofloxacin	Quinolones	EC <sub>50</sub>	2,970	Lutzhof et al. 1999
Bacteria					
<i>Soil Bacteria</i>	Oxytetracycline, macrolides and streptomycin	Antibacterials	Bacterial resistance measured in soil bacteria against antibiotics		Sengelov et al. 2003
<i>Soil microbial communities</i>	Tylosin	Macrolide	Affects structure of soil microbial communities		Westergaard et al. 2001
<i>Synechocystis</i> sp.	Erythromycin	Macrolide	Growth inhibition	1	Pomati et al. 2004
Invertebrates					
<i>C. dubia</i>	Sulfamethoxazole	Sulphonamide antibiotic	7 day EC <sub>50</sub>	210	Isidori et al. 2005
	Oxytetracycline	Tetracycline group	7 day EC <sub>50</sub>	180	Isidori et al. 2005
<i>D. magna</i>	Neomycin	Aminoglycoside antibiotic	EC <sub>50</sub>	90	Park and Choi 2008
<i>M. macrocopa</i>	Neomycin	Aminoglycoside antibiotic	EC <sub>50</sub>	740	Park and Choi 2008
Aquatic plants					
<i>L. minor</i>	Erythromycin	Macrolide	Growth inhibition in aquatic plant		Pomati et al. 2004
	Tetracycline	Tetracycline group	Growth inhibition in aquatic plant		Pomati et al. 2004

Table 2 (continued)

Test organisms	Compound	Antibiotic class	Measured unit	Concentration ( $\mu\text{g l}^{-1}$ )	Reference	
<i>L. Gibba</i>	Enrofloxacin	Quinolones	7 day $\text{EC}_{50}$	114	Robinson et al. 2005	
	Ampicillin	$\beta$ lactam	7 day $\text{EC}_{10}$	1,000	Brain et al. 2004	
	Neomycin	Aminoglycoside antibiotic	7 day $\text{EC}_{10}$	1,000	Brain et al. 2004	
	Sulfamethoxazole and levofloxacin	Various antibiotics	Phytotoxic effects		Brain et al. 2004	
	Fluoroquinolones	Quinolones	Affecting chloroplast replication		Brain et al. 2008	
	Tetracyclines	Various antibiotics	Affecting transcription and translation		Brain et al. 2008	
	Macrolides, Lincosamides, Aminoglycosides					
	Triclosan (Polychloro phenoxy phenol)	Antimicrobial	Affecting metabolic pathways such as folate and fatty acid biosynthesis		Brain et al. 2008	
	Animals					
		<i>Rainbow trout</i>				
<i>Artemia salina nauplii</i>	Sulphamethazole	Sulphonamide antibiotic	Inhibition of basal EROD activity		Laville et al. 2004	
	Various antibiotics		Depressed hatching rate, high mortality rate		Migliore et al. 1993; Wollenberger et al. 2000	
	Flumequine.	Quinolones	Fitness lost by altering the pigmentation		Brambilla et al. 1994	
<i>Vibrio fischeri</i>	Ampicillin	$\beta$ lactam	24 h $\text{EC}_{50}$	163,000	Backhaus et al. 2000	
	Norfloxacin	Quinolones	24 h $\text{EC}_{50}$	22	Backhaus et al. 2000	

**Table 3** Details of the molecules investigated in the present study

Common Name	Chemical Formula	Chemical Structure	CAS No.	Mol. Mass (g mol <sup>-1</sup> )
Ampicillin (AMP)	C <sub>16</sub> H <sub>19</sub> N <sub>3</sub> O <sub>4</sub> S		69-53-4	349.41
Ciprofloxacin (CIP)	C <sub>17</sub> H <sub>18</sub> FN <sub>3</sub> O <sub>3</sub>		85721-33-1	331.35
Gatifloxacin (GAT)	C <sub>19</sub> H <sub>22</sub> FN <sub>3</sub> O <sub>4</sub>		112811-59-3	375.39
Sparfloxacin (SPA)	C <sub>19</sub> H <sub>22</sub> F <sub>2</sub> N <sub>4</sub> O <sub>3</sub>		110871-86-8	392.41
Cefuroxime (CEF)	C <sub>16</sub> H <sub>16</sub> N <sub>4</sub> O <sub>8</sub> S		55268-75-2	424.37

Wazirabad and Okhla Barrage is “E”; only suitable for irrigation and industrial cooling. The water quality of the Yamuna River in this stretch is so bad that the photosynthesis processes are absent. BOD removal takes place mainly by the settling of organic matter. Samples were taken from Okhla STP and six different locations on the 22-km long stretch of the river passing through Delhi. The first sampling site was upstream of Wazirabad barrage near cremation ground. Sampling at this location reflects the water quality before receiving the wastewater discharges from Delhi; drinking water for Delhi is drawn

from here. The other five locations were selected to reflect the impact of wastewater discharge from various sources. Raw and treated sewage samples were taken from Okhla STP, which has the capacity to process 636 million liters per day (MLD). The plant operates to 75 % of its design capacity, with BOD, COD, and turbidity removal of 92.0, 92.9, and 95.2 %, respectively (Jamwal and Mittal 2010). The plant has several different units, with capacities ranging from 55 to 236 MLD. The study was carried out at 110 MLD old unit. Figure 1 shows the sampling points.

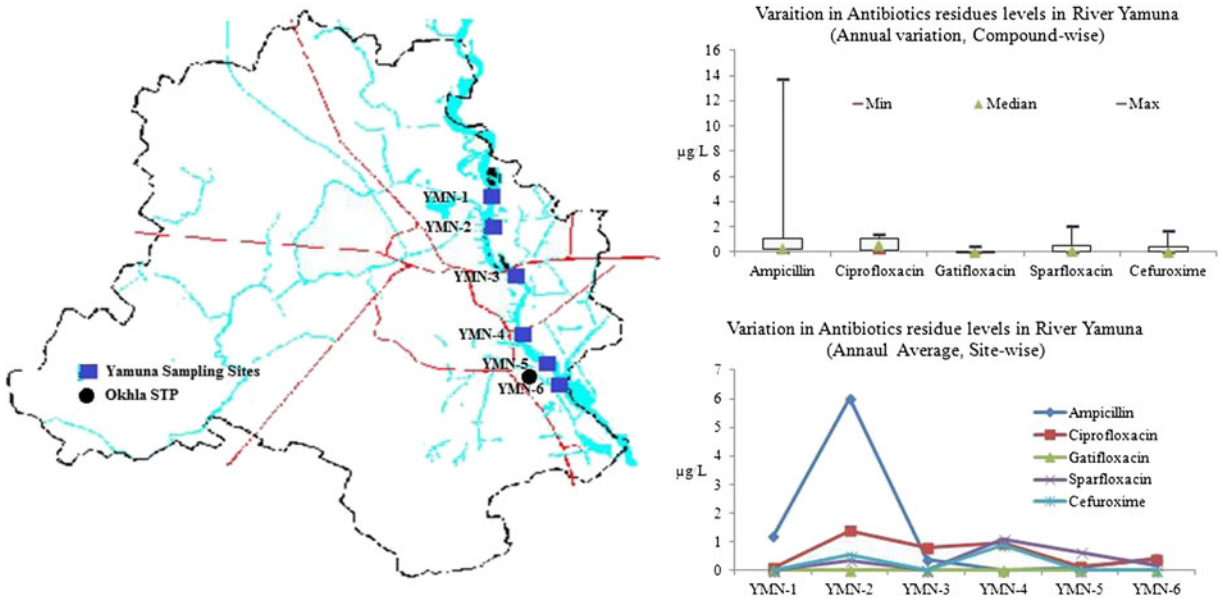


Fig. 1 Sampling points and the antibiotics residues concentration in Yamuna river

Sample collection

Two types of samples were collected. Type 1 was wastewater samples; type 2 was environmental samples from Yamuna River (Fig. 1). The sewage samples were taken from 110 MLD of the Okhla STP. The STP uses the activated sludge process. Samples were collected from influents (S1, Fig. 2) and effluents (S2, Fig. 2) of the STP. Type 1 samples were taken five times, and type 2 samples were taken thrice in winter, summer, and

monsoon seasons from the six environmental sampling locations on the river. Samples were collected in 5 L amber colored, food grade, unused plastic bottles. The bottles were washed with tap water and properly rinsed with distilled water. Before sampling, the bottles were rinsed twice with the sample water. All samples were collected as per APHA, AWWA 2000 (Page No 6-2, 6010 B). The collected sample bottles were kept in airtight iced plastic containers and were transported to the laboratory within 2–4 h of their collection. Samples

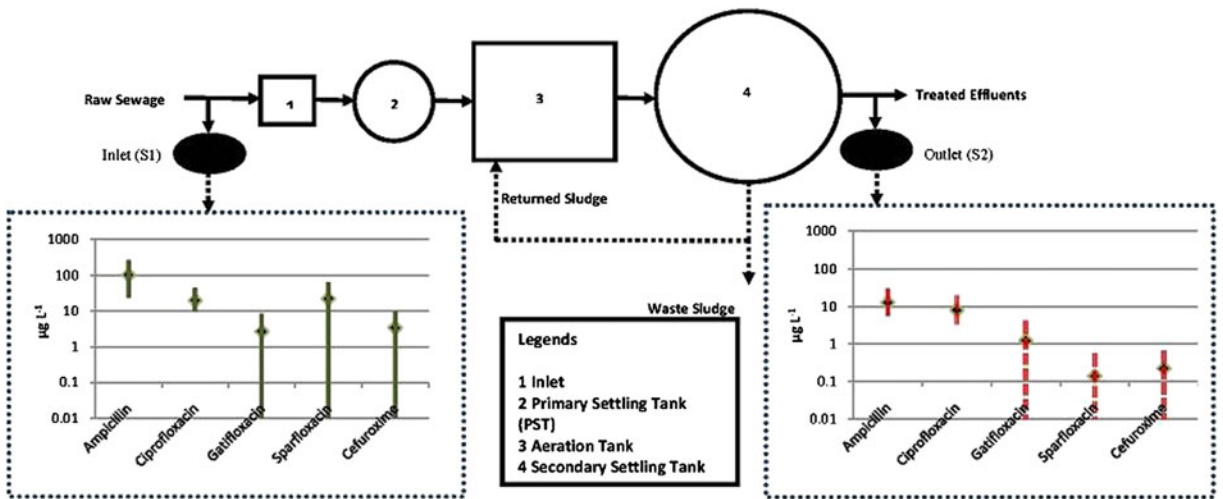


Fig. 2 Sampling points at different locations in STP, Okhla



were acidified to pH 3 in the field using formic acid. Collected samples were preserved at 4 °C until further analysis.

### Analytical procedure

#### *Extraction of antibiotics residues*

The samples were vacuum filtered through 0.7  $\mu\text{m}$  glass fiber filters to remove suspended matter. The extraction was performed in the same day to avoid any sample degradation. A solid-phase extraction (SPE) procedure was applied to the wastewater samples using commercial Oasis HLB (divinylbenzene/*N*-vinylpyrrolidone copolymer) cartridges (200 mg, 6  $\text{cm}^3$ ) from Waters (Milford, MA, USA). A vacuum manifold assembly (Milford, MA, USA) fitted with an external pump was used for this purpose. The SPE cartridges were preconditioned with 5 ml of methanol and 5 ml of Milli-Q LC-grade water (pH 3) at a flow rate of 3–5  $\text{ml min}^{-1}$ . Samples (500 ml) were loaded at a flow rate of 5–8  $\text{ml min}^{-1}$  followed by a washing step with 5 ml of water (pH 7). After that, the cartridges were dried by nitrogen stream for approximately 15 min and finally eluted with 4 $\times$ 2 ml of methanol at 1  $\text{ml min}^{-1}$ . The extracts were initially concentrated in Rotavapor® (Buchi, Switzerland) and finally evaporated to dryness by a gentle nitrogen stream. The residues were redissolved in 1 ml of mobile phase (1:1, 0.1 % aqueous TFA and ACN) for HPLC analysis.

#### *Chromatographic conditions*

Chromatographic separation of antibiotics were performed on Waters Spherisorb® ODS-2 (250 mm $\times$ 4.6 mm, 5  $\mu\text{m}$ ) HPLC column. Analyses were performed at a flow rate of 1.0  $\text{ml min}^{-1}$  at the ambient temperature. A gradient flow programming with binary pumps was used, containing solvent A (0.1 % aqueous TFA) and solvent B (ACN) as mobile phase during the analysis. The details of flow programming are given in Table 4. The injection volume was fixed to 20  $\mu\text{l}$  by using standard volume loop. All the compounds were eluted within 20 min, thus a chromatographic run was programmed for 30 min. The PDA detector was used for detection, and the chromatograms were extracted at two different wavelengths of 215 and 280 nm. The chromatograms were extracted at 215 nm for ampicillin, as lambda-max of ampicillin is around 215 nm, and

**Table 4** Gradients used to separate the molecules for (A) aqueous TFA and (B) ACN

Time (min)	Solvent		Flow rate ( $\text{ml min}^{-1}$ )
	(A) 0.1 % aqueous TFA	(B) ACN	
0	85	15	1.0
15	65	35	1.0
20	20	80	1.0
25	60	40	1.0
30	85	15	1.0

there was no absorbance at 280 nm shown in Figs. 3 and 4, which represents the UV absorbance by ampicillin at these two different wavelengths. The rest of the compounds show high UV absorbance at 280 nm than 215 nm (Fig. 4). The chromatographic and integrated data were recorded and processed by using Empower Software (Waters, USA). Quantification was performed using external calibration and peak area measurement. The details of antibiotic class, retention time, and wavelength of extraction are given in Table 5.

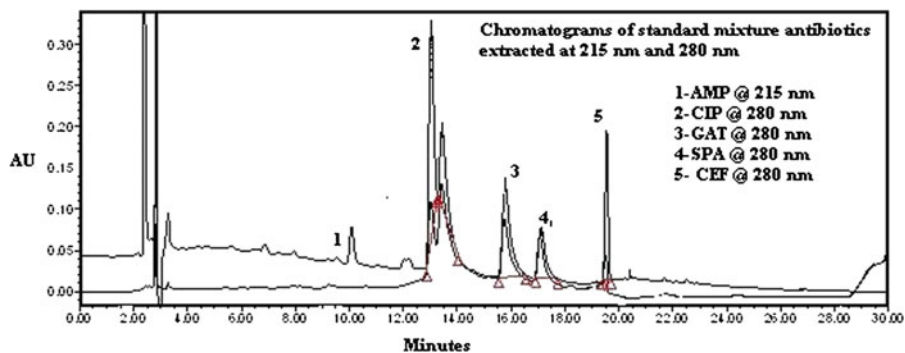
#### *Limit of quantification and linearity*

For concentration to be accepted as limit of quantification, the percent deviation from the nominal concentration (accuracy) must be  $\pm 20\%$ , and the relative standard deviation should be less than 20 %. Linearity was tested in the concentration range of 10–1,000  $\mu\text{g l}^{-1}$  (10, 50, 100, and 500  $\mu\text{g}$  and 1  $\text{mg l}^{-1}$ ). All the compounds showed good correlation with coefficients,  $r^2=0.98$ –0.99 (Table 5). The detection limits (DL) and quantification limits (QL) were calculated to be 10 and 30  $\text{ng ml}^{-1}$ , respectively, considering signal to noise (S:N) ratios. A S:N ratio of 3 was taken as DL, while a S:N ratio of 10 was taken as QL. The relative recoveries of antibiotics were calculated by comparing the peak areas for extracted antibiotics from spiked water and a standard solution of the antibiotic in deionized water.

#### Reagents and chemicals

Analytical grade (Merck, Darmstadt Germany) chemicals were used throughout the study without any further purification. The solvent used were of HPLC grade only. Milli-Q water was used to prepare all the reagents and calibration standards. The glassware was

**Fig. 3** Chromatogram of spiked antibiotics extracted at 215 and 280 nm



washed with dilute nitric acid (1.15) followed by several portions of distilled water. Standards of antibiotics were procured from Sigma-Aldrich Chemicals Private Ltd., Bangalore (India) and Dr. Ehrenstrofer GmbH, Augsburg (Germany). The working standards of antibiotics were prepared by dissolving the suitable amount of antibiotics in mobile phase. All solutions prepared for HPLC were passed through 0.2–0.6  $\mu\text{m}$  polypropylene filters (Millipore, EMD Millipore Corp., Billerica, MA, USA) before HPLC analysis.

## Results and discussion

### Residues recovery

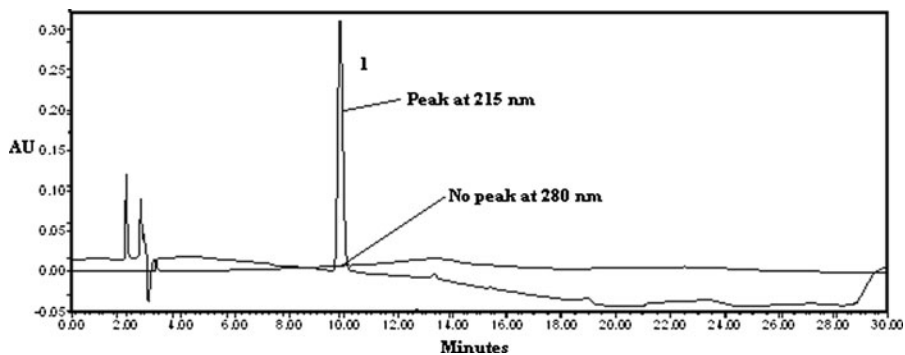
The test compounds are drawn from three groups of antibiotics,  $\beta$ -lactum, fluoroquinolones, and cephalosporins. These compounds exist as cationic species in acidic pH, zwitterionic species at neutral pH, and anionic species at basic pH. Rao et al. (2008) reported the highest recoveries for antibiotics with Oasis HLB as compared to Supelco C18, Lichrolut EN, and Isolute ENV at pH 3 for these groups. Thus, Oasis HLB cartridges were used for solid-phase extraction, retaining the compounds as cationic species. The relative analytical recoveries from

present method were checked by spiking different known concentration of antibiotics in the double-distilled water at acidic pH (pH 3). Five hundred milliliter of double-distilled water was spiked with 1, 10, 50, and 100  $\mu\text{g l}^{-1}$  concentrations and recovery was checked. The resulting recovery efficiencies (REs) (Table 5) categorize the antibiotics into low, medium, and high recoveries. RE for AMP was 25.5, relatively lower than previous reports. Rao et al. (2008) reported RE of 80–103 %, Jones-Lepp (2006) reported 77 $\pm$ 22 % recoveries, while Loganathan et al. (2009) reported recoveries of 50 % for antibiotics. The lower RE could be due to analyzing 500-ml samples, as antibiotic RE decreases with increasing sample volumes. Rao et al. (2008) reported reduction in RE of up to half when sample volume increased from 100 to 500 ml.

### Analysis of samples from STP

All targeted antibiotics were detected in the wastewater samples from Okhla STP. The antibiotic residues were less in the treated effluents than untreated influents (Fig. 2). The minimum and maximum concentrations detected in influents were for GAT and AMP, respectively. AMP and CIP were detected in each influent sample ( $N=5$ ), SPA was detected in four samples, while

**Fig. 4** Chromatogram of single spike ampicillin extracted at 215 and 280 nm



**Table 5** Recovery efficiency (RE) of various molecules obtained

Compound	Antibiotic class	Wavelength of extraction (nm)	RT (min)	R <sup>2</sup>	RE % ± SD	Recovery category
Ampicillin	β-lactam	215	10.1	0.9912	25.5±2.5	Low
Ciprofloxacin	Fluoroquinolone	280	13.2	0.9806	56.8±4.6	Medium
Gatifloxacin	Fluoroquinolone	280	15.8	0.9843	60.3±1.5	Medium
Sparfloxacin	Fluoroquinolone	280	17.1	0.9909	101.8±11.9	High
Cefuroxime	Cephalosporin	280	19.6	0.9957	108.8±13.7	High

GAT and CEF were detected in three samples. The mean residues concentration was 104.2 μg l<sup>-1</sup> for AMP, 20.1 μg l<sup>-1</sup> for CIP, 2.7 μg l<sup>-1</sup> for GAT, 22.5 μg l<sup>-1</sup> for SPA, and 3.4 μg l<sup>-1</sup> for CEF (Table 6). For effluents, the mean antibiotics residues concentration was 12.68 μg l<sup>-1</sup> for AMP, 8 μg l<sup>-1</sup> for CIP, 1.22 μg l<sup>-1</sup> for GAT, 0.14 μg l<sup>-1</sup> for SPA, and 0.22 μg l<sup>-1</sup> for CEF (Table 6). These are higher levels of antibiotics in wastewater than previously reported by some others (e.g. Yang et al. 2005; Xu et al. 2007; Heidler and Halden 2008; Loganathan et al. 2009) from different parts of the world, but lesser than those reported by Li et al. (2008) (see Table 1). Xu et al. (2007) detected residues up to 2.1 μg l<sup>-1</sup>, while Heidler and Halden (2008) reported similar levels of antibiotics to those reported here (i.e., 10.8 μg l<sup>-1</sup> in treated wastewater). Yang et al. (2005) reported concentrations of pharmaceutical residues around 1.1 μg l<sup>-1</sup>. Li et al. (2008) reported 389 mg l<sup>-1</sup> of penilloic acid, a degradation product of penicillin G, with 153 μg l<sup>-1</sup> of the parent compound also present. Residue levels in wastewater is a function of several factors, such as consumption rates, number of medical care units in the watershed, the general civic sensibility regarding

the medicine use, and disposal of unused and out of date medicines.

Antibiotics removal and loading estimations from STP

Mass balance estimates were used to investigate how well the STP removed pharmaceuticals from the wastewater, assuming the detected concentrations in the influents and effluents are representative of inflow and outflow masses of antibiotics from the system. The mass balance for each compound was obtained using the following equation:

$$\text{Removal Efficiency} = \frac{(C_i \times V_{\text{STP}}) - (C_e \times V_{\text{STP}})}{(C_i \times V_{\text{STP}})}$$

With removal efficiency expressed as percent

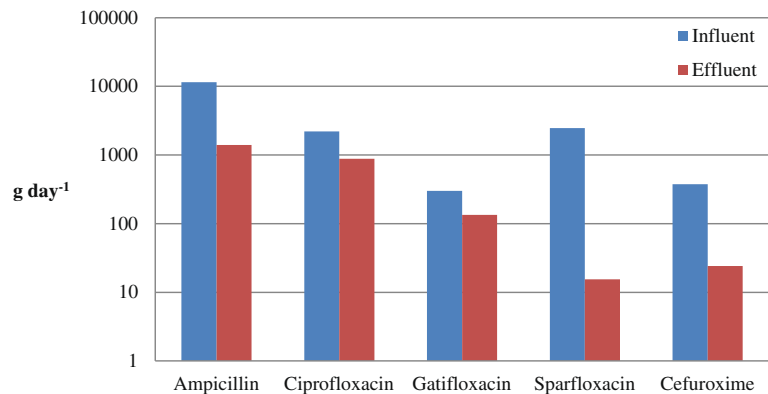
- C<sub>i</sub> Mean influent concentration
- C<sub>e</sub> Mean effluent concentration
- V<sub>STP</sub> Volume of STP (110 MLD)

The influent loadings varied from 301 to 11,462 g/day for the different antibiotics, and 15.4–1,395 g/day were released in the effluents (Fig. 5). Removal efficiencies are presented in Table 6 and varied from 55 to 99 %.

**Table 6** Antibiotics profiling and removal efficiency (% removal) of the STP

Compound	Concentration (μg l <sup>-1</sup> ) (N=5)						Removal efficiency (% removal)
	Influents			Effluents			
	Range	Avg	SD	Range	Avg	SD	
Ampicillin	23.5–263.3	104.2	98.11	6.3–27.1	12.68	8.38	88
Ciprofloxacin	9.7–45.4	20.06	15.10	2.9–17.7	8	6.33	60
Gatifloxacin	ND-8.4	2.74	3.88	ND-3.7	1.22	1.73	55
Sparfloxacin	ND-63.2	22.49	24.46	ND-0.5	0.14	0.22	99
Cefuroxime	ND-9.5	3.42	3.96	ND-0.6	0.22	0.30	94

**Fig. 5** Environmental loadings of antibiotics ( $\text{g day}^{-1}$ ) from a STP



Golet et al. (2003) suggested that sorption to particulates and subsequent removal in sewage sludge is the primary mechanism to reduce fluoroquinolone concentrations during secondary wastewater treatment. Castiglioni et al. (2006) found that the removal of pharmaceuticals in surface waters depended on their behavior in the particulate phase, supporting the removal by sorption. In a field study at a full-scale municipal wastewater treatment plant in Switzerland, Golet et al. (2003) reported a 49–60 % reduction in dissolved fluoroquinolone (ciprofloxacin and norfloxacin) concentrations during biological treatment. Partial elimination of macrolides in effluents from wastewater treatment plants has been reported by McArdell et al. (2003). Lin et al. (2009) reported varied removal efficiencies among WWTPs; high removal rates (72–100 %) were generally for non-steroidal anti-inflammatory drugs, estrogens, and caffeine, but some antibiotic groups (macrolides, penicillin, and imidazole) were not removed at all. Bendz et al. (2005) reported lesser removal efficiencies (<50 %) for antibiotics in STPs. Removal efficiencies for antibiotics appears to vary with plants, affected by their operations, geographic locations, and environmental factors. The estimated loadings were 1,395 g/day for AMP, 880 g/day for CIP, 134 g/day for GAT, 15.4 g/day for SPA, and 24.2 g/day for CEF, and the total loading from these compounds is 2,428 g/day. This is a rough estimate, and needs to be used with caution because suspended particulates of wastewater samples were not analyzed (we accounted only for antibiotics occurring in the dissolved fraction), and the samples are only from one unit of one STP. The mass balance for these five compounds suggests that effluent may contain hundreds of such compounds, as treatment does not seem to remove all of the influent concentrations, with grab samples with activated sludge process as treatment process.

#### Antibiotics levels in the Yamuna River

The Yamuna River receives effluents from 17 STPs and also carries sewage and industrial discharges from 17 stormwater drains. Stormwater drains in Delhi are highly polluted by discharge of domestic and industrial discharge. The river water samples taken across six different sites showed the presence of all targeted antibiotic residues in different seasons (Fig. 1). Antibiotics residue levels were lowest in monsoon, followed by summers, and maximum in winters, both in terms of frequency and concentrations. The concentration varied from 0.2 to 13.75  $\mu\text{g l}^{-1}$  for AMP, ND-1.44  $\mu\text{g l}^{-1}$  for CIP, ND-0.48  $\mu\text{g l}^{-1}$  for GAT, ND-2.09  $\mu\text{g l}^{-1}$  for SPA, and ND-1.7  $\mu\text{g l}^{-1}$  for CEF in winters. In winters, in river, AMP was found in all the six sampling sites, while GAT was found at three sites only. CIP, SPA, and CEF were present in samples from five sites. In summers, CIP was found in all the six sampling sites, while AMP was found at five sites only. SPA and CEF were present in samples from four and three sites, respectively, while GAT was not detected at any of the sampling site (Fig. 1). Antibiotics residue levels and occurrence frequency were reduced in monsoon. This may be observed due to the fact that monsoon brings huge freshwater to Yamuna River, especially in Delhi stretch which has very less freshwater flow throughout the year. Antibiotics levels in the Yamuna River are found at higher concentrations than reported for other rivers in Italy and Serbia (Table 1), while similar levels (lower micrograms per liter, up to 5.2  $\mu\text{g l}^{-1}$ ) of pharmaceutical residue (triclosan) have been reported in river water from the southern part of India (Ramaswamy et al. 2011). Higher concentrations levels of antibiotics have been reported in literature as follows: 11.92  $\mu\text{g l}^{-1}$  of sulfamethoxazole in the Llobregat River in NE Spain

(Munoz et al. 2009),  $10 \mu\text{g l}^{-1}$  of CIP in the Arc River in France (Feitosa-Felizzola and Chiron 2009),  $1.3 \mu\text{g l}^{-1}$  of CIP in the Brisbane River Australia (Watkinson et al. 2009), and  $80 \mu\text{g l}^{-1}$  of ERY in the Duhan River in Taiwan (Lin and Tsai 2009). Higher levels of antibiotics in Yamuna River water could be expected as the sampled section of the Yamuna River is one of the most contaminated river stretches in India. It receives around 3,000 MLD of sewage from various drains. Generally, wastewater treatment is not universal and the disposal rate of unused medicine is high across India. Though along the Delhi stretch of Yamuna, no specific trend in pharmaceutical residues was observed, but data revealed that one of the sites (YMN-2) was having the maximum concentration of these pharmaceuticals. There was a sharp increase in antibiotic residues levels from sites YMN-1 to YMN-2. This may be due to the fact that site YMN-1 is located upstream of a city where no major drain meets the river. Site 1 represents the water quality of the river before it receives the wastewater discharges from Delhi. Highest concentration and maximum number of antibiotics detected at this site (YMN-2; Fig. 1) could be justified on the basis of huge load of mixed sewage brought by Najafgarh drain (the largest drain in Delhi) at this site. In sites YMN-3 to YMN-6, changes were marginal in antibiotic residues levels. Marginal changes in antibiotic residues levels (YMN-3 to YMN-6) may be due to reduction by adsorption, degradation, and photolysis of antibiotic residues in the river and addition of more sewage (treated and untreated) by various drains to the river.

The detection of antibiotics residues (even at sub-nanogram per liter) is alarming for ecosystem sustainability. These compounds are specially engineered to show their effect at trace levels. There are no reports of direct effect on human beings from contaminated water but effects on other organisms have been documented. Kummerer (2004) reported the development of various resistant bacterial strains associated with discharges of antibiotics. Kim et al. (2008) and Oetken et al. (2004) reported death and decline in reproduction of standard test organisms like *V. fischeri*, *D. magna*, *M. macrocopa*, *O. latipes*, and some invertebrates. Alighardashi et al. (2009) reported acute sensitivity of sludge bacteria to erythromycin causing floc disintegration and breakage of filaments. Various aquatic toxicity data were summarized in Table 2. Antibiotic residues levels in untreated sewage were enough to show acute toxic effect to multiple test organisms (Tables 2 and 6), and as untreated

sewage also finds its ways to various drains and river Yamuna in Delhi, the river water could possibly have harmful ecotoxicological effects to aquatic organisms. Antibiotic residues levels detected in river Yamuna were in the similar ranges to show acute toxicity. Ciprofloxacin concentration in winters at site YMN-2 and YMN-3 ( $1.44$  and  $1.19 \mu\text{g l}^{-1}$ ) were in close proximity to cause growth inhibition to algal species *Microcystis aeruginosa* (Table 2, Halling-Sorensen et al. 2000). Algal species are very sensitive to antibiotics, and, as algae are the basis of the food chain, even slight decreases in algal populations could affect the nutrient cycle and food supply to higher trophic levels. Also note that the effects reported in the test organisms during toxicity tests are carried out under single-drug exposure. The observed effect concentration (EC) decreases when organisms are exposed to mixture of drugs. DeLorenzo and Fleming (2008) reported a 75 % reduction in  $\text{EC}_{50}$  in *Dunaliella tertiolecta* for mixture of drugs when compared to the same level of single drugs. Aquatic lives in the water bodies receiving treated sewage are exposed to a mixture of drugs residues, not just single compounds. Similarly, developments of antibiotic-resistance bacteria (Middleton and Salierno 2012; Shah et al. 2012) and risk to aquatic organisms (Zhang et al. 2012) have been correlated with pharmaceutical residues presence in wastewater from inadequate wastewater treatment systems.

## Conclusions

Pharmaceutical compounds are used in human medicine and animal treatment. They can reach the aquatic environment via sewage treatment plants. Too little is known about the occurrence and fate of antibiotics in the environment and the potential risk they pose to aquatic ecosystems. Fundamental data on the occurrence and levels of antimicrobials in different sections of the environment are needed. LC/MS or LC/MS/MS with SPE are often used for antibiotic determination. The advantages of LC/MS or LC/MS/MS over HPLC-PDA are better detection, lower detection limit and quantification limit, reduced matrix effect etc., but very high cost of the LC/MS or LC/MS/MS is a major hurdle in getting access to these types of instruments at the laboratories of developing countries. HPLC-PDA is relatively cheap and widely available at most of the research institutes, and, thus, researchers can

select among both the methods (LC/MS or HPLC-PDA) depending upon the facility available. Since STPs acts as point source discharges for these compounds, they are useful monitoring points to calculate loadings. This screening study represents the first measurement of antibiotics residues in aqueous environments in Delhi, India, and one of the few ever conducted in India. We evaluated the levels of antibiotics in influents wastewater at a STP, determined the effect of treatment (removable efficiencies ranged from 55 to 99 %), and measured high concentrations in the receiving water body, the Yamuna River, using the optimized HPLC-PDA method. The potential for ecotoxicological impact from these antibiotics makes it a priority to monitor these compounds more widely. A detailed study is needed to evaluate the total load of pharmaceutical compounds released from STPs, along with investigation of the physical, chemical, and biological processes in the receiving aquatic system such as adsorption on particulates, colloids, and organic matter in order to better assess the possible ecotoxicological risks. Significant gaps still exists in the understanding of the interaction between antibiotic residues, metabolites, and resistance promotion, and synergistic effect from organism exposure to multiple compounds in receiving water bodies.

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