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RESEARCH ARTICLE

Risk assessment of antibiotic residues in different water matrices in India: key issues and challenges

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Abstract Global detection of antibiotic substances in water matrices has considerably increased in the recent past. However, in India research on this issue is limited or generalised in the literature. Risks associated with the presence of antibiotics in the environment can be quantified using a hazard quotient (HQ) approach. Here, HQs were developed using the measured environmental concentration (MEC) approach for antibiotic residues in Indian water matrices previously reported in the literature. In the present study, environmental risk assessment, using the HQ index [HQ=measured environmental concentration (MEC)/predicted no effect concentration (PNEC)] for different antibiotics, was performed according to the guidelines of European Medicine Evaluation Agency (EMEA). MEC and PNEC levels were obtained from the literature. PNEC values were also calculated from EC_{50} using a safety factor when no PNECs were reported in the literature. HQs were obtained for industrial effluents ($HQ=10^4$) that were greater than any previously reported values. Ciprofloxacin, a fluoroquinolone antibiotic, seemed to present the greatest risk in India. The HQ indices for Indian water matrices were in the following order: industrial effluents>lake water>river water>hospital effluents>treated sewage ≃ groundwater. A very high HQ represents a potential environmental concern for aquatic environments in India and demands that immediate attention be devoted to regulating these compounds, especially in pharmaceutical industrial wastewater.

Keywords Antibiotic residues · Ecotoxicology · Risk assessment · Hazard quotient

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Introduction

India is the third largest producer of pharmaceutical chemicals after the USA and Europe, and its turnover is expected to reach US$74 billion per year by 2020 (CCI 2012). Production and use of large quantities of pharmaceuticals for human and veterinary applications could lead to the release of more pharmaceuticals substances into the environment (Kurunthachalam 2012). After administration to human and animals, up to 90 % of the antibiotics can be excreted unchanged (Hirsch et al. 1999) and reach to the aquatic environment. Reports on the environmental fate of pharmaceuticals in wastewater treatment plants (WWTP) shows that they may be partially removed during conventional treatment. Extremely high concentrations of antibiotics were found in treated sewage in India (Fick et al. 2009). Risk to aquatic organisms (Zhang et al. 2012) has been correlated with pharmaceutical residues present in wastewater. Microorganisms present in the aquatic environment are exposed to the multiple drug residues and their continual exposure may lead to mutations and development of new strains. Recent identification of antibiotic-resistance bacteria (Middleton and Salierno 2013; Shah et al. 2012) in the environment has added a new dimension to the risk posed by the presence of drug residues in the environment. It is probable that not only aquatic biota is affected, but continual exposure has also given rise to the presence of multi-drug resistance microorganisms which were not known earlier. Organisms respond to pharmaceutical exposure at trace environmental concentrations (Gullberg et al. 2011), which indicates that pharmaceutical residuals are of significant environmental concern (Li et al. 2010). Antibiotic resistance pathogens have been isolated from fishes from south east cost of India (Kumar et al. 2011). Antibiotic residues have now been reported in aquatic organisms (shrimps) from many southern states of India (Swapna et al. 2012) and thus organisms of higher trophic level are exposed to such residues which may

result irreversible loss to biodiversity. Ciprofloxacin is the most consumed antibiotic drug and as a consequence, fluoroquinolone resistance has been reported in both humans and animals in India (Sahoo et al. 2012). Residues of analgesic drug (diclofenac, ibuprofen, ketorofen, etc.) and antibiotics have been correlated with a decline in the population of Indian vultures (Oaks et al. 2004; Lemus et al. 2008; Taggart et al. 2009). Hence, the hazard engendered by antibiotic residue is not limited to aquatic organisms only, but also poses a serious threat to other organisms including humans. Continuous exposure of sub-therapeutic concentration to aquatic vertebrates and invertebrates showed almost negligible visible effects, but these effects could slowly accumulate to manifest themselves into a final irreversible condition noticed only after several generations affecting the sustainability of the aquatic life (Daughton and Ternes 1999). Since some of the groundwater resources were also reported to have antibiotic residues contamination, people may be exposed directly to antibiotic residues, however impacts of the same depends on the concentration levels. Furthermore, it should also be considered that co-existence of different antibiotics may synergise the ecotoxicological effects. Therefore, it is imperative to quantify the risk posed by pharmaceutical residues.

In India, pharmaceutical residues and their corresponding risk to the aquatic environment have not been well documented as most of the studies assessing environmental risk due to antibiotic residues are geographically limited to the USA and Europe (Hilton and Thomas 2003). As the patterns and volumes of antibiotic consumptions differ in different countries, the pharmaceutical residues' occurrence and their possible associated risks could be different in India. The objective of the present study was to determine whether antibiotic residues in different water matrices in certain regions of India pose potential ecological risks. Prioritisation and identification of the water matrices needing immediate attention of the policy makers for environmental pollution control and ecotoxicological hazard minimisation were also discussed.

Materials and methodology

There are several methods of assessing ecological risks (OECD 1995; US EPA 1998; European Commission 2003; Suter 2006). Hazard quotient (HQ) is one of the ways to express the ecological risk of a chemical compound (Hayashi 2007). The HQ is usually calculated as the ratio between the predictable environmental concentration (PEC) and predicted no effect concentration (PNEC) for pharmaceuticals residues. PEC levels for drugs depend on several factors such as type of drugs, sales figures, number of prescriptions, population density, market penetration of an individual drug, and consumption data. Generally, the PEC is calculated using the European Medicine Evaluation Agency (EMEA) guideline (EMEA Model 2001, EMEA Model 2003/2005) or European Union System for the Evaluation of Substances (EUSES) (Liebig et al. 2006). However, calculation of PECs can be difficult for a country like India where self-medication rates and over-the-counter (OTC) sale of medicines are very high (Greenhalgh 1987). Besides, there is a large-scale migration of people from rural to urban areas and most of them live in urban slums, lacking proper health and sanitation facilities. Poor health and sanitation facilities are expected to yield a higher rate of drug consumption. For such conditions, it is difficult to calculate the total consumption, and thus, the PEC could be different from real environmental concentrations. In such instances, the PEC can be replaced with the real-time concentration of drugs. Thus, use of measured environmental concentrations (MEC) is proposed in place of PEC.

An extensive literature survey was carried out for the occurrence of antibiotics residues in Indian water matrices, i.e., domestic wastewater, hospital wastewater, industrial wastewater, groundwater, and river water (Table 1). The highest reported concentrations of these antibiotics were used in this study to determine the maximum possible risk. HQs were calculated to identify whether these environmental concentrations of antibiotics pose any potential risk to non-target organisms. Uncertainty predominates in the science of risk assessment (Hokstad and Steiro 2006) and several factors may introduce uncertainty in assessing risk for antibiotics occurrence in the natural environment. For instance, since EC50 or LC50 values are generated from laboratory acute toxicity data obtained from exposure of a single drug for shorter durations (1–4 days), the values may not be representative enough of the real situation in the natural environment where multiple drugs coexists in lifelong exposure to aquatic species. So, it becomes difficult to generate unequivocal data for every chemical and every chemical combination for every specific situation (Jones et al. 2002). Risk assessment may involve high levels of uncertainty arising from data gaps and a safety factor was therefore considered. Table 2 illustrates the calculated PNEC values for antibiotic and test organisms. The PNEC values were obtained from the published literature and if no PNEC value was available for a given compound, the acute and chronic toxicity values (LC50 and EC50) were used to calculate the PNEC by considering the safety factors (F=1,000, for acute toxicity; F=10, for chronic toxicity) (Sanderson et al. 2003). Chronic data give much better insight into the "true" risk of chemical or chemical group, and significantly reduce uncertainty of risk assessment but chronic data for most of the chemicals/compounds are not available. Thus both chronic and acute toxicity values were considered for HQ estimation in the present study. Where multiple PNEC values were available or calculated for a particular antibiotic to the same species, the median value of the antibiotic was considered as PNEC. In risk calculation, the lowest PNEC value for a specific

Table 1 Levels of antibiotic residues reported from the water matrices of Indian subcontinent (all values are in μg l^{-1})

Sampling source	Hospital wastewater		Industrial wastewater		Lake water	River water		Ground water	Treated sewage
Compounds	UCT Hospital Ujjain	CRG Hospital Ujjain	CETP Hyderabad	CETP Hyderabad	Kazipeli Lake Hyderabad	Manjira and Tamariparani river Southern India	Yamuna river Delhi	Dug Wells Hyderabad	STP effluents Delhi
Amoxicillin	ND	ND	ND	ND	ND	ND	ND	ND	BDL–0.06
Ampicillin	ND	ND	ND	ND	ND	ND	0.2–13.75	ND	6.3–27.1
Ciprofloxacin	7.6–31	64.8–236.6	28,000–31,000	14,000	2,500–6,500	10.0–2,500	BDL–1.44	0.04–14	2.9–17.7
Citalopram	ND	ND	770–840	430	2.0–8.0	BDL–76	ND	BDL–1.4	ND
Citrizine	ND	ND	1,300–1,400	2,100	5–100	5.4–530	ND	0.6–28	ND
Enalapril	ND	ND	ND	2.2	BDL–1	BDL–1.5	ND	BDL–1.4	ND
Enrofloxacin	ND	ND	780–900	210	BDL–5	BDL–30	ND	BDL–0.07	ND
Erthromycin	ND	ND	1.0–10	ND	ND	ND	ND	ND	ND
Lomefloxacin	ND	ND	150–300	8.8	ND	BDL–1.1	ND	BDL–0.04	ND
Losarton	ND	ND	2,400–2,500	ND	ND	ND	ND	ND	ND
Methoxytindazole	2.5	1.4–3.8	ND	ND	ND	ND	ND	ND	ND
Metoprolol	ND	ND	800–950	4	1.0–7.0	BDL–0.24	ND	BDL–0.01	ND
Norfloxacin	5.7	20.6–22.8	390–420	25	60–520	BDL–4.7	ND	BDL–0.31	ND
Ofloxacin	66–73.2	1.5–7.5	150–160	55	5.0–11	0.18–10	ND	BDL–0.48	ND
Rantidine	ND	ND	90–160	ND	ND	ND	ND	ND	ND
Sulfamethoxozole	2.7–5.7	36.7–81.1	ND	ND	ND	ND	ND	ND	ND
Terbinafine	ND	ND	ND	0.12	BDL–15	0.03–0.2	ND	0.01–1.8	ND
Tindazole	13.6	30.4–88.8	ND	ND	ND	ND	ND	ND	ND
Trimethoprim	ND	ND	ND	4.4	ND	BDL–4	ND	BDL–0.06	ND
Triclosan	ND	ND	ND	ND	ND	3.8–5.16[a]	ND	ND	ND
Reference	Diwan et al. 2009	Diwan et al. 2010	Larsson et al. 2007	Fick et al. 2009	Fick et al. 2009	Fick et al. 2009; Ramaswamy et al. 2011[a]	Mutiyar and Mittal 2014	Fick et al. 2009	Mutiyar and Mittal 2013; Mutiyar and Mittal 2014

ND not determined, *BDL* below detection limit, *UCT* Ujjain Charitable Trust Hospital, *CRG* Chandrikaben Rashmikant Gardi Hospital, *CETP* combined effluent treatment plant

[a] Tamariparani river Tamilnadu (India)

Table 2 Calculated PNEC values from the reported LC_{50} and NOEC values

Antibiotics	Taxonomic group	Species	Type of study	UF used	PNEC		Reference
					Lowest (μg l^{-1})	Range (μg l^{-1})	
Amoxicillin	Bacteria	*V. fischeri*	Acute	1,000	1,320	1,320	Park and Choi 2008; Kim et al. 2007
	Algae	*M. aeruginosa*	Acute	1,000	0.006	0.006–0.0037	Holten-Lützhøft et al. 1999; Halling-Sørensen et al. 2000
	Algae	*S. capricornutum*	Acute	1,000	5	5–1,000	Holten-Lützhøft et al. 1999; Eguchi et al. 2004; Halling-Sørensen et al. 2000
	Algae	*R. salina*	Acute	1,000	3,108	3,108	Holten-Lützhøft et al. 1999
	Algae	*C. vulgaris*	Acute	1,000	1,000	1,000	Eguchi et al. 2004
	Algae	*L. gibba*	Acute	–		greater than the greatest concentration tested (1,000 μg l^{-1})	Brain et al. 2004
	Fish	*O. mykiss*	Acute	1,000	182.7	182.7	Laville et al. 2004
Ampicillin	Bacteria	*V. fischeri*	Acute	1,000	163	163	Backhaus and Grimme 1999; Park and Choi 2008
	Algae	*S. capricornutum*	Acute	1,000	1,000	1,000	Park and Choi 2008; Kim et al. 2007
	Algae	*L. gibba*	Acute	–	–	greater than the greatest concentration tested (1,000 μg l^{-1})	Brain et al. 2004
	Invertebrate	*D. magna*	Acute	1,000	2,300	1,000	Park and Choi 2008; Kim et al. 2007
	Fish	*O. Latipes*	Acute	1,000	1,000	1,000	Park and Choi 2008; Kim et al. 2007
Ciprofloxacin	Bacteria	Activated sludge bacteria	Acute	1,000	0.61	0.61	Halling-Sørensen et al. 2000
	Algae	*L. gibba*	Acute	1,000	0.698	0.698–1.796	Brain et al. 2004
	Algae	*S. capricornutum*	Acute	1,000	2.97	2.97	Halling-Sørensen et al. 2000
	Invertebrate	*D. magna*	Acute	1,000	60	60	Halling-Sørensen et al. 2000
	Fish	*G. holbrooki*	Acute	1,000	60	60–100	Halling-Sørensen et al. 2000
	Plants	*L. minor*	Acute	1,000	3.75	3.75	Martins et al. 2012
Citalopram	Arthropoda	*Caldocera*	Acute	1,000	0.8	800	Henry et al. 2004
Cetrizine	Invertebrate	*D. magna*	Acute	1,000	330	330	FDA-CDER 1996
Enalapril	Invertebrate	*D. magna*	Acute	1,000	354	354	http://sitem.herts.ac.uk/aeru/vsdb/Reports/1824.htm
	Fish	*P. promelas*	Acute	1,000	1,000	1,000	http://sitem.herts.ac.uk/aeru/vsdb/Reports/1824.htm
Enrofloxacin	Bacteria	*V Fischeri*	Acute	1,000	8.4	8.4	Hernando et al. 2006
	Algae	*M. aeruginosa*	Acute	1,000	0.049	-0.049	Robinson et al. 2005
	Algae	*P. subcapitata*	Acute	1,000	0.74	0.74	Robinson et al. 2005
	Invertebrate	*D. magna*	Acute	1,000	10	10	Bayer 1997
	Fish	*O. mykiss*	Acute	1,000	10	10	Bayer 1997
	Plant	*L. minor*	Acute	1,000	−0.106	0.106	Robinson et al. 2005
Erthromycin	Bacteria	*V. fischeri*	Acute	1,000	100	100	Isidori et al. 2005
	Algae	*P. subcapitata*	Chronic	10	2	2	Isidori et al. 2005
	Algae	*S. capricornutum*	Chronic	10	1.03	1.03	Yang et al. 2008
	Algae	*L. gibba*	Acute	–	–	greater than the greatest concentration tested (1,000 μg l^{-1})	Brain et al. 2004
	Invertebrate	*D. magna*	Acute	1,000	0.2	0.02–30.5	Sanderson et al. 2003; Lee et al. 2008; Isidori et al. 2005; Holten-Lützhøft et al. 1999
	Invertebrate	*C. dubia*	Acute	1,000	0.22	0.22–10.23	Isidori et al. 2005

Table 2 (continued)

Antibiotics	Taxonomic group	Species	Type of study	UF used	PNEC		Reference
					Lowest (μg l^{-1})	Range (μg l^{-1})	
	Fish	*D. rerio*	Acute	1,000	61.5	61.5–1,000	Lee et al. 2008; Isidori et al. 2005
	Rotifer	*B. calyciflorus*	Acute	1,000	0.94	0.94–27.53	Isidori et al. 2005
	Vertebrate	*T. platyurus*	Acute	1,000	17.68	17.68	Isidori et al. 2005
Lomefloxacin	Algae	*S. vacuolatus*	Acute	1,000	58	58	Halling-Sørensen et al. 2000
	Algae	*S. capricornutum*	Acute	1,000	20	20	Lanzky and Halling-Sørensen 1997
	Algae	*L. gibba*	Acute	–		greater than the greatest concentration tested (1,000 μg l^{-1})	Brain et al. 2004
Losarton	Invertebrate	*D. magna*	Acute	1,000	331	331	FDA-CDER 1996
Metronidazole	Algae	*Chlorella* sp.	Acute	1,000	12.5	12.5	Lanzky and Halling-Sørensen 1997
	Algae	*S. capricornutum*	Acute	1,000	39.1	39.1	Lanzky and Halling-Sørensen 1997
	Invertebrate	*D. magna*	Acute	1,000	1,000	1,000	Wollenberger et al. 2000
	Invertebrate	*D. magna*	Acute	1,000	250	250	Wollenberger et al. 2000
	Fish	*B. rerio*	Acute	1,000	500	500	Lanzky and Halling-Sørensen 1997
	Arthropoda	*A. tonsa*	Acute	1,000	100	100	Lanzky and Halling-Sørensen 1997
Metoprolol	Invertebrate	*D. magna*	Acute	1,000	100	1,000	Cleuvers 2003
	Algae	*D. subspicatus*	Acute	1,000	7.3	7.3	Cleuvers 2003
	Plant	*L. minor*	Acute	1,000	320	320	Cleuvers 2003
Norfloxacin	Algae	*P. subcapitata*	Chronic	10	20	20	Yang et al. 2008
	Algae	*S. vacuolatus*	Acute	1,000	69.6	69.6	Backhaus et al. 2000
	Algae	*L. gibba*	Acute	–		Greater than the greatest concentration tested (1,000 μg l^{-1})	Brain et al. 2004
Ofloxacin	Algae	*P. subcapitata*	Chronic	10	108	108–180	Isidori et al. 2005
	Invertebrate	*C. dubia*	Acute	1,000	10.5	10.5–28.9	Isidori et al. 2005
	Rotifer	*B. calyciflorus*	Acute	1,000	26.7	26.7–33.5	Isidori et al. 2005
Ranitidine	Algae		Acute	1,000	66	66	Sanderson et al. 2003
	Invertebrate	*D. magna*	Acute	1,000	63	63	Sanderson et al. 2003
	Fish	*Fish*	Acute	1,000	1076	1,076	Sanderson et al. 2003
Sulfa-methoxazole	Bacteria	*V. fischeri*	Acute	1,000	23.3	23.3–84	Ferrari et al. 2003; Isidori et al. 2005
	Algae	*C. meneghiniana*	Acute	1,000	1.25	1.25–2.4	Ferrari et al. 2003
	Algae	*P. subcapitata*	Chronic	10	36	36–74	Ferrari et al. 2003; Isidori et al. 2005
	Algae	*S. capricornutum*	Acute	1,000	0.146	0.146–1.53	Ferrari et al. 2004; Eguchi et al. 2004
	Algae	*L. gibba*	Acute	–		Greater than the greatest concentration tested (1,000 μg l^{-1})	Brain et al. 2004
	Invertebrate	*C. dubia*	Chronic	10	15.51	15.51–100	Nunes et al. 2005; Ferrari et al. 2003
	Invertebrate	*D. magna*	Acute	1,000	25.2	25.2–205.2	Ferrari et al. 2003; Isidori et al. 2005; Kim et al. 2007
	Invertebrate	*C. dubia*	Chronic	10	12.97	12.97–18.85	Isidori et al. 2005; Ferrari et al. 2003
	Fish	*D. rerio*	Acute	1,000	8	8–1,000	Isidori et al. 2005; Ferrari et al. 2003; Kim et al. 2007

Table 2 (continued)

Antibiotics	Taxonomic group	Species	Type of study	UF used	PNEC		Reference
					Lowest (μg l^{-1})	Range (μg l^{-1})	
	Rotifer	*B. calyciflorus*	Chronic	10	9.63	9.63–26.27	Isidori et al. 2005
Terbinafine	Algae	*P. subcapitata*	Acute	1,000	0.22	0.22	Palomaki 2010.
Tindazole	Invertebrate	*D. magna*	Acute	1,000	0.69	0.69	Lilius et al. 1994
Trimethoprim	Algae	*R. saline*	Acute	1,000	16	16	Holten-Lützhøft et al. 1999
	Algae	*M. aeruginosa*	Acute	1,000	112	112	Holten-Lützhøft et al. 1999
	Algae	*S. capricornutum*	Acute	1,000	25.5	25.5–110	Halling-Sørensen et al. 2000; Holten-Lützhøft et al. 1999; Eguchi et al. 2004
	Algae	*L. gibba*	Acute	–		Greater than the greatest concentration tested (1,000 μg l^{-1})	Brain et al. 2004
	Invertebrate	*D. magna*	Acute	1,000	60	60–167.4	Halling-Sørensen et al. 2000; Kim et al. 2007; Park and Choi 2008
	Fish	*B. Rerio*	Acute	1,000	100	100–795	Halling-Sørensen et al. 2000; Kim et al. 2007

taxonomic group was used to estimate the maximum possible risk posed by the antibiotics. The MEC/PNEC values were used to predict the possible risk posed by individual antibiotics to the various test organisms. If MEC/PNEC (HQ) >1 for a drug, there is risk to the aquatic organisms from that drug's residue (TGD 1996). The framework adopted for assessing the risk to the aquatic organisms by the antibiotics residues is shown in Fig. 1. HQs were developed for all water matrices from where the antibiotic residues were reported on the Indian subcontinent.

Results and discussion

Six sectors have been identified based on availability of secondary data and important components of the water environment. These sectors are hospitals, industries, sewage treatment plants (STPs), rivers, lakes, and groundwater. The HQ was calculated separately for these sectors.

HQ for hospital effluents

Residues of major fluoroquinolones, sulphonamides and tinidazoles were present in hospital wastewaters and the drains carrying the hospital effluents (Diwan et al. 2009) (Table 1). These residues were high enough to cause genotoxic effects and modify bacterial strains (Diwan et al. 2010). High HQs (1–219) were obtained for ciprofloxacin, and for many test species ciprofloxacin residues could pose a serious hazard (HQ >1, possible risk), including bacteria, algae, invertebrates, and fish (Fig. 2). Among antibiotics, ciprofloxacin showed the highest HQ (219, Fig. 2) to test species as a very high concentration of ciprofloxacin (236.6 μg l^{-1}) was present in the hospital effluents. This may be because of the fact that ciprofloxacin is one of the most widely used antibiotics. Ciprofloxacin is among the leading quinolones of choice in hospitals (Githinji et al. 2011), and thus, high residues of this drug could be found in the hospital effluents. The residue levels of ofloxacin, sulfamethoxazole, and tinidazole also had a high HQ for one or more test species while methoxytindazole and norfloxacin (HQ <1) had insignificant environmental risks (Fig. 2). Presence of antibiotic residues in hospital effluents is a reason for concern, as 66 % of the drugs reported for Indian hospitals effluents had a HQ >1. Antibiotic resistance in *Escherichia coli* isolated from hospital wastewater showed that hospital effluents pose significant environmental risks (Diwan et al. 2010). Diverse uses of multiple antibiotics could reduce the concentration of individual compounds in wastewater, but the hazard associated with the discharge of a cocktail of drugs could be also high as new bacterial strains with multiple-drug resistance could develop. A proper WWTP could effectively reduce (80–85 %) the antibiotic residues (Duong et al. 2008). Furthermore,

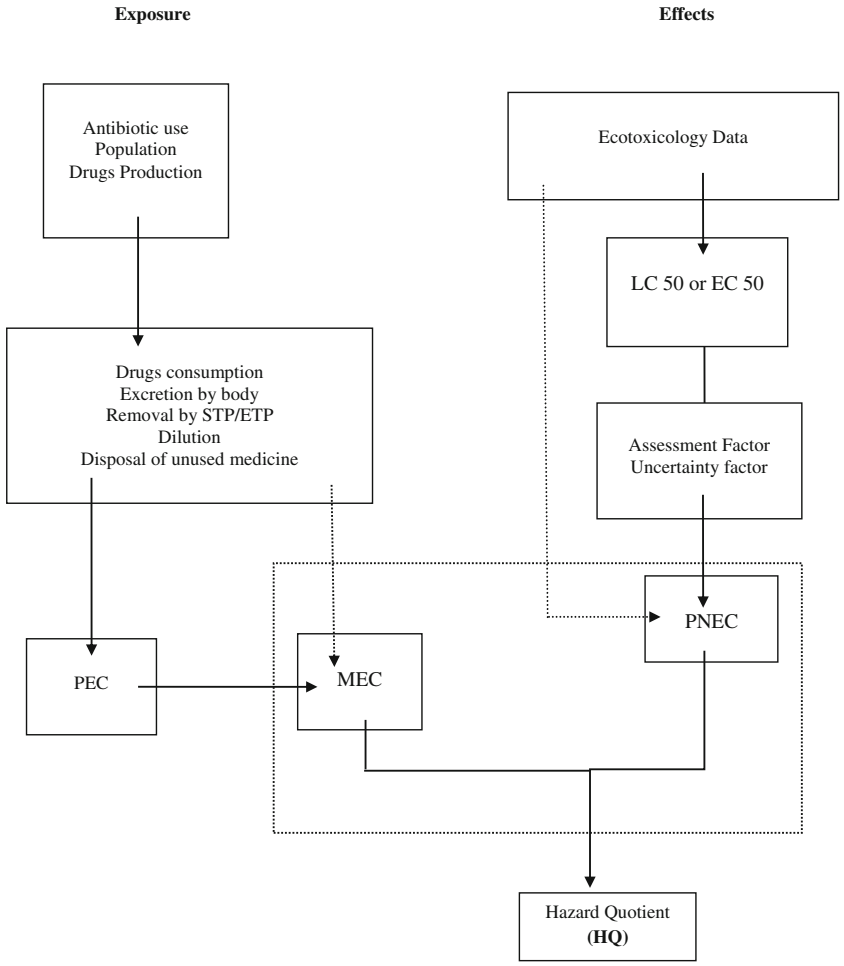

Fig. 1 Antibiotic risk assessment and hazard quotient (*HQ*) development framework for the aquatic environment

awareness of the importance of safe disposal of leftover medicines could further reduce their levels in hospital wastewater.

HQ for industrial wastewater

Pharmaceutical industries in India are located in various clusters in the provinces of Andhra Pradesh, Gujarat, Maharashtra and Goa. Pharmaceutical industrial effluents can have high active pharmaceutical ingredients (API) especially in India and China (Larsson et al. 2007; Lin and Tsai 2009). The effluent from a WWTP in Patancheru (Hyderabad, India) has been reported to pollute the region's waters with the highest levels of pharmaceutical residues ever detected in the aquatic environment (Lubik 2009). There is a need to adopt measures to control the discharge

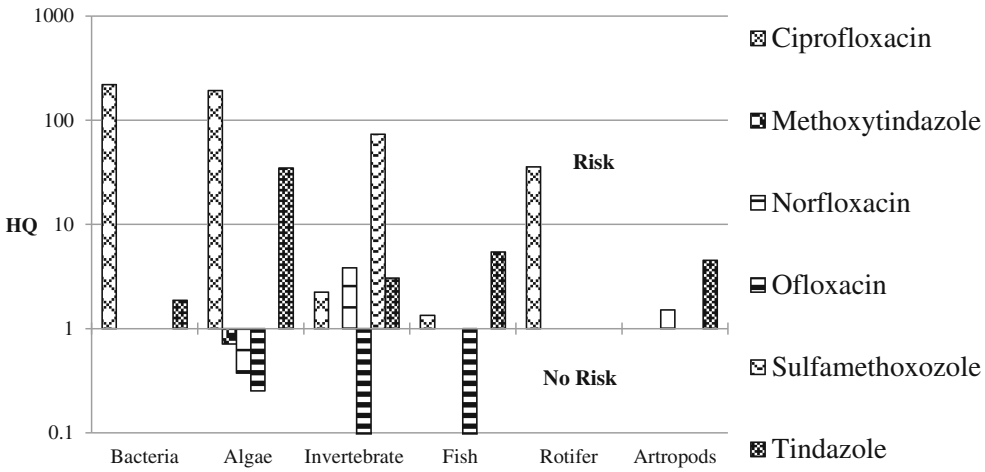

Fig. 2 Hazard quotient (*HQ*) for pharmaceutical residues present in the hospital effluents

of high API into the surrounding aquatic environment. Effluents from Hyderabad's pharmaceutical industrial cluster (India) are reported to have extremely high levels of antibiotics (Table 1). Lack of transparency in the production chain and weak environmental regulations in India are the main reasons for high APIs in the Indian aquatic environment (Table 1). Stricter discharge standards in the European Union (EU) and the USA have resulted in reduced levels of APIs (EU=ND–0.2 μg l^{-1}, Tamtam et al. 2008; USA=ND–0.85 μg l^{-1}, Karthikeyan and Meyer 2006) reaching their water matrices (Larsson and Fick 2009). HQs developed for antibiotic residues present in the effluents from the WWTPs in India pose severe risk (High HQ) to most of the test species (Fig. 3). The maximum HQ value was for ciprofloxacin (HQ=36,885), which is the greatest HQ ever reported for an industrial effluent, to the best of the author's knowledge. Improper disposal of pharmaceutical effluents has been the major source of these micro-pollutants in the environment. The high levels of these compounds in wastewater may lead to the development of antibiotic-resistance in microbes present in the aquatic environment. It is a major challenge for producers and regulatory agencies in India. There is an increasing awareness that environmental bacteria comprise an important reservoir of drug resistance genes. The most obvious risk associated with high levels of broad-spectrum antibiotics reported in the WWTP effluents is that it induces the development of antibiotic-resistant microbes (Fick et al. 2009). Dilution of pharmaceutical industrial effluents in small to medium rivers may not completely remove the associated hazards, as toxic effects on test organisms were reported from 60 to 500 times distilled water-diluted pharmaceutical industrial effluent as well (Larsson et al. 2007; Gunnarsson et al. 2009). Similar high values of HQ in antibiotic residues (Fig. 3) showed that effluents from pharmaceutical production units pose serious ecological and environmental risks.

HQ for river water

Antibiotic residues could flow into the river either through the discharge of domestic sewage or through industrial effluents to the river. Evidence for these chemicals appearing in natural waters through municipal and industrial wastewater discharge has been gathered (Park and Choi 2008; Kookana et al. 2011). Park and Choi (2008) developed a HQ for pharmaceutical residues in Korean surface waters and showed that the HQ was less than one, suggesting that their potential environmental impact may be low for Korean rivers. However, the situation of the Indian rivers is entirely different as these rivers receive the industrial discharge containing high levels of pharmaceutical residues. Few studies have been reported for antibiotic residues in natural waters of the Indian subcontinent, but reports available showed very high antibiotic residues levels in Indian rivers (Larsson et al. 2007; Fick et al. 2009; Ramaswamy et al. 2011). Alarmingly high levels of antibiotic residues were present in the tributaries of Manjira River (Isakavagu–Nakkavagu Area) and Tamariparani River, Tamilnadu (Table 1). The Manjira River in Isakavagu–Nakkavagu area receives effluents from pharmaceutical industries in Patancheru, Hyderabad. The estimated HQ for the antibiotic residues in these rivers showed extreme high risk (HQ=25–4,098) posed by ciprofloxacin (Fig. 4). Other fluoroquinolones also pose high risks to test organisms. Previously, high levels of triclosan with HQ values such as 4.7, 39.2 and 1,543 have been reported for the Vellar River, Kaveri River, and Tamariparani River (Ramaswamy et al. 2011). Unlike the high risks indicated by high HQs for antibiotic residues for these Indian rivers, low potential ecological impact of pharmaceutical residues has been reported for Korean rivers by Han et al. (2006) (HQ <1) and Park and Choi (2008) (HQ=0.002–13.8). Pharmaceutical industrial

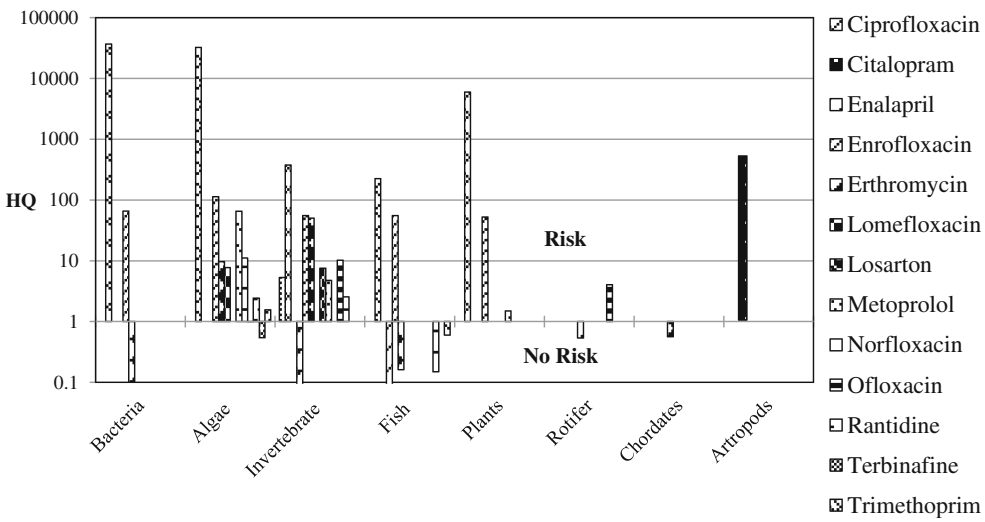

Fig. 3 Hazard quotient (*HQ*) for pharmaceutical residues present in pharmaceutical industrial effluents

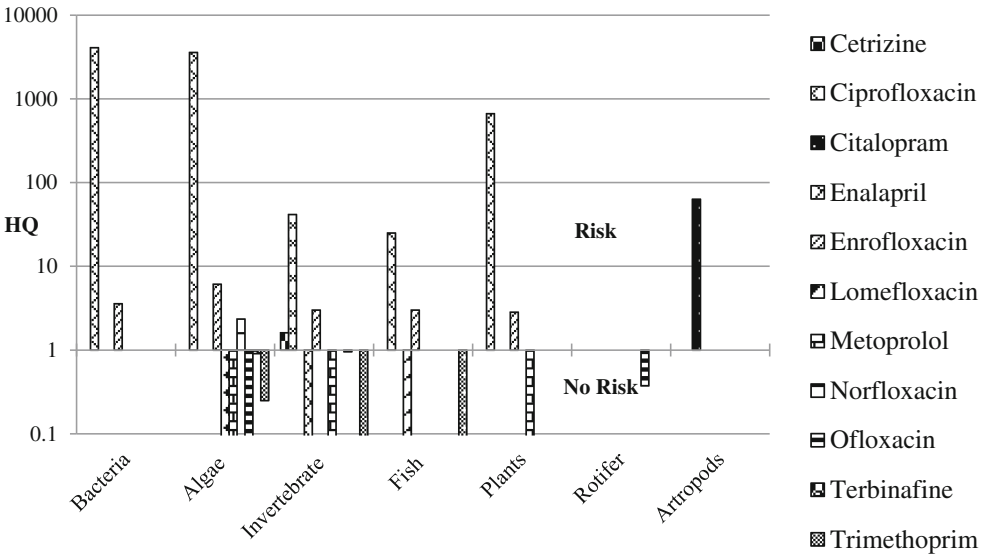

Fig. 4 Hazard quotient (*HQ*) for pharmaceutical residues present in river water receiving pharmaceutical industrial effluents

discharges were likely major contributors of pharmaceutical chemicals into the tributaries of Manjira River. The Manjira, Tamariparani, Kaveri, and Vellar Rivers received effluents from several pharmaceutical industries and thus were expected to contain higher levels of antibiotic residues (Table 1). Recently, high levels of other pharmaceuticals, non-steroid anti-inflammatory drugs (up to 600 ng l^{-1}) have also been reported from Kaveri, Vellar, and Tamiraparani Rivers in southern India (Shanmugam et al. 2013). The agency responsible for pollution control in the country, the Central Pollution Control Board (CPCB), does not have any specific standards based on API for the disposal of drug-laden wastewater. Thus, high volumes of API reach to the Indian rivers via wastewater disposal due to weak effluent discharge standards. However, CPCB's recently announced decision to modify the discharge standards for pharmaceutical industry may help in resolving the issue (www.cpcb.nic.in/upload/Tenders/Tender_134_PHARMA.pdf).

It should be noted that the extreme high HQ for the reported river water is due to discharge of pharmaceutical laden wastewater to the rivers and it is not a general situation for other rivers. One of the worst polluted river in Northern India, Yamuna river in Delhi, was reported to have very less antibiotic residues (Mutiyar and Mittal 2014) compared to the Manjira, Tamariparani, Kaveri, and Vellar Rivers (Fig. 5). The investigated stretch of the Yamuna river receive huge amount of treated and untreated domestic wastewater but still the pharmaceutical residues levels were comparably low. It can be inferred that pharmaceutical residues could be higher in smaller rivers having less flow, as large rivers have remarkable self-purification abilities and could purify themselves from the pharmaceutical residues, either by dilution, solar radiation, and degradation by the inherent microorganisms or by adsorption into suspended particulates. Thus, high APIs have so far not been reported from large rivers like Ganga and Godavari.

HQ for lake water

Lakes reported to have high concentrations of antibiotic residues such as the Kazipeli Lake of Hyderabad are in the

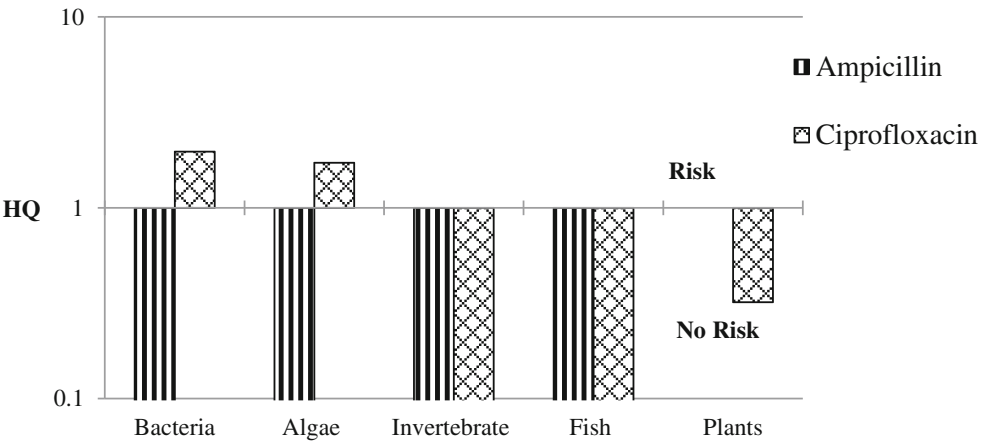

Fig. 5 Hazard quotient (*HQ*) for pharmaceutical residues present in Yamuna river, Delhi (without major pharmaceutical industrial discharge)

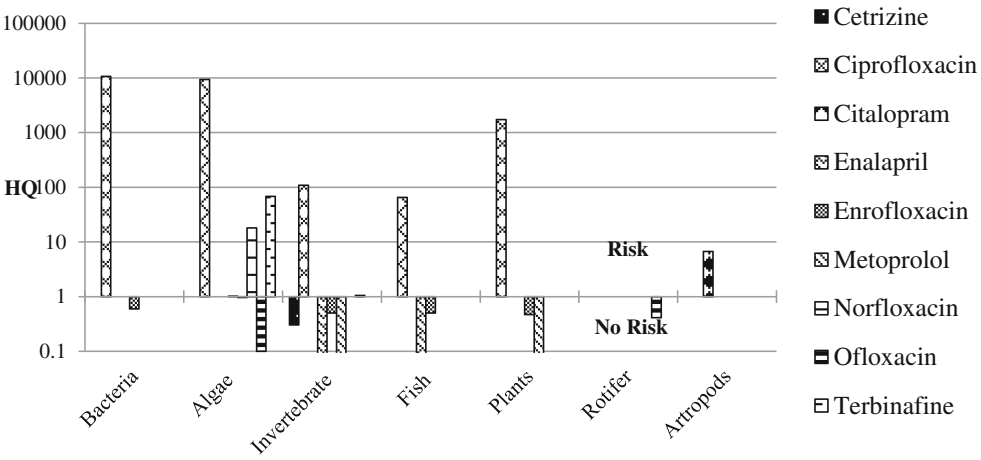

Fig. 6 Hazard quotient (*HQ*) for pharmaceutical residues present in water from lake (being fed with industrial discharge)

vicinity of the highly polluted Manjira River (Isakavagu–Nakkavagu area) and are generally fed by the API-laden water from these rivers (Table 1). The HQ developed for the lake water showed that, except for enalapril and metoprolol, all other antibiotics posed minor to severe risks depending upon their MEC and PNEC (Fig. 6). The maximum HQ value was for ciprofloxacin, as the concentration of this antibiotic was reported to be the highest in the lake water as well (Table 1). The high HQ of the lake water for test organisms showed that the lake environment is not suitable for the sustenance of aquatic life and could help develop antibiotic resistance in microbes. It is made clear here that no study has been carried out so far to assess the harmful impacts and in depth scientific studies are required to strengthen the claim.

HQ for groundwater

Groundwater samples from villages around Hyderabad were found to have very high levels of antibiotic residues (Table 1). This may be due to the groundwater recharge by the contaminated Kazipeli Lake water and water from the Manjira River (Isakavagu–Nakkavagu area). The HQ values developed for groundwater showed that it poses mild to high risk for ciprofloxacin (HQ=0.1–23), citalopram (HQ=1.2) and terbinafine (HQ=8.2) (Fig. 7). However, the test species do not have a natural habitat in the contaminated groundwater, and thus, are not directly exposed to the antibiotic residues. However, the contamination of groundwater could pose a potential risk to rural populations of a developing country like India as it still depends on groundwater as a drinking water source (Mutiyar et al. 2011). Thus, the people dependent on groundwater from this area are consuming very low doses of pharmaceuticals daily (35 $\mu g\ l^{-1}$ of ciprofloxacin, 70 $\mu g\ l^{-1}$ of citrizine, 9.25 $\mu g\ l^{-1}$ of gatifloxacin, 4.75 $\mu g\ l^{-1}$ of enoxacin, etc.) considering 2.5 l of daily drinking water requirement. However the exposure of pharmaceuticals by oral route is much lesser than acceptable daily intake (ADI) for pharmaceuticals as direct consumption does not exceed ADI. However, chronic exposure of multiple drugs to human beings may results visible or invisible harmful effects and should be properly explored by conducting scientific studies. Furthermore, it is very difficult to remediate the groundwater after contamination and the contaminated groundwater could remain in the aquifers for decades. Therefore, controlling the pollution of groundwater should be of prime concern.

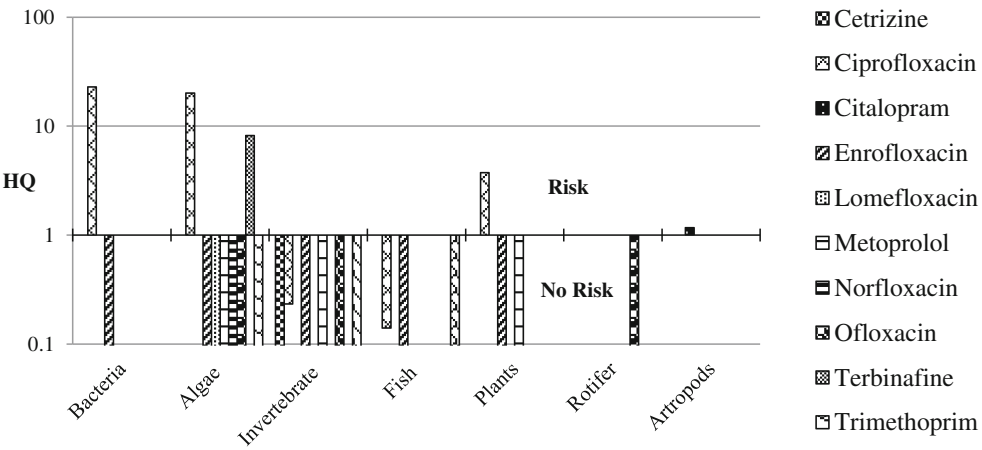

Fig. 7 Hazard quotient (*HQ*) for ground water from Hyderabad area

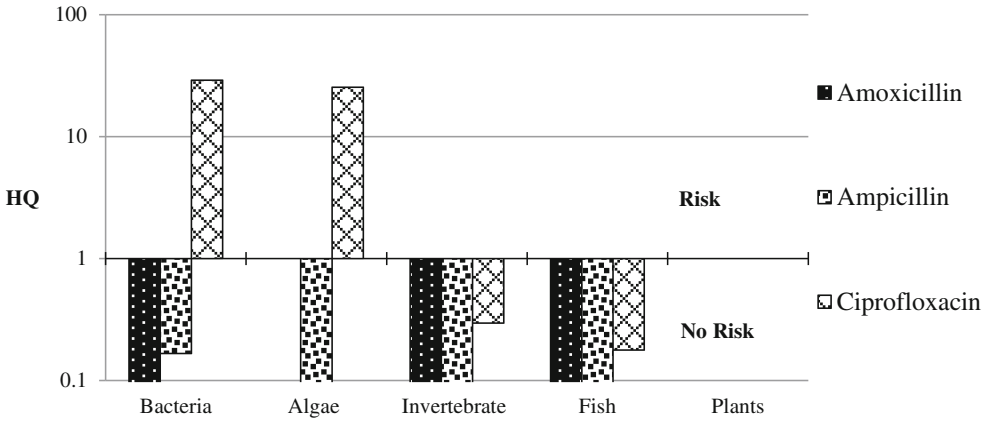

Fig. 8 Hazard quotient (*HQ*) for treated sewage

HQ for treated sewage

Antibiotics enter sewage effluents via urine and faeces and improper disposal of unused medicines. As STPs cannot completely remove pharmaceuticals, APIs are frequently detected in the treated sewage (Kim and Carlson 2007; Ying and Kookana 2007). Several studies have reported the presence of antibiotic residues in domestic sewage from developed countries, but very few studies have been carried out for developing countries including India. The presence of antibiotic residues in domestic sewage in India was first reported by Mutiyar and Mittal (2013), who found traces of ciprofloxacin, ampicillin and amoxicillin in the treated sewage samples from Delhi (Table 1). The estimated HQ values for the antibiotic residue levels in the treated sewage of the city showed that such residues posed a mild environmental risk. Among the detected compounds, ciprofloxacin was the only one with a HQ value of greater than 1 (Algae=25.4, Bacteria=29.0; Fig. 8), and posed a potential threat to environments receiving these effluents (Yamuna river). It is also to be noted that in Delhi, Yamuna river has almost zero dilution potential as no fresh water flows through river Yamuna (Delhi area) most of the times in a year.

Sector prioritisation on the basis of HQ data

Different types of water matrices, namely hospital effluents, industrial effluents; river water, lake water, groundwater, and treated sewage were explored in the present study. These matrices may have different geographical extents, varied concentrations of antibiotic residues, and pose different risks to natural environment and human beings. These matrices can be prioritised based on the potential risk posed by these water matrices. Water matrices with the highest HQ values are of greatest public concern. Among different targeted water matrices, i.e., hospital effluents, industrial effluents, river water, lake water, groundwater and treated sewage, the highest HQ values were obtained for pharmaceutical industrial wastewater, indicating a sector with top priority for environmental preservation and pollution prevention. Ciprofloxacin residues showed maximum HQs (Figs. 2, 3, 4, 5, 6, 7, and 8) for all targeted water matrices, which may be due to the fact that ciprofloxacin is the highest prescribed antibiotic in India (Diwan et al. 2010). Thus, ciprofloxacin could be used as representative molecules among the antibiotics for the purpose of a hazard-based prioritisation. It is evident from Table 3 that ecological hazards due to antibiotics residues for different water matrices varied as pharmaceutical industrial effluents>lake water>river water (receiving pharmaceutical industrial effluents)>hospital effluents>groundwater ≃ treated sewage> Yamuna river (receiving domestic wastewater). Here it was observed that river and lake water (from Hyderabad area) present higher ecotoxicological risk comparing to hospital wastewater or sewage wastewater. This was due to the fact that the rivers and lakes in this area receive pharmaceutical industrial wastewater.

In the present study, high HQ value for pharmaceutical industrial effluents (225–36,885), lakes (65–10,656), rivers (0–4,098), and groundwater (0.1–23) could be attributed to

Table 3 HQ values for Ciprofloxacin for different organisms and water matrices

	Hospital effluents	Pharmaceutical industrial effluents	River water	Yamuna river	Lake water	Ground water	Treated sewage
Bacteria	219.3	36,885.2	4,098.4	2.0	10,655.7	23.0	29.0
Algae	192.0	32,281.2	3,586.8	1.7	9,325.7	20.1	25.4
Invertebrates	2.2	375.0	41.7	0.0	108.3	0.2	0.3
Fish	1.3	225.0	25.0	0.0	65.0	0.1	0.2

industrial discharges. Based on the reported concentrations and high HQ values, the inclusion of some antibiotics in Priority Pollutants Lists is advised, especially for industrial wastewater. Reference values for these compounds may be assessed and included in Indian water quality guidelines, MINAS (for industrial wastewater) and IS: 10500 (for drinking water). The control of the disposal of industrial effluents from pharmaceutical manufacturing units should be given priority as it poses maximum hazard.

The estimation of HQs and their use for risk assessment based on acute ecotoxicity data has occasionally been criticized because it has certain limitations as high uncertainity factor (UF) for acute toxicity data and different endpoints observed during the toxicity studies. The non-availability of the chronic data for all the compounds compel us to depend on the acute data. Most of the toxicity data are derived for a single compound while in natural environment mixture of chemicals/chemical groups exists together thus to overcome these shortcomings, chronic studies with mixture of compounds should be carried out. Within these limits HQs derived by acute toxicity are still highly useful for screening the drugs for possible risks. Ecotoxicological significance of antibiotics in water has not been closely examined, but it can only be surmised that these substances have the potential to adversely affect the aquatic biota (e.g., bacteria, algae, invertebrates, fish, plants) that are continuously exposed to the varying concentrations and cocktail of drugs. Bacterial species have maximum HQs from antibiotic residues and accordingly, development of antibiotic resistance bacteria is reported in different parts of India (Diwan et al. 2010; Kumar et al. 2011; Sahoo et al. 2012). Low antibiotic concentrations in aquatic environments play an important role in maintaining resistance in bacterial populations (Gullberg et al. 2011). The present study supports the findings that such high concentration of antibiotics possibly triggers the development of multidrug resistant bacteria in the Indian aquatic environment, as the NDM-1 positive bacteria was recently reported in Delhi's environment (Walsh et al. 2011). Flaherty and Dodson (2005) concluded that a cocktail of drugs negatively affected *Daphnia magna*'s growth and reproduction and the exposure duration played a critical role in inducing toxicity. Pharmaceutical residues in aquatic environment provide lifelong exposure to aquatic organisms, and thus, their occurrences in aquatic environment are of high concern. Similar toxicity observations, i.e., harmful effects on algae, fishes and other aquatic organisms were reported by Sanderson et al. (2004) and Zounkova et al. (2010).

Conclusion

Studies on the presence of antibiotic residues in the environmental matrices from India are limited. However, existing studies reported extremely high-residue concentrations. Such high levels of APIs pose serious environmental and ecological risks. Hazard identification based on acute toxicity data showed that the relative sensitivity of the considered taxa to antibiotics and their toxicity was bacteria>algae>invertebrate>fish. Similar trends of toxicity due to pharmaceutical residues have been previously reported for different water matrices in Spain by Gros et al. (2010). Bacterial species, being the most sensitive, can develop resistance to drugs that may lead to the failure of currently used antibiotics. Most of the HQ values assigned to the antibiotics reported in the extant literature for India are based on single-grab sample analyses. This clearly demonstrates the need for water quality monitoring and in-depth research in different water matrices using composite and periodical samplings. Water resources around pharmaceutical industrial clusters should be screened first for antibiotics residues. More data on environmental levels of pharmaceutical residues will be helpful in assessing the actual environmental risk, not only to aquatic organisms, but also to human beings. Various exposure routes could be determined only after having reliable and extensive data on the antibiotics occurrences.

The present study has helped in ranking the antibiotics residues contamination in different water matrices and the prioritisation of the sectors seeking urgent attention. The pharmaceutical sector should adopt the Good Manufacturing Practices (GMP) and target zero discharge. Furthermore, industries should be more transparent regarding their environmental discharge and be more open to adopting modern technologies to reduce environmental impacts.

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