Concurrence of antibiotic resistant bacteria (ARB), viruses, pharmaceuticals and personal care products (PPCPs) in ambient waters of Guwahati, India: Urban vulnerability and resilience perspective

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HIGHLIGHTS

- Antibiotics resistance was not correlated with the prevalence of PPCPs and *E. coli*.
- Risk quotient in the urban water was higher than reported for other Indian rivers.
- PMMoV was a good faecal pollution indicator due to prevalence, specificity and detectability.
- As, Co and Mn appear to be inducing antibiotic resistance in E.coli.
- Wetland in the urban area exhibited the least pollution and better resilience for ARB.

GRAPHICAL ABSTRACT
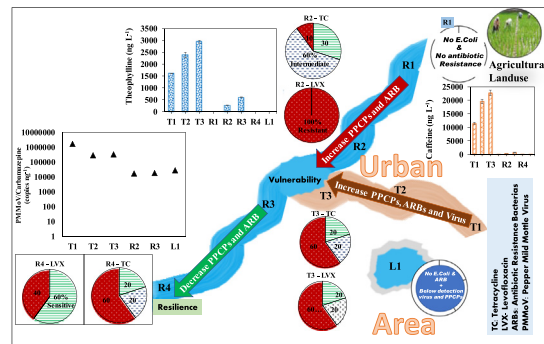
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ABSTRACT

Multi-drug resistant microbes, pathogenic viruses, metals, and pharmaceuticals and personal care products (PPCPs) in water has become the crux of urban sustainability issues. However, vulnerability due to pollutant concurrences, source apportionment, and identification of better faecal indicators needs to be better understood. The present study focuses on the vulnerability of urban Guwahati, the largest city in Northeastern India, through analyzing the concurrence of PPCPs, enteric viruses, antibiotic resistant bacteria, metal, and faecal contamination in water. The study strives to identify a relevant marker of anthropogenic pollution for the Indian scenario. Samples from the Brahmaputra River ($n = 4$), tributary Bharalu River (an unlined urban drain; $n = 3$), and Ramsar recognized Lake (Dipor Bil; $n = 1$) indicate caffeine > acetaminophen > theophylline > carbamazepine > crotamiton for PPCPs and pepper mild mottle virus (PMMoV) > aichi > hepatitis A > norovirus GII > norovirus GI for enteric viruses. PMMoV was the better indicator of faecal pollution due to its prevalence, specificity and ease of detection. Antibiotic resistance was neither correlated with the prevalence of PPCPs nor *E. coli*. As, Co and Mn appear to be inducing antibiotic resistance in *E. coli*. While the risk quotient of the urban drain (Bharalu River) indicates one order higher magnitude than reported for other Indian rivers, the Lake exhibited the least pollution and better resilience. The concurrence of pollutants and multi-drug resistant *E. coli*, owing to the complete absence of

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wastewater treatment, puts the city in a highly vulnerable state. Pollution is being regulated only by the dilution capability of the Brahmaputra River, which needs to be further researched for seasonal variation.

1. Introduction

Around 844 million people lack essential drinking-water facilities, and at least 2 billion use drinking water contaminated with feces (WHO, 2016). Emerging contaminants, such as pharmaceutical and personal care products (PPCPs), endocrine disrupting compounds (EDCs) and their transformation products, are (even at trace levels) of great concern for human health and the aquatic ecosystem (Gogoi et al., 2018). PPCPs designed for maximum biological activity at low concentrations and long-term treatment can be toxic to humans and aquatic organisms if present in ambient waters and/or administered when not required (Ebele et al., 2017; Tong et al., 2011). Owing to inefficient removal by wastewater treatment plants (WWTPs), PPCPs are detectable in streams around the world (Kolpin et al., 2002). The condition is more severe in developing countries like India where urban areas do not have sufficient or advanced treatment processes, as well as due to high population growth and unregulated sale, use, availability, and accessibility of antibiotics. Asia consumes 65,400 tonnes of acetaminophen (ACE), making it the largest consumer in the world and India consumes nearly 1500 tonnes per month (Archana et al., 2016). India is ranked 3rd and 13th in the world in pharmaceutical production and consumption, respectively (Mutiyar et al., 2018). At the same time, pathogenic viruses, responsible for the waterborne diseases are a serious concern for public health (Hamza et al., 2014, Kuroda et al., 2015). Global consumption of antimicrobials is expected to increase by 63,151 to 105,596 tonnes (67%) between 2010 and 2030 (Van Boeckel et al., 2015). Antibiotic consumption in India increased from 3.2 to 6.5 billion tonnes between 2000 and 2015 (Klein et al., 2018). Moreover, there is no regulation of antibiotic use in dairy, poultry or animal husbandry in India (GARP, 2011). With urbanization, the disease profile in the developing world is shifting toward lifestyle-associated diseases, i.e. cardiovascular diseases and diabetes, leading to corresponding changes in the consumption of pharmaceuticals (Mohapatra et al., 2016).

Although much literature is available on PPCPs, to our knowledge their concurrence with viruses, faecal bacteria, and antibiotic resistant bacteria (ARB) in surface water is largely unreported and thus their interrelationships are yet to be undrstood. The present study focuses on prevalent PPCPs (based on type, use and prevalence), viruses, and ARBs. PPCPs in the present study include acetaminophen (ACE), caffeine (CAF), theophylline (THEO), carbamazepine (CMZ) and crotamiton (CTMT), used respectively as an antipyretic, stimulant, respiratory disease treatment, anticonvulsant and antipruritic. PPCP selection is further based on differential persistence and potential as sewage markers in receiving waters (Mohapatra et al., 2016; Yang et al., 2013). ACE, CAF and THEO were chosen among the labile group reported as suitable indicators of untreated wastewater (Madoux-Humery et al., 2013), whereas CMZ and CTMT were selected based on their conservative properties and persistence, more suitable for tracking the fate of persistent pollutants (Daneshvar et al., 2012; Nakada et al., 2008). Four virus types, i.e. pepper mild mottle virus (PMMoV), Hepatitis A, Aichi Virus and noroviruses (NoVs) were analysed to detect their presence in sampled water matrices. Instead of measuring concentrations of various antibiotics in water, bacteria isolated from the samples were tested for antibiotic resistance to three fluoroquinolones (LVX-levofloxacin, CIP-ciprofloxacin, NFX-norfloxacin) and three nonfluoroquinones (KM-kanamycin, ST-sulfamethoxazole, and TC-tetracycline).

Among the selected PPCPs, CAF, a purine methylxanthine alkaloid, is a widely consumed psychoactive drug (Sinija and Mishra, 2009) that can serve as a marker of domestic wastewater contamination (Buerge et al., 2003; Sauvé et al., 2012; Yang et al., 2013). CMZ is a benzodiazepine derivative used for its antiepileptic and psychotropic activity, for severe pain associated with neurological disorders (Kosjek et al., 2009), and is a conservative wastewater-specific indicator (Gasser et al., 2010). India ranks second in CMZ consumption (115.5 tonnes in 2007) after the US and first in Asia (Zhang and Geißen, 2010). To our knowledge only nine studies have reported data on PPCPs in Indian rivers (Balakrishna et al., 2017). One study determined the occurrence and fate of some commonly used antibiotics in the Yamuna River (Mutiyar et al., 2018) and another highlighted the occurrence of PPCPs in the Ganges river (Sharma et al., 2019). To our knowledge there are no published reports on these emerging pollutants, ARB or viruses in the Brahmaputra River.

The presence of ARB is a serious concern as it directly affects the treatability of patients with infectious diseases. In the U.S. there is resistance to at least one family of antibiotics in 70% of all hospital-acquired infections (Marti et al., 2013). The World Health Organization (WHO) recently endorsed a global action plan to tackle antimicrobial resistance, including the essential drug-resistance trend (WHO, 2016), and the Indian government launched the National Action Plan on Antimicrobial Resistance (Ministry of Health and Family Welfare, 2017). Among the faecal microorganisms, human enteric viruses (e.g., noroviruses and hepatitis A) are major cause of gastroenteritis worldwide (Fletcher et al., 2013; Fong and Lipp, 2005), leading to high risk of morbidity and mortality among young children, the elderly, and immunocompromised patients (Gallimore et al., 2004). However, faecal indicator bacteria like *E. coli* and/or total coliform faecal do not always correlate with the presence of viruses (Gibson et al., 2012; Wu et al., 2011). Thus it is imperative to investigate viral contamination of surface water for a precise understanding of risks associated with faecal contamination.

Most published studies focus on scanning urban waters for a wide spectrum of analytes. Pollution, especially metals, induces antibiotic resistance, but conventional water quality parameters like electrical conductivity (EC), total dissolved solids (TDS), major ions, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are less discussed in the context of ARB prevalence (Baquero et al., 2008; Economou and Gousia, 2015; Honda et al., 2016; Larsson et al., 2007; Threedeach et al., 2012). *E. coli* has become pathogenic and is no longer indicative of faecal contamination. PMMoV may be a better indicator (Fiore, 2004; Kuroda et al., 2015) but needs to be tested further. Interesting questions are being explored, as in the present study, and/or need further research. Does faecal contamination directly correspond to ARB occurrence? In a country like India, where viral infections are often mistaken as bacterial disease, does virus prevalence have any relation to PPCPs and ARBs? Urban waters are becoming major sinks of PPCPs, but is there capacity to deal with this problem? And what is the status of vulnerability to such pollution in a city without WWTPs?

In light of the above, the present objectives were to: 1) understand the vulnerability and resilence of urban surface waters in Guwahati, Northeastern India, which has no wastewater treatment facility; 2) investigate the occurrence of PPCPs, viruses, and faecal bacteria in surface waters; 3) determine the interrelationship between prevalence and antibiotic resistance of *E. coli*, as well as concurrence with PPCPs, viruses and metal pollution; and 4) evaluate the utility of PMMoV as a faecal indicator and tracer in surface water.

2. Methodology

2.1. Study area

Guwahati, the largest city in Assam Province, is the gateway and transit point of communication and transportation for seven sister states in northeastern India and thus selected among the Smart Cities Mission by Indian government (Fig. 1). Migration for jobs, facilities, business opportunities and education has resulted in rapid and unplanned growth, with a population of about 968,549 (2011 census) in a limited geographical space of about 328 km^2 (metropolitan area). The city is transected by the Brahmaputra River, a perennial transboundary tropical river that crosses four countries (India, China, Bangladesh and Bhutan), and is fourth largest in the world in average discharge at its mouth (Pervez and Henebry, 2015). The Brahmaputra experiences annual floods from June to September. Guwahati contains a freshwater wetland (Dipor Beel), recognized as the World Heritage Site under the Ramsar Convention. A Brahmaputra tributary, the Bharalu traverses the dense urban sprawl and is now restricted to serving as an unlined urban drainage. Despite being within 200 km of the most rainy location in the world (Cherrapunji, Meghalaya), Guwahati is facing a severe freshwater availability issue owing to heavy dependency on groundwater with unprecedented depletion in last two decades and reported arsenic pollution (Bhattacharya and Borah, 2014; Hazarika and Nitivattananon, 2016).

2.2. Surface water sampling

Eight surface water ($n = 8$) locations in Guwahati (Fig. 1) were sampled on 25 June 2017. Sampling was carried out based on a preliminary survey and strategic locations: the Brahmaputra River before entering the city (R1); just before mixing with the urban drain (Bharalu tributary) (R2); after downstream mixing (R3); and 10 km downstream of Guwahati city (R4). T1, T2, and T3 locations were sampled on the Bharalu tributary representing upstream (T1) to the confluence point with the river (T3). Although four samples were obtained from each corner of Dipor Beel based on similarity in in situ parameters, only one location was selected to sample the representative lake water. Overall, four river, one lake and three drain sampling locations were selected

based on the screening for DO, ORP, TDS, EC, pH and HCO$_3$ at 12, 16 and 10 locations on the river, lake and drain, respectively.

At every location, three samples were taken to prepare a composite which was then analysed for conservative PPCPs (crotamiton and carbamazepine) and labile PPCPs (acetaminophen, theophylline and caffeine), faecal bacteria (E. coli and total coliform), and viruses (hepatitis A, norovirus GI and GII, aichi and PMMoV). Sensitivity of isolated E. coli to six antibiotics (3 quinolones, 1 tetracycline, 1 aminoglycoside, and 1 sulphonamide) was determined. Temperature, pH, electrical conductivity (EC), oxidation-reduction potential (ORP) and dissolved oxygen (DO) were measured in situ using a Hanna 981A multiprobe. and bicarbonate was determine by titration (APHA et al., 2005). As, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn were quantified by ICPMS (PerkinElmer NexION® 2000).

2.3. PPCP extraction and analysis

2.3.1. Extraction

Water samples (500 mL) were collected in glass bottles and analysed in duplicate for PPCPs. Supplementary Fig. S1 shows a schematic of the PPCP analysis. Analytical standards were purchased from Wako Pure Chemicals (Osaka, Japan). Isotope-labelled and deuterated internal standards, including acetaminophen (acetyl-^{13}C$_2$, 99%; ^{15}N, 98%) caffeine (3-methyl-^{13}C, 98%), carbamazepine (D$_{10}$, 98%) were from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA), and crotamiton (D$_7$, 90%) was supplied by Hayashi Pure Chemicals (Osaka, Japan). Stocks of 1000 mg L^{-1} were prepared in ICP-MS grade methanol (Wako) for each PPCPs and stored at −20 °C.

The water samples were filtered through a glass fiber filter (GF/F, 0.7 μm) and refrigerated at 4 °C. Samples were spiked with the specific internal standard (Table S2) for each PPCP before solid phase extraction. Extractions were conducted manually using a SPE suction manifold (GL Sciences Inc., Tokyo, Japan). A 200 mg, 6 cc Oasis HLB cartridge (Waters Corp., Milford, MA, USA) was preconditioned with 5 mL CH$_3$OH and 5 mL Milli Q water (pH 4). The samples were passed through the cartridge at 10 mL min^{-1} followed by air for 30 s. Cartridges were then washed with 2 × 5 mL Milli Q water (pH 4) and analytes eluted with 2 × 5 mL CH$_3$OH at 1 mL min^{-1}. Eluents were dried under a gentle stream of nitrogen gas at 40 °C, reconstituted in a 1 mL mixture of
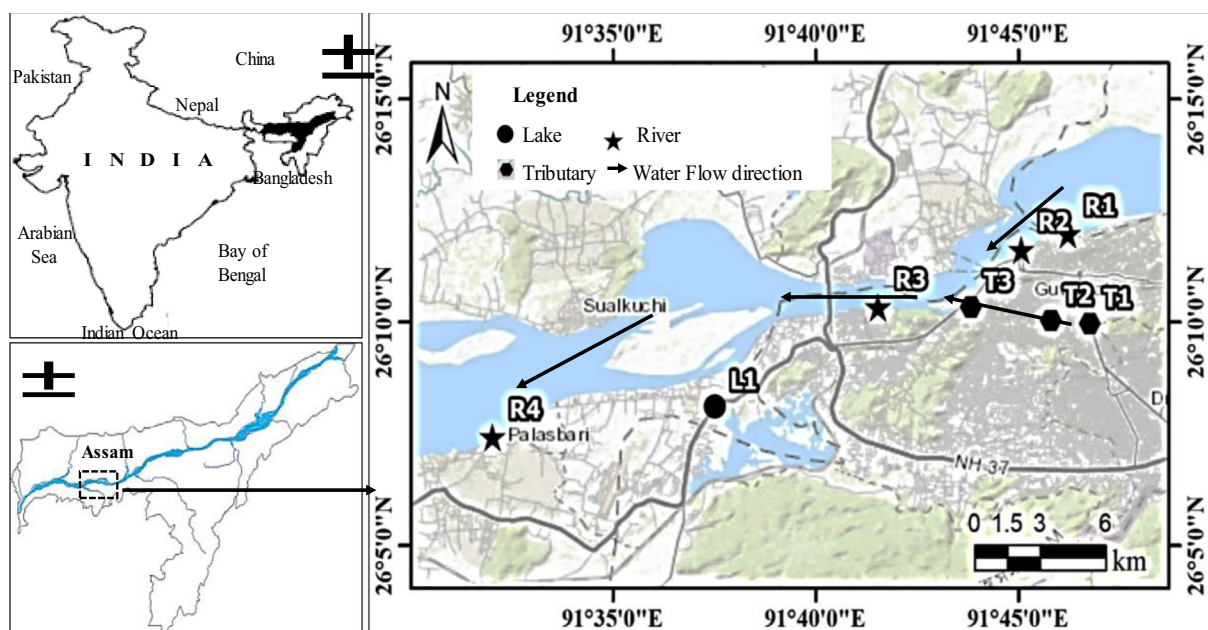

Fig. 1. Locations of surface water sampling in Guwahati city: Bharalu (a tributary of the Brahmaputra river) through the populated urban area of Guwahati city (T1–T3); the Brahmaputra river (R1–R4); and Deepor lake (L1) in Assam, India.

50:50 (v/v) methanol:water, and stored at −20 °C until analysis. Analytical quality assurance data showed 56 to 102% recovery of each compound (active ingredient) from a spiked matrix.

2.3.2. Analyses

An Ultra Performance Liquid Chromatography (UPLC) equipped with a Hypersil GOLD™ column (150 mm × 2.1 mm × 5 μm; Thermo Fischer Scientific, Massachusetts, USA) was used at 40 °C to separate the target compounds, which were detected and quantified by Orbitrap Fourier Transform Mass Spectrometry (Exactive, Waltham, Massachusetts, USA) using analytical standards. Injection volume was 10 μL. Solvents A and B were water with 0.1% formic acid and methanol with 0.1% formic acid, respectively, similar to that described by Kosma et al. (2014). Run time was 53 min at a flow rate of 0.2 mL min^{-1}. Data were acquired in two simultaneous full scans (positive ion mode) over a mass range of 150–300 for molecular ions and 50–200 for fragment ions. In-source collision-induced dissociation (CID) at 20 eV was used to produce fragment ions from molecular ions. Detection and confirmation of target compounds were based on mass-to-charge ratio (m/z) and retention time, with criteria of 5 mg L^{-1} mass tolerance and a 0.3 min retention time window. One molecular ion $[M + H]^+$ and at least one fragment ion were acquired for each compound. Retention time and m/z of each compound are given in Table S1 and for internal standards in Table S2.

2.3.3. Analytical performance

SPE recovery from the spiked matrix was 56 to 102%, depending on compound (active ingredient). Quantification was performed using internal calibration curves of 10–100 ng L^{-1} or 100–1000 ng L^{-1}, depending on compound and concentration. Linearity was confirmed for all compounds. Matrix effects, including signal suppression and enhancement, were compensated by use of internal standards. The limit of detection (LOD) was the lowest detectable concentration with a signal-to-noise ratio of at least 3:1, whereas limit of quantification (LOQ) was the lowest detectable concentration with a signal-to-noise ratio of at least 10:1. When LOQ was below the lowest concentration in the calibration curve, the lowest concentration was considered as the LOQ. Concentrations below the lowest concentration of the standard were calculated by interpolating between that concentration and the origin. LODs were 0.17–46 ng L^{-1} and LOQs were 10–153 ng L^{-1} (Table 2).

2.4. Virus measurements

2.4.1. Virus concentrations

Water samples were concentrated for viruses using a negatively charged membrane (Katayama et al., 2002) (Fig. S2). In the first step, 200 μL of 2.5 M MgCl$_2$ was added to 50 mL of water samples to obtain a final concentration of 25 mM and then passed through a negatively charged membrane (HA, 0.45-μm pore size, 47 mm diameter, 9.6 cm^2 area; Millipore, Japan). The membrane was rinsed with 200 mL of H$_2$SO$_4$ (0.5 mM, pH 3.0) to elute the cations, followed by 5 mL of 1 mM NaOH (pH 10.5) and collected in a 5 mL tube containing 25 μL of 100 mM H$_2$SO$_4$ (pH 1.0) and 50 μL of 100× TE Buffer.. The samples were further concentrated by ultrafiltration (Ultracel YM-50, MWCO 50 kDa, Millipore) to obtain final volumes of approximately 600 μL as per the manufacturer's protocol. The prepared samples were subjected to viral RNA extraction, reverse transcription, and qPCR.

2.4.2. Viral RNA extraction, reverse transcription and quantification

Viral RNA was extracted using a QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Reverse transcription (RT) was carried out using a high capacity cDNA reverse transcription kit (Applied Biosystems, Tokyo, Japan). RT thermal conditions were: 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 min. Real-time PCR (qPCR) was conducted using 20 μL of a reaction mixture containing 5 μL of cDNA, 10 μL of TaqMan Gene Expression Master Mix (Applied Biosystems, Tokyo, Japan), 1 μL each of 10 μM forward primer and reverse primer, 0.5 μL of 5 μM TaqMan probe, and 2.5 μL of nuclease-free water. Primer and probe sequences are listed in Table S3. Real-time PCR was conducted using Step OnePlus real-time PCR system (Applied Biosystems) with cycling at 95 °C for 10 min, followed by 50 cycles at 95 °C for 15 s and 60 °C for 1 min. The calibration curve for quantification of viral genomes was generated by amplifying ten-fold serial dilutions (1.0×10^0 to 1.0×10^4) of a plasmid DNA containing the target sequence.

2.5. Antibiotic resistance of E. coli

2.5.1. Isolation of E. coli

Strains of E. coli were isolated and separated from the collected samples using the membrane filtration method. Samples were diluted by 10-fold steps using phosphate-buffered, 0.8–0.85% NaCl solution. A culture media was prepared in distilled water using Chromocult® Coliform Agar ES (Merck, Japan) at 34.5 g L^{-1} and autoclaved at 121 °C and 15 psi for 15 min. After adding 4 mL of diluted sample to 36 mL of buffer solution, it was poured into 37 mm monitor kits (Advance Toyo, Tokyo, Japan) consisting of a filter paper with a pad to absorb the culture media. After incubating for 22–24 h at 35.5 °C, the Petri dishes were removed and E. coli colonies identified by a dark blue/violet colour.

2.5.2. Antibiotic susceptibility test

The sensitivity of E. coli to six antibiotics, viz. levofloxacin (LVX), ciprofloxacin (CIP), norfloxacin (NFX), kanamycin monosulphate (KM), tetracycline (TC) and sulfamethoxazole (ST), were tested using the Kirby-Bauer (KB) disc diffusion method (Honda et al., 2016). Isolated E. coli were cultured in NaCl solution at 35 ± 2 °C to obtain turbidity equivalent to McFarland No. 0.5, corresponding to a cell density of 1.5 × 10 CFU mL^{-1}. The E. coli cultures were smeared on 5 mL of Tryptic-Soy Broth (EIKEN Chemical Co., Ltd., Tokyo, Japan) in sterile Petri plates (90 mm diameter) with KB discs containing the antibiotics. Plates were incubated at 37 °C for 18–22 h. The susceptibility of isolated E. coli was characterized as resistant, intermediate or sensitive based on inhibition zone diameter.

2.6. Quality assurance/quality control (QA/QC) and statistical analyses

To determine if contamination occurred during transport, blanks in the same type of bottle were analysed prior to sampling. Duplicate analysis of samples was conducted to check accuracy and precision. To ensure instrument sensitivity and check cross-contamination, blanks were run for each batch of five samples. Signals were considered significant if the signal-to noise ratio was >3:1. SPSS 21 (IBM) was used for Principal Component Analysis (PCA) and hierarchical cluster analysis (HCA) after normalization by obtaining z-scores for each parameter. Varimax rotation, an orthogonal rotation mode, was used to generate non-related PCs. Results were represented on a 3-dimension PCA diagram. Cluster analysis was conducted using the Ward method to show proximity among the analysed parameters of all samples.

2.7. Estimation of ecological risk

To estimate the potential ecological impact of the selected PPCPs, ecological risk was determined by calculating risk quotient (RQ; Sharma et al., 2019). Maximum measured concentrations (MC$_{RW}$) were divided by the corresponding predicted no effect concentration (PNEC) for algae, Daphnia magna, and fish [Eq. (1)]. For a worst-case scenario, the maximum detected concentration from any of the sampling sites was divided by the lowest PNEC reported in the literature [Eq. (2)].

$$RQ = MC_{RW}/PNEC \qquad (1)$$

$$PNEC = (EC_{50} \text{ or } LC_{50})/AF \qquad (2)$$

EC$_{50}$ (effective concentration reducing a biological process by 50%) and LC$_{50}$ (lethal concentration for 50% of the organisms) values were obtained from the literature (Table S4; Huschek et al., 2004; Sharma et al., 2019; Watanabe et al., 2016). An uncertainty factor of AF = 1000 was used to account for intra- and interspecies variability in sensitivity (Mutiyar et al., 2018). Risk to aquatic organisms was divided into three classes: low risk (RQ < 0.1), moderate risk (0.1 < RQ < 1), and high risk (≥1) (Sharma et al., 2019).

3. Results and discussion

Table 1 shows in situ properties and metal concentrations in river, drain and lake samples. Major observations were as follows: i) the Drain had the highest EC, with EC$_{River}$ < EC$_{Lake}$ < EC$_{Drain}$; ii) the Drain showed the impact of septic wastewater, evident from negative redox values, while the river and lake redox were positive; iii) lake water had the highest pH and temperature, and lowest HCO$_3^-$, likely attributable to lack of flow and biological activity; iv) HCO$_3^-$ was highest at the upstream river location, implying better flow, weathering and CO$_2$ dissolution; and v) concentrations of certain metals (Zn > Cu > Pb) were much higher than Co > Cd ≥ As, but there was no clear difference in metal contamination among the different surface waters.

3.1. Comparative PPCP occurrence and variability

PPCP concentrations in surface water samples are summarized in Table 2. Occurrence and concentration frequencies (number among total analysed, showing concentrations above LOD) of the five analysed PPCPs were CTMT (25%) < CMZ (50%) < ACE (63%) = THEO (63%) < CAF (100%). Lake water samples contained only two of the PPCPs (CMZ and CAF), while river samples contained ACE, THEO and CAF. In the Bharalu Tributary concentrations of ACE and THEO were both on the order of 10^3 but the maximum ACE concentrations was almost twice that of THEO. In contrast, CAF concentrations were on the order of ~10^4, five to eight times higher than THEO and ACE. In the Brahmaputra River, concentrations of THEO and CAF were on the order of 10^2, one tenth those of the urban drain tributary. Interestingly, the urban wetland (Dipor Beel) had the best water quality on the basis of PPCP concentrations. PPCPs detected in the Bharalu tributary were similar to the Najafgarh drain discharging into the Yamuna River in Delhi (Mutiyar et al., 2018).

Variations in PPCP concentrations illustrated the dilution capability of the river and significance of urban sources of PPCPs, evident from concentration trends (R1 < R4 < R2 < R3 in the river and T1 < T2 < T3 in tributaries; Fig. 2). Most of the PPCPs were not detected on both ends of the sampled Brahmaputra River locations, indicating a major dilution effect. This can be attributed to high discharge during the

monsoon period (July 2017 sampling) and seasonal variation should be investigated for tropical conditions. In contrast, urban drains showed an additive effect for all PPCPs except CTMT, which remained the same along the drain. Overall PPCP results reflect drug use in the city as a stimulant (mainly tea) > antipyretic > respiratory drugs > anticonvulsant > antipruritic, similar to national drug use in India. Lifestyle impacted PPCP concentrations, evident from higher CAF in the Brahmaputra River than the Ganges (Peteffi et al., 2018). CAF is not likely amenable to significant sorption, volatilization or sedimentation in surface water (Benotti and Brownawell, 2007; Bradley et al., 2007; Buerge et al., 2003; Richardson and Bowron, 1985). Although CMZ was much higher in the Llobregat river in Spain (~3090 ng L^{-1}) than in surface water in India and other places throughout the world (Ginebreda et al., 2010), it is the primary anticonvulsant and antiepileptic drug in India (Mutiyar et al., 2018). About 72% of CMZ is metabolized after an oral dose and excreted through urine, while the rest is excreted unaltered in feces (Zhang et al., 2008). CMZ is not rapidly photodegraded but dilution may decrease concentrations (Tixier et al., 2003). Crotamiton was detected in the Bharalu Tributary but is not widely used in India and its concentration was <LOD in the Brahmaputra River and Lake samples.

As PPCP abundance is highly related to consumer behavior, drug regulation, lifestyle, and WWTP availability, a table illustrating PPCP concentrations in ambient waters of different countries/regions was prepared (Table 3). With small differences, the findings are consistent with the observation that rivers in developed countries contain less PPCPs than in developing countries, primarily attributable to better medical regulations rather than availability of treatment facilities. In India, PPCP pollution is highest in the Yamuna River, followed by the Ganges and Brahmaputra, underlining the impact of urban effluents and dilution by the river. Rivers with huge discharges like the Mississippi, Ganges and Brahmaputra effectively dilute PPCPs, while they can be more prominent in smaller rivers (Linden et al., 2015).

3.2. Vulnerability through ecological risk

ACE, THEO, CAF, CMZ, and CTMT RQs, derived for algae, daphnia and fish, are summarized in Table S4 and represented in Fig. 3. The outcome of such analyses is of course dependent on the nature of the bioassays used to calculate PNECs; i.e., what organisms are used, whether tests are acute or chronic, and whether they include sensitive and relevant endpoints. The CAF RQ for algae is high (1515) for Bharalu compared to 49.5 for the River Ganges and 176 for the Yamuna River. The RQs (except CAF for algae) were generally ≪1, implying negligible risk of acute/chronic toxicity to these aquatic organisms. The CMZ RQ < 0.1 implies no health risks for aquatic organisms (Fig. 3). The highest CMZ concentrations in rivers were (ng L^{-1}) 75 (Bharalu), 16 (Ganges), 1346 (Yamuna), and 128 (Kaveri). Only the Yamuna River water seems to have potential harmful effects of CMZ on fish (Triebskorn et al., 2007; Contardo-Jara et al., 2011). Chronic low concentrations affect aquatic organism

Table 1
Sampling locations, in situ water quality parameters, and metal concentrations.

	Sample ID	Location name	Temp. °C	pH	EC ms cm^{-1}	DO mg L^{-1}	ORP mV	HCO$_3^-$ mg L^{-1}	Cu µg L^{-1}	Pb µg L^{-1}	Zn µg L^{-1}	Cd µg L^{-1}	As µg L^{-1}	Co µg L^{-1}	Mn mg L^{-1}	Ni mg L^{-1}	Cr mg L^{-1}
Tributary	T1	Junali	29.5	6.91	221	6.6	−50	125	12.20	6.77	30.84	0.15	0.05	3.40	0.03	0.29	0.38
	T2	Bhangagarh	30.4	7.02	487	3.1	−147	110	13.07	6.94	59.35	0.19	0.45	3.80	0.21	0.32	0.38
	T3	Bharalumukh gate no 9	31.0	6.64	648	1.8	−112	105	13.60	6.91	31.63	0.19	0.19	3.46	0.03	0.27	0.39
River	R1	Kharguli	28.4	6.98	110	7.3	28	170	13.45	6.86	47.24	0.21	0.10	3.44	0.03	0.26	0.39
	R2	Uzanbazar	27.8	6.54	114	6.3	45	130	14.25	6.69	24.53	0.20	0.95	4.04	0.80	0.30	0.39
	R3	Pandu port	28	6.93	114	6.1	114	100	13.30	7.18	26.23	0.29	0.15	3.44	0.05	0.30	0.36
	R4	Palashbari ward no. 1	28.2	7.4	119	5.4	67	120	13.09	8.43	44.58	0.66	0.06	3.44	0.05	0.29	0.38
Lake	L1	Deepor beel	33.7	8.01	188	6.2	67	85	15.03	7.30	52.26	0.46	0.25	3.47	0.03	0.32	0.41

Brahmaputra River (R), Bharalu tributary of Brahmaputra (T).

Table 2
Concentration of PPCPs, viruses and faecal bacteria at different sampling locations, their detection limit, detection rate, and concentration range.

Particulars	PPCPs[a]					Virus[b]					Faecal bacteria[c]	
Sample ID	ACE	THEO	CAF	CMZ	CTMT	Hepatitis A	Norovirus GI	Norovirus GII	Aichi	PMMoV	E. coli	Total coliform
T1	2156	1625	11,445	9	<LOD	10.6	0.2	2.6	170	14,557	3.9	4.7
T2	4064	2384	19,577	53	8	41.8	0.6	3.5	385	15,236	3.8	4.6
T3	5967	2939	22,733	75	8	52.5	0.2	5.8	567	23,738	4.0	4.6
R1	<LOD	<LOD	35	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	15.2	<LOD	1.5
R2	6	277	410	8	<LOD	<LOD	<LOD	<LOD	<LOD	137	1.3	2.1
R3	6	609	805	9	<LOD	<LOD	<LOD	<LOD	<LOD	164	1.4	2.4
R4	<LOD	<LOD	47	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	15.7	1.7	2.4
L1	<LOD	<LOD	54	3	<LOD	<LOD	<LOD	<LOD	<LOD	82.1	<LOD	1.4
Limit of detection (LOD)	20	46	17	0.17	1	10	10	10	10	10	0	0
Limit of quantification (LOQ)	100	153	100	10	10	–	–	–	–	–	–	–
Detection rate (%)	62.5	62.5	100	75	25	37.5	37.5	37.5	37.5	100	75	100
Concentration range (ng L^{-1})	<LOD –5967	<LOD –2939	35–22,733	<LOD –75	<LOD –8	<LOD –52.5	<LOD –0.6	<LOD –5.8	<LOD –567.1	15.2–23,738	<LOD –4.0	1.4–4.7

ACE – acetaminophen, THEO – theophylline, CAF – caffeine, CMZ – carbamazepine, CTMT – crotamiton.
For PPCPs, underlined concentrations = below limit of quantification (LOD).
[a] PPCPs concentration (ng L^{-1}).
[b] Virus concentration (copies mL^{-1}).
[c] Faecal bacteria concentration log (CFU mL^{-1}).

function, genotoxicity, behavior, reproduction, and metabolism. The effects of PPCPs on the aquatic environment can be synergistic or antagonistic. The fish population was decreased 50% in rivers of Switzerland and correlated with increase in organic micropollutant level (Burkhardt-Holm et al., 2002). A combination of drug pollutants could lead to concentration addition (Cleuvers, 2003), which can be fatal in
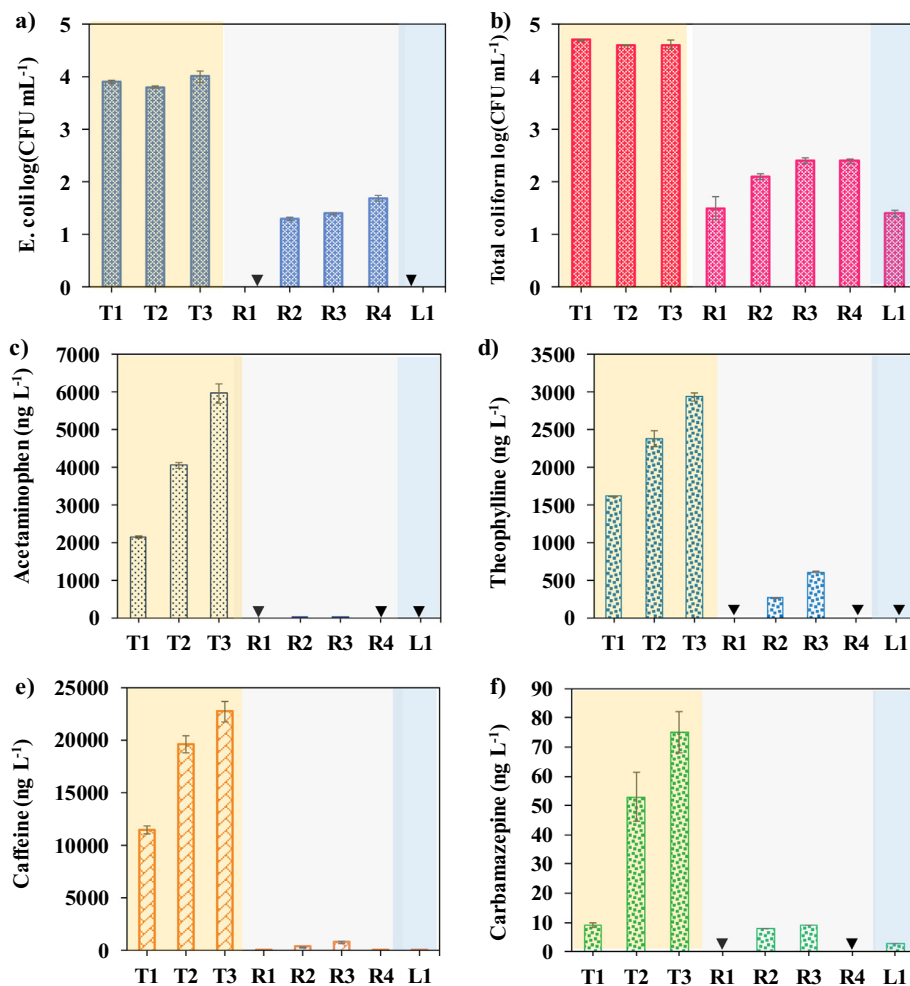
Fig. 2. Variation along the stream of: a) *E. coli*; b) total coliform; c) acetaminophen; d) theophylline; e) caffeine; and f) carbamazepine along the Bharalu tributary (T1–T3, upstream to downstream) and the Brahmaputra river (R1–R4, upstream to downstream), and lake (L1) Black inverted triangle indicates value less than detection limit. Black inverted triangle indicates values below the detection limit. Error bars indicate standard deviations of the means.

Table 3
Comparison of PPCPs (acetaminophen, caffeine, carbamazepine) in several surface waters in the world (concentration unit ng L^{-1}).

	Surface water	Min	Max	Reference
Acetaminophen	Mississippi River, USA		ND	Boyd et al., 2003
	Pontchartrain Lake, USA		ND	
	Streams of Iowa, USA	–	1950	Kolpin et al., 2004
	Elbe river, Europe	–	66	Wiegel et al., 2004
	UK surface water	–	264	Bound and Voulvoulis, 2006
	Mississippi river, USA	24.7	65.2	Zhang et al., 2007
	Guadalquivir River, Spain	–	2694.5	Robles-Molina et al., 2014
	Yamuna River, India	<LOD	1565	(Mutiyar et al., 2018)
	Ganges River, India	<LOD	4.17	(Sharma et al., 2019)
	Present study	<LOD	5967	
Caffeine	Ramos River, Brazil	–	357,000	Ferreira Da Silva et al., 2005
	Tributary of Maggiore, Italy	0.6	1056	Loos et al., 2007
	Maggiore Lake, Italy	–	124	
	Mississippi river, USA	<LOD	38	Zhang et al., 2007
	Mediterranean Sea, South coast of France	13	107	Togola and Budzinski, 2008
	Barigüi River, Brazil	ND	753,500	Froehner et al., 2010
	Atibaia River, Brazil	174	127,092	Montagner and Jardim, 2011
	Drinking water in Missouri, USA	2.5	225	Wang et al., 2011
	Guadalquivir River, Spain	–	233	Robles-Molina et al., 2014
	Sinos River, Brazil	150	16,720	Linden et al., 2015
	Sinos River, Brazil	42	28,439	Peteffi et al., 2018
	Yamuna River, India	<LOD	2640	(Mutiyar et al., 2018)
	Ganges River, India	17.5	743	(Sharma et al., 2019)
	Present study	35	22,733	
Carbamazepine	Surface water, Berlin	–	1075	Heberer, 2002
	Detroit River, Canada	0.3	0.8	Hua et al., 2006
	Jamaica Bay, New York	5	35	Benotti and Brownawell, 2007
	Vantaa river, Finland	21	80	Vieno et al., 2007
	Mississippi river, USA	42.9	113.7	Zhang et al., 2007
	US Surface Water	4.1	51	Benotti et al., 2009
	Rivers near Madrid in Spain	–	184	Alonso et al., 2010
	Llobregat River, Spain	80	3090	Ginebreda et al., 2010
	Pearl River, China	15.6	43.1	Zhao et al., 2010
	Yangtze Estuary, China	17	675	
	Kaveri River, Southern India	–	128	Ramaswamy et al., 2011
	Tamiraparani River, Southern India	–	12.2	
	Vellar River, Southern India	–	5.72	
	Yamuna River, India	<LOD	1346	(Mutiyar et al., 2018)
	Ganges River, India	<LOD	16.1	(Sharma et al., 2019)
	Present study	<LOD	75	

LOD – limit of detection, ND – not detected.

water systems with negligible flow like lakes or wetlands. For mixtures of compounds, the actual risk to the aquatic environment can be many times greater than the worst-case scenario for a single PPCP, thus Hazard Quotient (HQ) or RQs values should be used with caution when defining ecological risk posed by PPCPs. Overall, Daphnia seems more sensitive than algae and fish to all PPCPs except CAF in all of the water systems compared.

3.3. Prevalence of faecal bacteria and viruses

India is a tropical country and likely to have high microbial densities in surface water, but to our knowledge there is no scholarly study on the abundance of viruses. Distributions of faecal bacteria and viruses in the surface water samples are summarized in Table 2. The detection rate was 88% for *E. coli* and 100% for total coliform and PMMoV. Except for
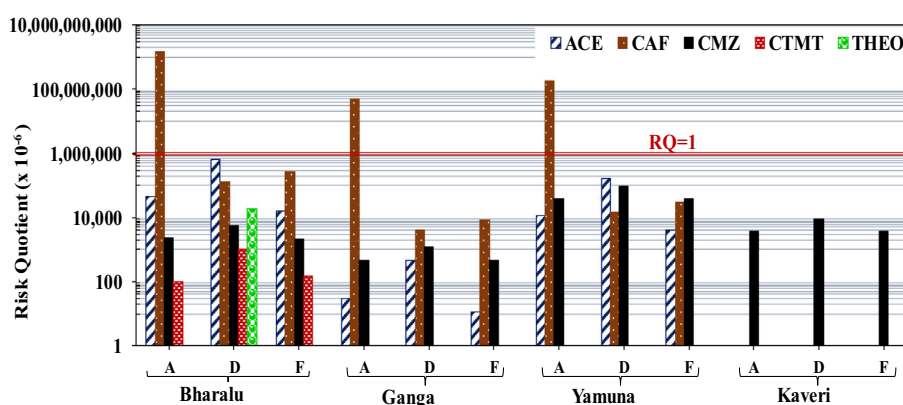
Fig. 3. Risk quotients (RQ; logarithmic scale) for PPCPs in the Bharalu Tributary and comparison with the Ganges River (Sharma et al., 2019), Yamuna River (Mutiyar et al., 2018), and Kaveri River (Ramaswamy et al., 2011) with respect to acute toxicity to algae (A), daphnia (D), and fish (F).

PMMoV, no viruses were detected in river or lake waters. The urban drain (Bharalu Tributary) had a prevalence of hepatitis, norovirus GI, aichi, and PMMoV. Again, based on coliform and virus concentration, the condition of Dipor Bill was better than others. *E. coli* and total coliform in urban drains were two times higher than that of river and four times that of Dipor Beel. This implies that while there is high inactivation of *E. coli* by attenuation in the river, among other differences, the wetland receives less faecal/sewage wastes (Hamza et al., 2011a).

The virus count was T1 < T2 < T3 in tributaries (Fig. 4), except for norovirus GI, attributable to the fact that as the drain progresses toward the river it collects wastewater from multiple urban sources and thus virus prevalence increases from T1 to T3. The order of abundance of viruses in the tributaries was PMMoV > Aichi 1 > hepatitis A > norovirus GII > norovirus GI. PMMoV was abundant with a maximum count of 23,738 copies mL^{-1}, which is an order of magnitude higher than reported in Japan (2 to 2900 copies mL^{-1}; Haramoto et al., 2013), as well as in German river water samples (3 to 1100 gen.equ. mL^{-1}; Hamza et al., 2011b). Faecal-oral routes potentially transmit Aichi viruses through the intake of food and water. Aichi 1 is globally distributed and has been reported in many environmental samples, such as sewage, surface water, groundwater, and shellfish (Kitajima and Gerba, 2015). Hepatitis A ranged from 10.6 to 52.5 copies mL^{-1} and is a serious public health concern in many countries (Ahmad et al., 2018). Interestingly, the highest counts of Norovirus GI (0.6 copies mL^{-1}) and Norovirus II (5.8 copies mL^{-1}) were at different locations

within the drain. This is the opposite from Japan where Norovirus II (0.34 copies mL^{-1}) had a lower count than GI (0.27–33 copies mL^{-1}) (Haramoto et al., 2012). Resulta indicate that the urban drain flowing through Guwahati city is highly polluted, which significantly deteriorates the water quality of the Brahmaputra River. High concentrations of pathogenic viruses pose health risks to humans. The current occurrence of disease-causing bacteria and viruses in surface water is alarming and emphasize the need for remedial measures to prevent undesirable outcomes.

3.4. Antibiotic sensitivity of isolated E. coli

The sensitivity of isolated *E. coli* to six antibiotics is illustrated in Fig. 5. While no *E. coli* could be isolated at the R1 location (before the river enters the city), the R2 location in the city but before mixing with Bharalu drain water exhibited up to 100% resistance to all antibiotics except tetracycline. Interestingly, ARB were less at location T3 (tributary sample just before mixing with the river) than R2 and R3 (location 2 km downstream after mixing of the tributary and river) but higher than R4 (12 km downstream after mixing). This implies that the river carries highly resistant microbes then receives additional *E. coli* with less resistance. Thus there is a net decrease in resistance by location R4. Location R3 exhibited 100% resistance to LVX, CIP, NFX, ST, and TC, except for KM, which decrease at R4 except for TC (that decreased to 60%). The increase in resistance to TC is in agreement with
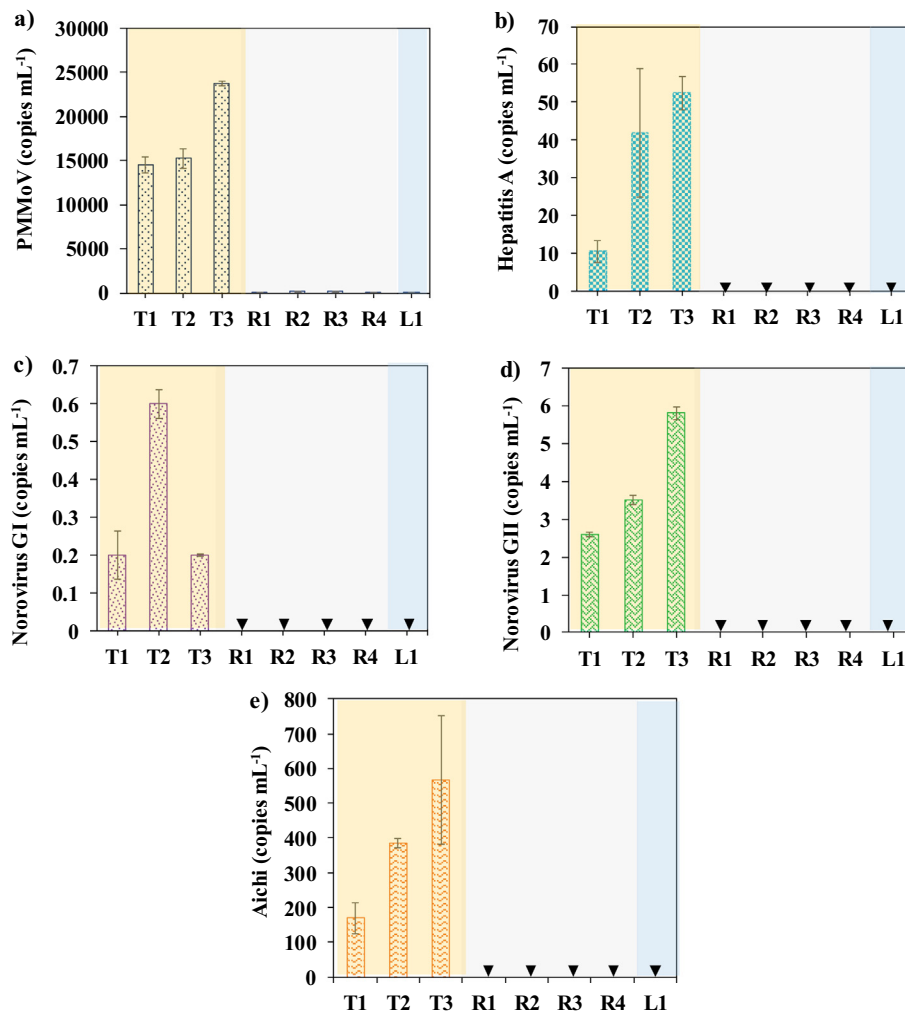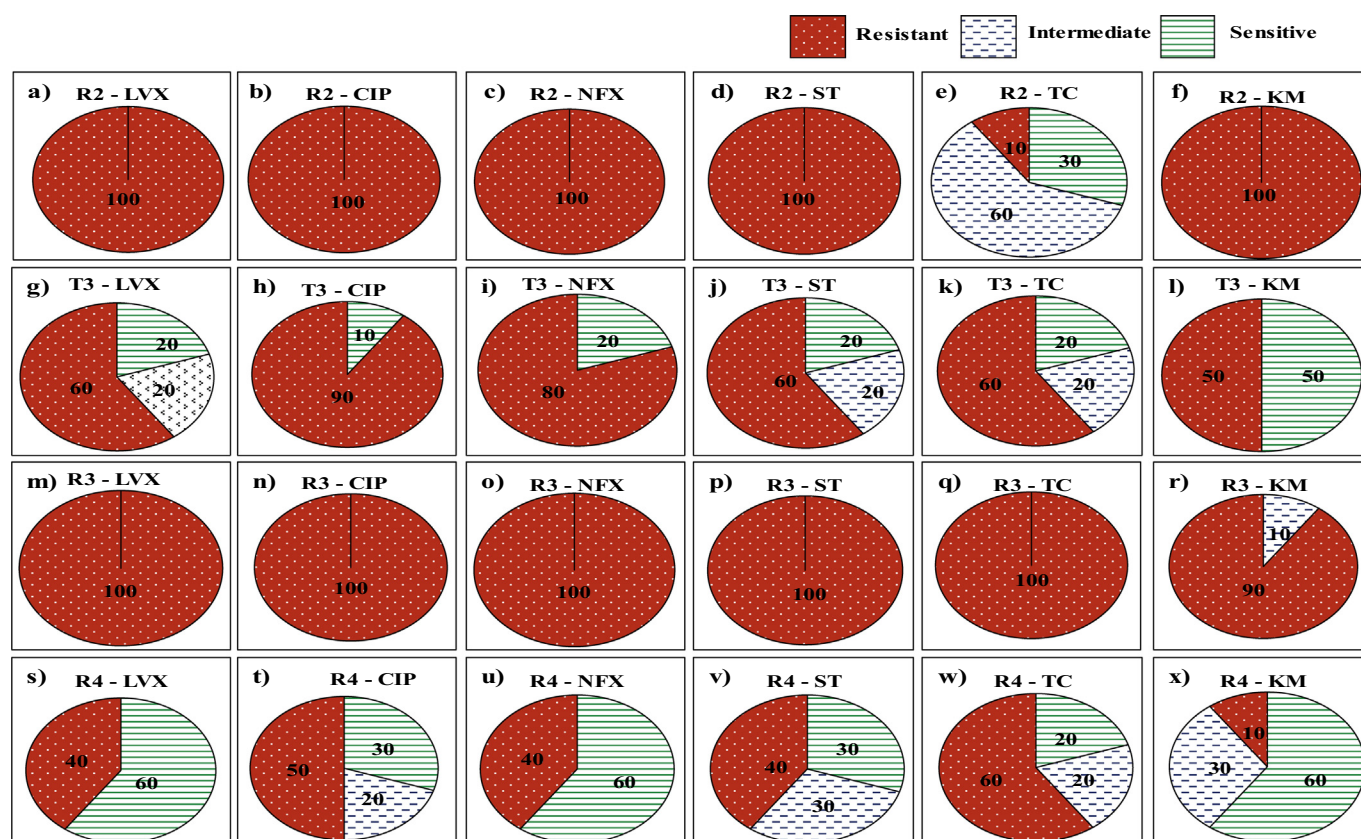

Fig. 4. Concentrations of viruses at the sampling locations: a) PMMoV; b) Hepatitis A; c) Norovirus GI; d) Norovirus GII; and e) Aichi. Error bars indicate standard deviations of the means. The black inverted triangle indicates value is below the detection limit.

Fig. 5. Pie chart showing levels of resistance (resistant, intermediate, sensitive) for six types of antibiotics at four sampling locations (R2, T3, R3 and R4): last point of the river before mixing (a to f); last point of the tributary before mixing of river (g to l); river after mixing of the tributary (m to r); and river downstream after the city (s to x). LVX = levofloxacin; CIP = ciprofloxacin; NFX = norfloxacin; KM = kanamycin; ST-sulfamethoxazole; and TC = tetracycline.

Kim et al. (2007), who showed that bacteria species resistant to TC have been frequently detected in surface water and wastewater.

Correlation analysis was used to show relationships among the variables LVX, CIP, NFX, KM, ST, TC, *E. coli* and total coliform (Table S5). LVX correlated positively with CIP (0.89) and NFX (0.94), likely due to cross-resistance within this class of fluoroquinolone (FQ) antibiotics. There is a significant correlation among NFQs as well; ST and TC showed a correlation of 0.99. Among the cross-correlations between FQs and NFQs, only NFX showed moderate correlation with ST and TC. *E. coli* and total coliform showed no significant correlations. Resistance to any specific class of antibiotics is mainly dependent on frequency of use (Ferreira Da Silva et al., 2007; Threedeach et al., 2012), and lack of adequate treatment of sewage effluent (Marathe et al., 2017). Lack of treatment results in release of untreated effluent into the river (Schijven et al., 2016). Thus policy focusing on reduction of resistant bacterial

loads in wastewaters and control of the release of antimicrobial agents from biomedical and farm waste can be a first step forward.

3.5. Concurrence of virus, PPCPs and faecal bacteria

The concurrence of PPCPs, viruses and faecal bacteria in different surface waters is shown in Table 4. Although they showed a similar spatial distribution, i.e. higher in Bharalu tributary followed by the river and lake, there was some difference in the distributions of PPCPs, viruses and *E. coli*. This may be because of non-specificity to human sources of pollution and because PPCPs are more directly associated with raw sewage (Liu and Wong, 2013). THEO and CAF were detected at locations R2 and R3 but viruses and faecal bacteria were not present, implying differences in persistence of PPCPs in the various water systems. *E. coli* is present in both human and animal feces and apart from raw sewage, the

Table 4
Concurrence of PPCPs, virus, faecal bacteria in different surface water.

	ACE	THEO	CAF	CMZ	Hepatitis A	Norovirus GI	Norovirus GII	Aichi	PMMoV	*E. coli*
THEO	0.99									
CAF	0.98	0.99								
CMZ	0.98	1.00	0.91							
Hepatitis A	0.96	0.98	1.00	1.00						
Norovirus GI	0.00	0.09	0.25	0.19	0.27					
Norovirus GII	0.97	0.94	0.88	0.91	0.87	−0.25				
Aichi	1.00	1.00	0.98	0.99	0.97	0.05	0.96			
PMMoV	0.96	0.97	0.98	0.82	0.74	−0.44	0.98	0.88		
E. coli	0.87	0.92	0.93	0.33	0.25	−0.87	0.70	0.46	0.96	
Total coliform	0.84	0.90	0.91	0.59	−0.97	−0.50	−0.72	−0.89	0.92	1.00

ACE – acetaminophen, THEO – theophylline, CAF – caffeine, CMZ – carbamazepine.

bacteria can contaminate surface water through urban and agricultural runoff during heavy rainfall due to sanitary sewer and combined sewer overflows (McLellan et al., 2007). Faecal bacteria also tend to have a high susceptibility to inactivation by natural attenuation processes, while viruses are more persistent but present in low numbers, except PMMoV. This warrants a need to identify better sewage markers applicable in different regions of the world.

3.6. PMMoV use as sewage marker in India

To check the feasibility of PMMoV as a sewage marker under Indian conditions, regression of PMMoV with different PPCPs, virus and faecal bacteria was evaluated (Fig. 6). In general, R^2 values for PMMoV with PPCPs were high, i.e. ACE (0.95), THEO (0.95) CAF (0.95) and CMZ (0.81). This suggests that PMMoV, with high abundance, easy detection and high source specificity to human faecal material (Kuroda et al., 2015), can indicate the presence of PPCPs. Concentration ratios of

PMMoV to CMZ for different locations (Fig. 7a) and versus CAF/CMZ (Fig. 7b) are plotted for the Bharalu tributary and Brahmaputra River to delineate source(s) of PMMoV and CMZ (Kuroda et al., 2015) and compare the fate of PMMoV and CAF. The effect of dilution is nullified by assuming CMZ does not attenuate in surface water. As PMMoV, CAF and CMZ in surface water are presumably derived from domestic wastewater discharge, the similar reduction of PMMoV and CAF relative to CMZ suggests comparable persistence of PMMoV and CAF in the surface water. This is also attributed to the fact that while CMZ behaves conservatively in surface water (Nakada et al., 2008), the fate of viruses and CAF is mainly controlled by temperature, sedimentation, resuspension, solar radiation and adsorption (Brookes et al., 2004; Buerge et al., 2003). Thus, a high ratio of CAF to CMZ can be an indication of a higher proportion of raw sewage and lack of wastewater treatment, as in the three locations (Fig. 7b). While CAF can be used as a tracer of recent faecal contamination, CMZ seems a promising tracer of cumulative persistent contaminants, and PMMoV is a promising for both faecal and PPCP
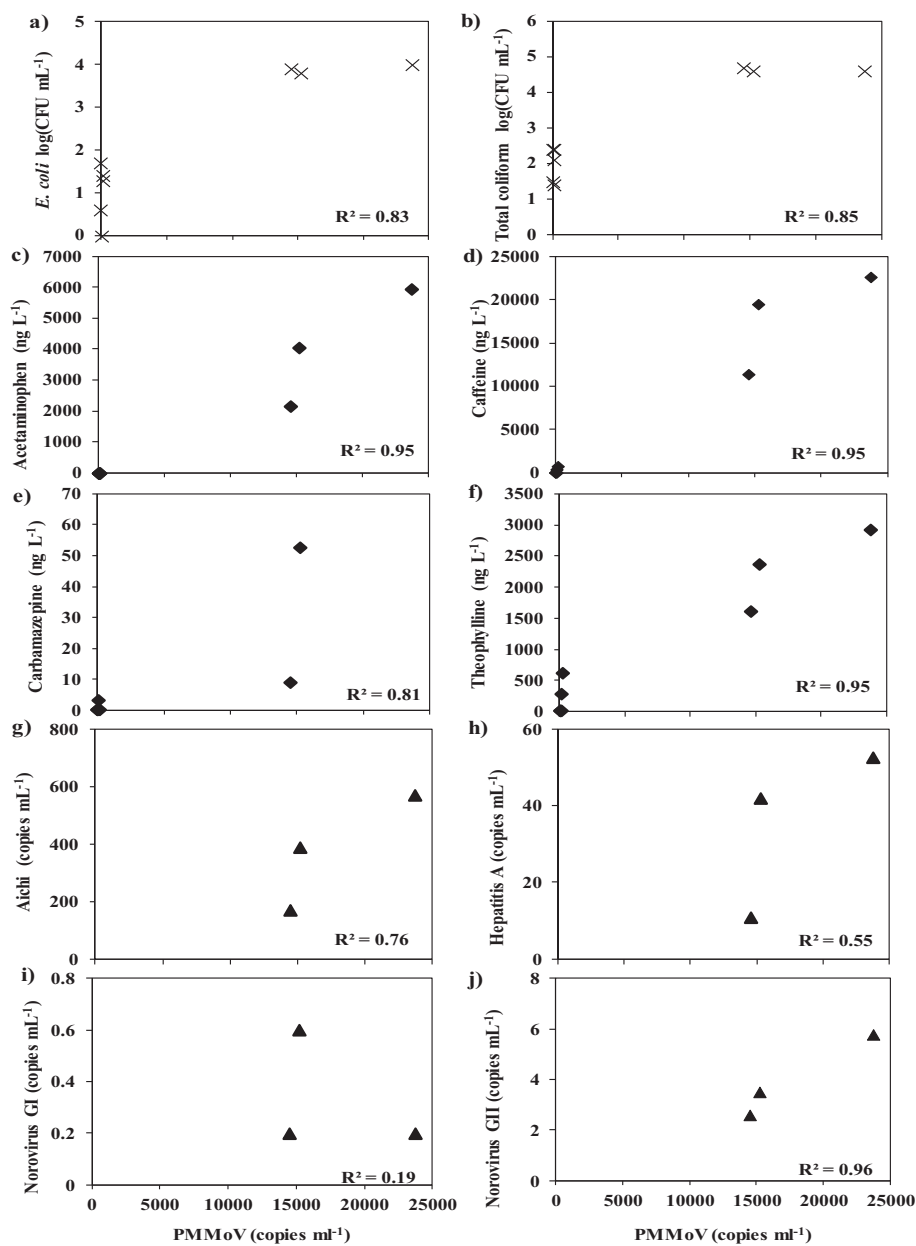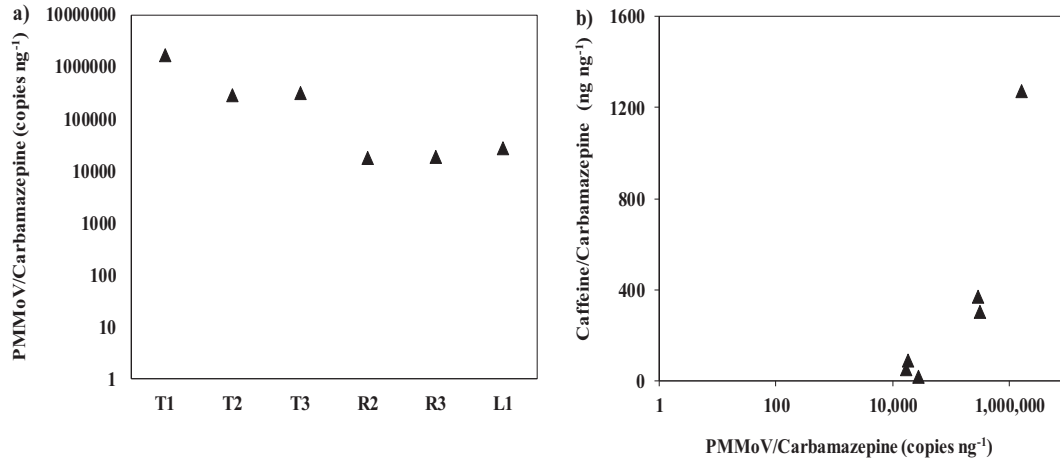

Fig. 6. Scatter plots of: a) *E. coli*; b) total coliform; c) acetaminophen; d) caffeine; e) carbamazepine; f) theophylline; g) aichi; h) hepatitis; i) oroviruNus; and j) norovirus GII in relation to PMMoV to show its potential as a marker of anthropogenic faecal pollution.

Fig. 7. Concentration ratios of PMMoV to carbamazepine plotted with (a) sampling locations in the Deepor Beel, Bharalu Tributary and Brahmaputra River, and (b) ratios of caffeine to carbamazepine.

pollution. This suggests PMMoV can be used for sensitively identifying and tracking faecal-polluted water and thus control water pollution based on the precautionary principle (Colson et al., 2010).

3.7. Principal component and cluster analysis

Fig. 8 (Table S6) shows a 3-D display of principal component loadings and a dendrogram of in situ parameter metal, PPCPs, virus, faecal coliform and antibiotic resistance obtained from hierarchal cluster analyses. Principal component analysis delineated two closely related groups, one (dark red circles) consists of PPCPs (ACE, THEO, CAF,

CMZ) and viruses (hepatitis A, norovirus GI, norovirus GII, aichi, PMMoV); and another (purple colour) of antibiotics (LVX, CIP, NFX, KM, ST, TC), followed by loadings for metals and other in-situ parameters. Cluster analysis identified three major groups (Fig. 8b), with the bottom first group comprising one cluster of EC, PPCPs (ACE, THEO, CAF, CMZ), virus (hepatitis A, norovirus GII, aichi, PMMoV) and a second cluster of faecal coliform (*E. coli*, total coliform and norovirus GI) of human disease and drug use origin. This is followed by a second group comprising two clusters, i.e. antibiotic resistance (LVX, CIP, NFX, KM, ST, TC) and metals (As, Co, Mn). A third group consists of in situ parameters (pH, Temp, HCO₃, ORP, DO) and metals (Cu, Pb, Zn, Cd, Ni, Cr). The
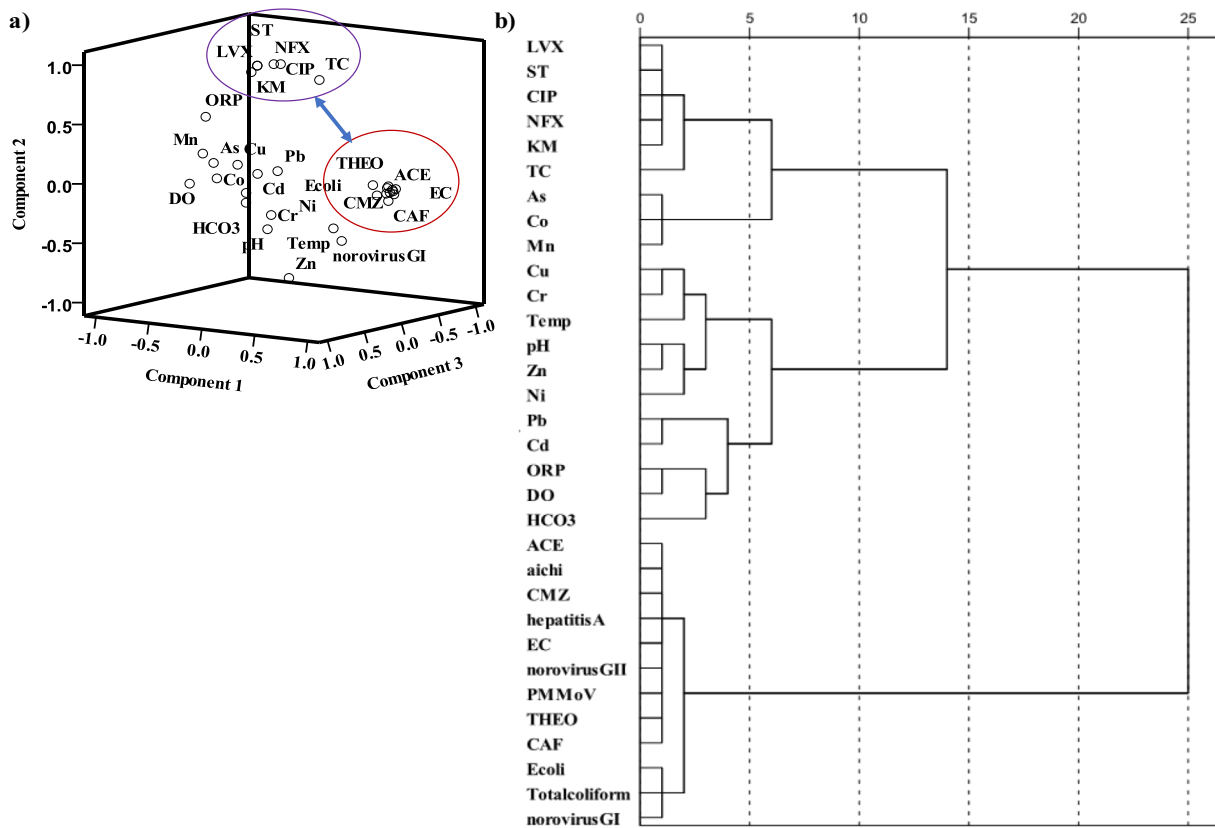
Fig. 8. (a) Three-dimensional principal component loadings and (b) dendrogram illustrating the distance of in situ metal, PPCPs, virus, faecal coliform and antibiotic resistance obtained from hierarchal cluster analyses. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

results substantiate that viruses align more closely with PPCP concentrations followed by microbial count rather than resistance of *E. coli*. Yet ARB prevalence is somehow also related to cross-resistances, as well as contamination by metals like As, Co and Mn which correlate with water quality parameters such as pH, ORP, DO and metals of industrial origin. Thus PPCP, faecal and virus contamination together comprise a different set of pollution propositions than conventional water quality parameters and metal pollution that causes antibiotic resistance in microbes present in surface waters. That is probably why the city wetland exhibited relatively poor water quality on the basis of metal pollution but had less faecal, viral and PPCPs pollution. This observation stresses the need for changes in ambient water quality criteria from the BOD, COD and EC-based standard that still prevails in India.

4. Conclusion

The present study not only points out the vulnerability of an urban area without treatment facilities but also emphasizes the resilience of urban waters, especially large rivers that have decontamination potential by virtue of dilution of PPCPs, viruses, faecal bacteria and antibiotic resistance in coliform bacteria strains. Results showed that while the urban stretch becomes infected with microbes displaying 100% resistance to levofloxacin, ciprofloxacin, norfloxacin, kanamycin monosulphate and sulfamethoxazole, upstream and downstream river channels do not possess many anthropogenic molecular markers. The level of contamination of PPCPs was considerably lower in the lake and PPCPs were not detected in upstream and downstream locations of the Brahmaputra River, implying strong self-purification processes, adsorption and sedimentation. Viruses were not detected in river or lake samples, except for PMMoV which showed high potential as a molecular marker for faecal pollution in proximity to urban areas. This information is useful for further determinations of the water quality status of surface water in the Anthropocene. Future work may also focus on the bacterial strains present in biofilms of water network systems, the corresponding selection pressure from treatment methods for antibiotic resistance, as well as seasonal variations in dilution capacities of major tropical rivers. As the water quality of the lake was comparatively better than other aquatic bodies, it may be used as a safe source of drinking water. In general, it is expected that treated water may also contain antibiotic resistant bacterial genes because the water treatment process can enhance antibiotic resistance and accelerate transfer of pathogenic genes among bacterial strains. Therefore, the first step should be controlling the widespread use of antibiotics through the regional, local and national attention of scientists, policymakers, and medical practitioners. Additionally, process-based standardized methods and guidelines/criteria are needed for indirect and direct assessment and monitoring of the status and ecological risks associated with these emerging contaminants.

Acknowledgment

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.scitotenv.2019.133640.

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