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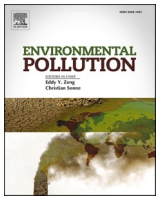

Presence of emerging organic contaminants and microbial indicators in surface water and groundwater in urban India☆

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ABSTRACT

This study presents a first combined assessment of emerging organic contaminants (EOC) and antimicrobial resistance (AMR) indicators in the South Indian city of Bengaluru from multiple sources, addressing a knowledge gap on EOCs and AMR occurrences and relationships in different water sources in urban India. A unique approach in this study was to combine the detection of EOCs with an assessment of the AMR-indicating class 1 integron-integrase gene, *intI1*. Twenty-five samples collected from groundwater, local surface waters, and tap water imported from the Cauvery Basin were screened for 1499 EOCs. A total of 125 EOCs were detected at concentrations per compound of up to 314 μg/L. Concentrations for a range of contaminants were higher than those previously detected in Indian groundwaters. High concentrations of Per- and polyfluoroalkyl substances (PFAS) were detected with up to 1.8 μg/L in surface water and up to 0.9 μg/L in groundwater. Calculated risk quotients indicated potential AMR development caused by high concentrations of azithromycin, fluconazole, and sulfanilamide in surface waters that have little protection against sewage inflows. Surface waters that have recently undergone environmental restoration (e.g., removing silted bottom layers and enhancing protection against encroachments and sewage inflows) had lower EOC detections and risk of AMR development. Specific EOC detections, e.g., the ubiquitous detection of the sweetener sucralose (in use since ~2000), indicated recent groundwater recharge and a contribution of imported Cauvery River water for recharge. This study highlights the need for monitoring and water protection, the role of EOCs as potential drivers of AMR, and the success of surface water protection measures to improve freshwater quality.

1. Introduction

Protecting water resources and reducing pollution is essential for maintaining healthy ecosystems and ensuring safe human consumption and use of water (Vörösmarty et al., 2010), and is an ongoing challenge due to human activities introducing various anthropogenic pollutants. The agricultural revolution of the twentieth century alleviated pressures on global food supplies but led to water quality degradation through the use of agrochemicals (Lapworth et al., 2022; Misstear et al., 2022; UN, 2022). More recently emerging organic contaminants (EOC), including pharmaceuticals, lifestyle products, and industrial compounds have been detected in surface waters and groundwaters, particularly in urban environments (Lamastra et al., 2016; Lapworth et al., 2018; Sorensen et al., 2015). EOCs pose threats to aquatic ecosystems and human health via their own toxicity, and by contributing to antimicrobial resistance (AMR) development and spread (Andrade et al., 2020; Antimicrobial Resistance Collaborators, 2022; Stanton et al., 2022). Antimicrobials

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Abbreviations:

AMR	antimicrobial resistance
EOC	emerging organic contaminants
intI1	class 1 integron-integrase gene
MEC	Measured environmental concentration
PFAS	per- and polyfluoroalkyl substances
PNEC	Predicted no effect concentration
$PNEC_R$	Predicted no effect concentration for resistance
PFOS	Perfluorooctane-sulfonate
PPCPs	pharmaceuticals and personal care products
RQ	Risk quotient
SEC	specific electrical conductivity
SPE	solid phase extraction
UK	United Kingdom
WWTP	wastewater treatment plant

and other compounds, including antibiotics, metals and disinfectants, are linked to the preferential survival of AMR microorganisms (Alderton et al., 2021), and increased gene exchange, leading to the spread of AMR to clinically important pathogens (Wang et al., 2019). Water environments act as a source, sink, and significant pathway of AMR (Liguori et al., 2022), which is exacerbated in the presence of high antimicrobial concentrations (Stanton et al., 2022). In rapidly developing urban environments, water quality protection is particularly challenging due to diverse pollution sources (Lapworth et al., 2017) and limited attenuation compared to natural environments (Foster et al., 1999). Some of the fastest urban developments are seen in Asian cities, such as Bengaluru, which had a population rise of the urban district from 5.7 million in the 2001 census to 8.5 million in 2011 (GoI, 2012). Formerly known as the *City of Lakes* due t o its large number of cascading urban water 'tanks' (man-made artificial lake structures), the surface water area in Bengaluru has now declined due to competing land use, but tanks remain an important feature of the city's blue-green infrastructure. However, the water quality of these tanks is highly variable. In 2017, the Environment Management and Policy Research Institute (EMPRI) tested 305 tanks for physico-chemical parameters and bacteriological contaminations and reported poor quality in a majority (86%) of the sites (EMPRI, 2018). Thirty-three tanks were identified as requiring immediate restoration (*rejuvenation*) which was initiated in recent years. Aside from removing silted bottom layers from the tanks, enhanced protection against encroachments and sewage inflows were the key measures to improve water quality.

Groundwater pollution has been assessed in Bengaluru in terms of major ions (e.g., Gulgundi and Shetty, 2018; Naveen et al., 2018; Pius et al., 2012; Raghavendra et al., 2018), metals (e.g., Cumar and Nagaraja, 2011; Shankar, 2009; Singh et al., 2010) and the presence of faecal indicators such as *Escherichia coli* (e.g., DMG, 2011; Jessen et al., 2021; Prakash and Somashekar, 2006; Sheeba et al., 2017). However, there are few data on potential EOC concentrations and their relationship to AMR in Bengaluru's surface water and groundwaters. To date, only three studies (Gopal et al., 2021; Iyanee et al., 2013; Nozaki et al., 2023) have characterised and assessed the risk from selected EOCs in Bengaluru surface waters, and no study has investigated groundwaters despite that fact that EOCs have been detected in other environmental matrices such as in the air (e.g., Chakraborty et al., 2016; Chakraborty et al., 2021), fish (Nozaki et al., 2023) and soil (Chakraborty et al., 2015; Chakraborty et al., 2016). Similarly, few studies have sampled these environments in India (Das et al., 2023; Kristiansson et al., 2011; Rutgersson et al., 2014), and particularly Bengaluru, for AMR indicators. Skariyachan et al. (2013) investigated the presence of resistant bacteria in the Byramangala Reservoir, yet this only used culture-based approaches (i.e., did not employ a quantitative molecular approach to identify AMR indicator genes) and did not examine the relationship between AMR and EOCs. Recent studies have employed risk assessment methods (for example, those derived from European environmental risk assessments (EMA, 2006; 2018; European Commission, 2001)) to assess the risk that antimicrobial concentrations pose to selecting AMR. Risk assessment-style approaches, for example, have been undertaken in the UK (Hayes et al., 2022), France (Haenni et al., 2022) and globally, including India (Wilkinson et al., 2022). However, there remains a research need to combine this risk assessment-based approach with the molecular analysis of AMR indicators, to elucidate the effects of different water source types and EOC concentrations on AMR in India, specifically Bengaluru.

The objective of this study was to undertake a combined assessment of urban EOC concentrations and AMR indicators from different water types, including river water, tank water, tap water and groundwater in India (Bengaluru). Using a broad screening for a total of 1499 EOCs, we aimed to fill the research gap in providing the first scoping study on EOCs entering the groundwater system in the urban environment in Bengaluru. We hypothesised that source type of the sampled waters will have a distinct effect on EOC detections and concentrations. We also aimed to understand the impacts of tank rejuvenation and assess the effects of EOC concentrations and source type on the prevalence of the AMR proxy, *intI1*. To assess the risk EOCs found pose for selection of AMR, we used an environmental risk assessment approach.

2. Study location and methods

2.1. Study location

2.1.1. Surface hydrology and water supply

Bengaluru, located in the southeastern part of Karnataka (Fig. 1A), is characterised by an undulating morphology with three major river valleys, Vrishabhavathi Valley, Hebbal Valley and Kormangala-Challaghatta Valley (Pavithra et al., 2021). These valleys host a number of cascading natural and man-made tanks that capture and store monsoonal rainfalls. Tank waters and other surface waters, such as the Vrishabhavathi River flowing through the city's western part, are impacted by wastewater effluent (Aravinda et al., 2015; EMPRI, 2018), and are no longer used for public water supply. However, Vrishabhavathi River discharges into Byramangala Reservoir, which is used for agricultural irrigation. Particularly in the city's peripheral areas, numerous drilled boreholes tap the fractured basement aquifer and serve as irrigation and potential drinking water source, although the aquifer has been described as overexploited (Mohan Kumar et al., 2011). Despite the heavy withdrawals, groundwater is only able to cover about 50% of the city's demand (Sinha et al., 2023; Tomer et al., 2020). The remaining demand is covered by an elaborate water import scheme developed in the 1970s that transfers water c.100 km from the Cauvery River (Suresh, 2001) and supplies households through a piped water supply network after filtration and chlorination treatment. Temporary storage of the piped water is present both on household-level as well as in larger municipal storage sites, and the piped distribution system is mainly serving the central parts of Bengaluru.

2.1.2. Hydrogeological setting

Bengaluru predominantly sits on Precambrian gneisses and granites of the Peninsular Gneissic Complex (Krabbendam and Palamakumbura, 2018), with local granitic intrusions in the central part, such as Lalbagh Park. The aquifer system features a shallow zone dominated by weathered and partially weathered bedrock, forming a saprolite with varying permeability and thickness (Skrikanta Murthy, 2011). A particularly thick saprolite of about 60 m depth is located near Vrishabhavathi Valley (Hedge and Subhash Chandra, 2012). At depth, groundwater is governed by flow though fractures in the crystalline bedrock. Fractures are mostly observed at depths of tens to over a hundred metres with decreasing likelihood at greater depths. However, boreholes have

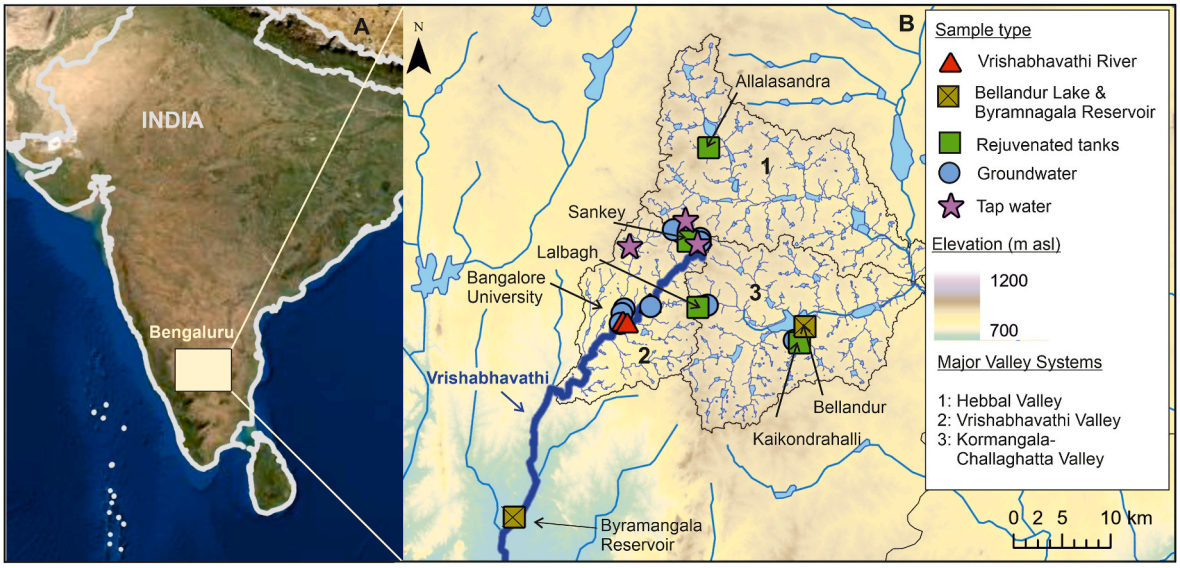

Fig. 1. Map of (A) location of the study area, and (B) sampled sites, including water courses (Vrishabhavathi River highlighted), sampled surface water locations, and elevation profile of the study site. Data sources: Esri, Maxar, Earthstar Geographics, and the GIS User Community (background map), USGS SRTM (elevation data), Natural Earth (outlines).

recently been drilled to depths beyond 300 m, indicating some potential for occasional deeper fracturing (Hedge and Subhash Chandra, 2014).

Groundwater levels vary between a few to about 70 m below ground level and are impacted locally by abstraction (Brauns et al., 2022). Annual fluctuation of groundwater levels reflects rainfall (c. 800 mm/year) patterns, with the shallowest groundwater levels in August/September (monsoon), and the deepest levels around April, at pre-monsoonal onset (Sekhar et al., 2018). Historically tanks were considered an important source of groundwater recharge (EMPRI, 2018). However, recent research suggests that piped mains water leakage is a more significant source for groundwater recharge, particularly in the more densely piped inner-city (Brauns et al., 2022; Sekhar et al., 2018; Tomer et al., 2020) and that shallow groundwater may discharge to some of the tanks during the post-monsoonal peak in groundwater levels (Brauns et al., 2022).

2.2. Methods

2.2.1. Sample collection and storage

A total of 25 pre-monsoonal water samples were collected during a 9-day sampling campaign in March 2018 in different areas of Bengaluru (n = 24) and at Byramangala Reservoir (n = 1), situated approximately 15 km southeast of the city along the Vrishabhavathi River (Fig. 1B). Surface water samples with likely impact from domestic/industrial effluent were obtained from Vrishabhavathi River (n = 3), Bellandur Lake (n = 1) and Byramangala Reservoir (n = 1). Additionally, samples were collected from the rejuvenated tanks Allalasandra, Lalbagh, Kaikondrahalli and Sankey (n = 4, Fig. 1B). All surface water samples were collected as grab samples from approximately 20–30 cm below the water surface. Groundwater samples (n = 13) were collected via in-situ pumps from one well and 12 boreholes constructed to a depth of 37–305 m. Mains water samples (n = 3), representative of imported Cauvery Water, were taken directly from home storage or—in one location—from the tap. All groundwater sites were operational and had been pumped on or within the day(s) immediately prior to sampling. Samples were collected after additional purging directly before sampling until the point of stabilisation of specific electrical conductivity (SEC), pH and temperature readings from field meters.

Samples for EOCs were collected in 500 mL glass bottles sterilized via autoclaving. Prior to sampling, plastic tubing was removed from pumps, and special attention was taken by the sampler to minimize risks of contamination, e.g., no skin products were used. A procedural blank was taken to inform about any background contamination. Samples were stored refrigerated before extraction. Solid phase extraction (SPE) of the unfiltered samples onto pre-conditioned sorbent Oasis® HLB cartridges was undertaken within one day of sample collection for most (n = 21) samples, and within a maximum of 4 days for those samples with very high turbidity to allow particulate matter to settle before extraction (n = 4). Extracted samples were stored at 4 °C, except during transportation to the United Kingdom, UK (24 h at ambient room temperature). For further details on sample extraction methods please refer to the supplementary information (S1).

Samples for molecular analyses were taken at each site. Samples for molecular analyses were collected by positive pressure filtration of 0.3–13.5 L of water onto 0.22 μm Millipore® Sterivex™ polyethersulfone cartridges. Approximately 2–3 mL of DNA/RNA Shield (Zymo Research, USA) was added for preservation prior to shipping to the UK. Prior to analysis, samples were frozen at −20 °C.

2.2.2. Chemical analysis

All EOC samples were screened for a total of 1499 EOCs at the National Laboratory Service (NLS) at Starcross near Exeter, UK using an Agilent 6540 Ultra- High-Definition (UHD) Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS system. To assess instrument performance, an isotopically labelled internal standard Carbutamide-d9 (CAS 1246820-50-7) was added to each of the pre-conditioned SPE cartridges. The target compounds were analysed in a blank and at a concentration of 0.1 μg/L, and the obtained response factor was then used to create a single point calibration curve. The estimation of concentrations was based on quant ion response and the response of internal standards (see supplementary information S1 for more details). Detection limits (LODs) for the target compounds ranged from 0.001 to 10 μg/L for quantified compounds, with 350 compounds being detectable as presence/absence only. A full list of measured compounds, including LODs is provided in Supplementary Information Table S1. Two compounds, trinexapac and carbary l were detected in similar concentrations in the procedural blank as in some water samples and were subsequently excluded from the data analysis. Triclosan was detected in the procedural blank at a very low concentration (0.003 μg/L) close to the LOD and mostly in order(s) of magnitude lower than in the samples (minimum 0.004; maximum 2.1, median 0.09 μg/L). Consequently, triclosan is included in the data interpretation for all samples exceeding the background concentration.

2.2.3. Quantification of intI1 prevalence

DNA was extracted from the Sterivex filters using the Qiagen PowerWater kit (Mo Bio Laboratories Inc., Carlsbad, CA) according to the manufacturer's instructions. DNA quantity and quality were assessed using the NanoDropTM 8000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and the Qubit® dsDNA BR (broad range) Assay Kit with Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA).

The integron-integrase gene, *intI1*, was chosen for qPCR analysis as it acts as a generic biomarker of anthropogenic pollution and proxy of AMR (Gillings et al., 2015). To calculate the relative prevalence of this gene in the microbial community, the *intI1* gene copy number was divided by the 16S rRNA gene copy number for each sample.

To determine the prevalence of *intI1* in the water samples, a hydrolysis probe-based qPCR approach was used to quantify absolute copy number of both the 16S rRNA and *intI1* genes. Prior to the analysis of the samples, qPCR of serially diluted DNA from a representative set of samples indicated that a dilution of 1 in 2 (1:1 DNA:molecular grade water) was optimal to avoid PCR inhibition. Reactants consisted of 10 μL LightCyler 480 Probes Master Mix (Roche Molecular Systems, Inc), 2 μL of 10X concentrated primer-probe mix (resulting in a final concentration of 1 μM of each primer and 0.2 μM of probe in reaction), 5 μL diluted DNA template and PCR grade water to a total volume of 20 μL. The thermal cycling conditions were as follows: 95 °C for 3 min, 40 cycles of 95 °C for 10 s and 60 °C for 30 s. The primers and probes used were optimised in previous studies and their sequences are shown in Table S 2. qPCR was performed in duplicate in a 96-well plate in the LightCylcer 480 Instrument II (Roche Molecular Systems, Inc.). For every plate, qPCR standards (10^1 to 10^8 copies/μL) and negative controls were also run in duplicate. Standards were a constructed gBlock synthetic gene fragment (Integrated DNA Technologies, Coralville, IA, USA), and were used to create a standard curve, from which sample gene copy number was calculated. To pass quality control, the reaction efficiency had to fall between 90 and 110% and technical duplicates had to have Ct values within 0.5 cycles of each other.

2.2.4. Data analyses and risk assessment of AMR selection

Risk quotients (RQs) were computed to evaluate the level of risk associated with the concentrations of EOCs in this study to contribute to AMR development. This method looks at the relationship between a predicted no effect concentration for the development of AMR ($PNEC_R$) and measured environmental concentrations (MECs) (Sengar and Vijayanandan, 2022). The AMR Industry Alliance publishes a list of PNECs as part of their antibiotic discharge targets, which chose the lowest PNEC for each antimicrobial from either PNEC-Environment (PNEC-Env) values (ecotoxicology-based data) (Brandt et al., 2015; Le Page et al., 2017) and PNEC-Minimum inhibitory concentration (PNEC-MIC) values (AMR-based data) (AMR Industry Alliance, 2023; Bengtsson-Palme and Larsson, 2016). RQs were calculated for compounds that were both quantified in this study and included on the AMR Industry Alliance list of PNECs or in the list of $PNEC_R$s created by Bengtsson-Palme and Larsson (2016). RQs $\geq$1 indicate a significant risk of selection of AMR (Hayes et al., 2022; Singer et al., 2019).

Data wrangling, statistical analyses, and graphical presentation for most figures was done using R in RStudio version 2023.09.0 + 463 and the packages tidyr, ggpattern and RColorBrewer. Linear models ("lm" function) were run to assess the relationship between *intI1* prevalence and the type and concentration of EOCs. Fig. 1 was produced using ArcGIS 10.8.2.

3. Results

3.1. Emerging organic contaminants

A total of 125 EOCs were detected in concentrations per compound ranging from 0.001 to 314 μg/L. EOCs were detected in each of the analysed samples, with 10–84 detected compounds per sample. Table 1 provides detection frequencies and summary statistics for detected compounds in sub-categories of medical/veterinary, agrochemical, industrial, and lifestyle compounds, which will be discussed in more detail in subsections. Twenty-six of the detected compounds were present in over a third of all samples, with the artificial sweeteners sucralose and saccharin, and the anticonvulsant carbamazepine being the three most frequently detected (92%, 80% and 84%, respectively).

The occurrence of EOCs by sample source varied substantially in terms of total number of detected compounds, with the highest number of compounds detected in samples from Vrishabhavathi River, Bellandur Lake and Byramangala Reservoir, and the lowest numbers of detections at the rejuvenated lakes and in tap water (see Fig. 2A). The number of detections in groundwaters (n = 10–34 per sample, and 62 compounds in total) was low in comparison to the more polluted surface waters, but higher than those tap water or the rejuvenated tanks. A c. 90 m deep borehole within 20 m of Vrishabhavathi River had the highest number of EOC detections; however, no overall correlation could be identified between the number of detected EOCs and the distance of boreholes from surface water bodies (or with borehole depth).

Higher concentrations were typically detected in the local surface waters (Fig. 3), and much lower ones in groundwaters (maximum 3.2 μg/L for sucralose) and tap water (maximum of 0.1 μg/L for the herbicides 2,4-D-Dichlorophenoxyacetic-acid and diuron).

3.1.1. Medical and veterinary compounds

Medical and veterinary compounds constituted the highest number of detected EOCs (n = 69, Table 1), with 21 of these being detected at mean concentrations >0.5 μg/L. The antidiabetic metformin was detected with the highest maximum concentration (300 μg/L). The largest subgroup was antibiotics (n = 12 compounds, highlighted in bold in Table 1), followed by anticonvulsants (n = 9) which were detected in all but two water samples (Fig. 3). Of the 12 detected antibiotics (highlighted in bold in Table 1, and separately listed in Table S 3 with additional information on occurrence by source type), sulfamethoxazole was the most abundant (28% detection) and had the highest maximum (2.3 μg/L, measured at Bellandur Lake), mean and median concentration. Medical/veterinary compounds made up the largest proportion (60%) of detected compounds in the more heavily polluted water sources (Byramangala Reservoir, Bellandur Lake and Vrishabhavathi River) and in the rejuvenated tanks (45%, Fig. 2B). A majority (n = 39) of the 69 medical/veterinary compounds detected in this study were uniquely present in samples from Vrishabhavathi River, Bellandur Lake and Byramangala, with typically higher concentrations than those in the rejuvenated tanks. Similarly, none of the antibiotics detected at Vrishabhavathi River or Bellandur Lake were present in the rejuvenated tanks (Table S 3). However, four of the antibiotics (sulfamethoxazole, dapson, sulfanilamide and sulfamerazine) were also detected in groundwaters, and one (sulfamerazine) also in one of the tap water samples.

3.1.2. Agrochemicals

Agrochemical compounds (herbicides, insecticides and fungicides) were the second-largest group detected in this study (n = 40, Table 1). However, only 12 of these were detected with mean concentrations >0.5 μg/L. The herbicides atrazine, its metabolite desethyl-atrazine, and the insecticide degradate fipronil-sulfone were uniquely detected in the majority of tap water samples and in groundwaters from the densely-piped areas near the Indian Institute of Science (IISc) and near the Sankey tank, but not in surface water of the tank. Similarly, the metabolite fipronil-sulfone was uniquely detected in groundwater and tap water; however, its parent compound fipronil was detected at Vrishabhavathi River and Byramangala Reservoir, but not in tap waters. Pesticide concentrations of 0.1 μg/L, used as a threshold for permissible concentrations of an individual compound in the EU Drinking Water Directive (European Union, 2020), were exceeded for 21 compounds in surface waters (concentrations up to 313.5 μg/L, but only up to 1.4 μg/L in rejuvenated tanks), 7 compounds in groundwater (concentrations up

Table 1
Name, percent detection, and summary statistics on concentrations of detected compounds by compound categories (Agrochemical, industrial, medical/veterinary, and lifestyle products). The name of compounds belonging to the group of antibiotics are highlighted in bold.

Compound name	Percent Detected (%)	Minimum (μg/L)	Maximum (μg/L)	Mean (μg/L)	Standard deviation (μg/L)	Median (μg/L)
Agrochemicals (n = 40)						
2,4-Dichlorophenoxyacetic acid	32	0.027	2.720	0.941	1.026	0.485
2,6-Dichlorobenzamide	40	0.001	0.013	0.005	0.004	0.005
2-Phenoxypropionic acid	28	1.300	145.000	49.329	57.355	12.000
4-Phenoxybutyric acid	44	0.026	313.500	52.719	95.593	0.160
Atrazine	16	0.002	0.028	0.014	0.011	0.013
Atrazine desethyl	20	0.002	0.018	0.007	0.006	0.005
Azoxystrobin	8	0.110	0.120	0.115	0.007	0.115
Bendiocarb	8	0.220	0.400	0.310	0.127	0.310
Bromoxynil	12	0.003	0.011	0.008	0.004	0.011
Buprofezin	4	0.045	0.045	0.045	–	0.045
Carbendazim Azole	40	0.023	1.300	0.367	0.417	0.208
Carboxin	44	0.005	0.036	0.015	0.011	0.009
Chlorantraniliprole	16	0.001	0.160	0.059	0.075	0.037
Chlorpyrifos	4	0.055	0.055	0.055	–	0.055
Climbazole	24	0.013	0.420	0.156	0.159	0.084
Clothianidin	4	0.010	0.010	0.010	–	0.010
Desmetryn Simetryn	4	0.037	0.037	0.037	–	0.037
Dichlorvos	12	0.077	0.810	0.556	0.415	0.780
Dimethoate	24	0.005	0.610	0.220	0.219	0.153
Diuron	76	0.001	20.000	2.212	4.979	0.100
Fenthion sulfoxide Mesulfenfos	4	0.013	0.013	0.013	–	0.013
Fenuron N N Dimethyl N phenylurea	56	0.015	0.260	0.100	0.082	0.074
Fipronil	20	0.002	1.400	0.320	0.608	0.020
Fipronil Sulfide	20	0.001	0.013	0.004	0.005	0.003
Fipronil sulfon M B46136	20	0.002	0.035	0.011	0.013	0.008
Flubendiamide	40	0.001	0.100	0.012	0.031	0.002
Griseofulvin	16	0.015	0.320	0.098	0.148	0.029
Imidacloprid	20	0.013	1.300	0.378	0.533	0.230
Ketoconazole	4	0.028	0.028	0.028	–	0.028
Malathion	20	0.071	0.250	0.177	0.066	0.195
Monocrotophos Azodrin	20	0.032	0.600	0.360	0.271	0.530
Monuron	44	0.026	1.300	0.325	0.368	0.210
Phenoxyacetic acid	4	0.240	0.240	0.240	–	0.240
Propoxur baygon	4	0.150	0.150	0.150	–	0.150
Terbutryn	4	0.300	0.300	0.300	–	0.300
Thiabendazole	4	0.044	0.044	0.044	–	0.044
Thiacloprid	8	0.055	0.082	0.069	0.019	0.069
Thiamethoxam	24	0.002	0.250	0.093	0.108	0.046
Triazophos	8	0.075	0.120	0.098	0.032	0.098
Tricyclazole	32	0.005	0.062	0.028	0.024	0.022
Industrial (n = 12)						
1,4,5,6,7,7-Hexachloro-5-norbornene-2,3-dicarboxylic acid	8	0.026	0.120	0.073	0.066	0.073
Bisphenol S	32	0.013	19.000	5.156	6.750	2.325
Perfluoro Heptanoic Acid	4	0.006	0.006	0.006	–	0.006
Perfluoro Hexanoic Acid	16	0.010	0.020	0.016	0.004	0.017
Perfluoro Octanoic Acid PFOA	40	0.005	0.450	0.085	0.158	0.009
Perfluoro Pentanoic Acid	8	0.014	0.017	0.016	0.002	0.016
Perfluorobutane sulfonate	72	0.014	1.800	0.420	0.456	0.250
Perfluorohexane sulfonate	48	0.010	0.280	0.079	0.082	0.048
Perfluorooctane sulfonate PFOS	24	0.012	0.510	0.132	0.201	0.024
Tonalide Fixolide	68	0.005	1.300	0.239	0.365	0.040
Triclocarban	20	0.470	5.800	2.504	1.991	2.050
Triclosan	44	0.004	2.050	0.589	0.766	0.090
Lifestyle products (n = 4)						
Acesulfame Acesulfame K	64	0.180	32.000	3.128	7.780	0.920
Cotinine	28	0.023	18.000	9.380	7.333	11.000
Saccharin	80	0.005	63.500	9.759	22.900	0.012
Sucralose	92	0.058	205.000	20.049	44.042	2.000
Medical/veterinary (n = 64)						
10,11-dihydro-10,11-dihydroxy Carbamazepine	40	0.330	3.500	1.565	1.239	1.005
Acetaminophen Paracetemol	16	1.200	38.000	19.550	16.224	19.500
Albendazole[a]	16	–	–	–	–	–
Amisulpride	20	0.044	0.099	0.080	0.023	0.093
Amitriptyline	4	0.018	0.018	0.018	–	0.018
Atazanavir	8	0.033	0.039	0.036	0.004	0.036
Atenolol	20	2.400	6.500	4.140	1.847	3.400
Azithromycin	4	0.087	0.087	0.087	–	0.087
Boldenone Dehydrotestosterone	4	0.073	0.073	0.073	–	0.073
Carbamazepine	84	0.011	3.000	0.415	0.704	0.180
Celecoxib[a]	4	–	–	–	–	–

(*continued on next page*)

Table 1 (*continued*)

Compound name	Percent Detected (%)	Minimum (μg/L)	Maximum (μg/L)	Mean (μg/L)	Standard deviation (μg/L)	Median (μg/L)
Cetirizine	28	0.680	17.000	6.069	5.962	3.100
Cimetidine	4	1.500	1.500	1.500	–	1.500
Clarithromycin	20	0.027	0.220	0.080	0.079	0.050
Clobazam Urbadan	8	0.098	0.350	0.224	0.178	0.224
Clopidogrel	24	0.002	0.034	0.021	0.013	0.024
Clopidol	24	0.002	0.008	0.005	0.002	0.005
Codeine	20	0.064	0.270	0.159	0.087	0.120
Cyclophosphamide	4	0.013	0.013	0.013	–	0.013
Dapson	12	0.001	0.087	0.040	0.043	0.033
Dextrorphan Levorphanol d form	20	0.105	0.370	0.213	0.104	0.170
Diazepam	8	0.039	0.250	0.145	0.149	0.145
Diclofenac	32	0.004	2.800	0.799	0.901	0.645
Estazolam	4	0.002	0.002	0.002	–	0.002
Fexofenadine	36	0.024	0.960	0.224	0.331	0.071
Fluconazole I Diflucan	16	0.870	2.200	1.253	0.634	0.970
Flunitrazepam	4	0.230	0.230	0.230	–	0.230
Flunixin	4	0.031	0.031	0.031	–	0.031
Flurazepam	4	0.091	0.091	0.091	–	0.091
Furosemide	16	0.010	0.240	0.075	0.111	0.024
Gabapentin	8	0.410	13.000	6.705	8.902	6.705
Gliclazide	4	4.700	4.700	4.700	–	4.700
Hydrochlorothiazide	36	0.003	37.000	9.704	14.194	1.100
Ibuprofen	48	0.004	28.000	5.442	8.366	1.140
Lamotrigine	40	0.001	0.150	0.043	0.050	0.022
Levamisole	4	0.110	0.110	0.110	–	0.110
Lidocaine Diocaine	56	0.001	0.640	0.159	0.234	0.016
Lincomycin	4	0.007	0.007	0.007	–	0.007
Losartan	16	0.110	0.690	0.325	0.253	0.250
Medroxyprogesterone	16	0.340	5.700	3.648	2.401	4.275
Mefenamic acid	24	0.033	24.000	5.277	9.268	1.500
Meloxicam	4	0.120	0.120	0.120	–	0.120
Metformin	16	1.100	300.000	167.775	137.970	185.000
Metoprolol[a]	16	–	–	–	–	–
Miconazole	4	0.009	0.009	0.009	–	0.009
Morphine	20	0.100	0.260	0.168	0.076	0.130
Norfluoxetine[a]	12	–	–	–	–	–
Ofloxacin[a]	20	–	–	–	–	–
Oxcarbazepine	28	0.008	1.100	0.538	0.426	0.570
Phenobarbital	48	0.048	4.800	0.854	1.345	0.410
Phenytoin	4	0.071	0.071	0.071	–	0.071
Praziquantel	4	1.100	1.100	1.100	–	1.100
Ractopamine	8	0.045	0.160	0.103	0.081	0.103
Ranitidine	12	0.210	0.770	0.503	0.281	0.530
Rifaximin	8	0.420	0.540	0.480	0.085	0.480
Roxithromycin	4	0.055	0.055	0.055	–	0.055
Salbutamol Albuterol	24	0.012	0.250	0.149	0.084	0.175
Simvastatin	4	1.300	1.300	1.300	–	1.300
Sotalol	20	0.069	0.260	0.200	0.080	0.240
Sulfamerazine	8	0.007	0.073	0.040	0.047	0.040
Sulfamethazine	16	0.039	0.660	0.243	0.290	0.137
Sulfamethoxazole	28	0.012	2.300	0.513	0.803	0.320
Sulfanilamide	12	0.010	0.018	0.015	0.004	0.017
Telmisartan	52	0.002	1.300	0.283	0.422	0.130
Terbutaline	20	0.240	0.420	0.340	0.068	0.340
Topiramate	8	0.040	2.500	1.270	1.739	1.270
Tramadol	44	0.001	1.000	0.338	0.421	0.015
Trimethoprim	16	0.064	0.260	0.129	0.089	0.097
Venlafaxine	8	0.150	0.310	0.230	0.113	0.230

[a] Qualitative (present/absent) detection only.

to 0.45 μg/L) and 2 compounds in tap water (diuron and 2,4-dichlorophenoxyacetic-acid, concentrations of 0.1 μg/L for both compounds). The recommended concentration for the sum of pesticide concentrations (0.5 μg/L) was exceeded for all local surface waters (max $\sum$ 871 μg/L) and 3 of the groundwaters, but not in tap waters. The majority (59%) of detected EOCs in tap waters were agrochemicals, however, the total count of individual agrochemicals (n = 11) was similar to that of the other water sources (n = 8–22).

3.1.3. Industrial compounds

Despite Bengaluru's high degree of industrialization, only 12 industrial compounds were detected (Table 1). Bisphenol-S, and the antibacterial disinfectants triclocarban and triclosan, both of which are linked to AMR development, were detected with mean concentrations >0.5 μg/L. Bisphenol-S had the highest maximum concentration of all industrial compounds (19 μg/L, measured at Vrishabhavathi River). The industrial compounds with the highest detection frequency across all water sources were perfluorobutane-sulfonate (72%) and tonalide (68%).

Industrial compounds were primarily detected in the local surface waters, but also in groundwaters and one tap water sample (Figs. 2 and 3). Most industrial compounds detected in groundwaters (n = 7 out of 11) were surfactants belonging to the group of per- and polyfluoroalkyl substances (PFAS) such as perfluorooctane-sulfonate (PFOS),

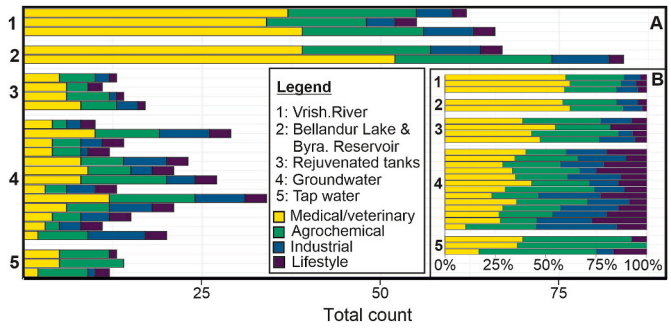

Fig. 2. Bar plots showing A) number of detected compounds by EOC group in each water sample, and B) percentage contribution of each EOC group to total detections for each sample. Note that samples are group according to source type.

perfluorohexane-sulfonate, and perfluorobutane-sulfonate (detection frequency in groundwaters was 54%, 85%, and 92%). Three of these compounds were only detected in groundwater. Fewer individual PFAS (n = 3–4) were detected in the local surface, with those found at Vrishabhavathi River, Bellandur, Byramangala Reservoir generally in higher concentrations than in groundwater (up to 1.8 μg/L) and those in the rejuvenated tanks at slightly lower or similar concentrations as in groundwater (Table S 4). There were no PFAS detections in tap water. In tap water, only 3% of detected compounds were industrial compounds and no surfactants (PFAS) were detected.

3.1.4. *Lifestyle compounds*

Even though the screening included a broad range of lifestyle compounds, only the three artificial sweeteners included in the analysis screen (sucralose, saccharin and acesulfame-K) and the nicotine metabolite cotinine were detected. All three sweeteners were ubiquitous with detection frequencies of 92%, 80%, and 64%, respectively, and high maximum concentrations ranging from 32 to 205 μg/L (highest value in the Vrishabhavathi River). Cotinine concentrations ranged 0.02–0.04 μg/L in groundwater and tap water, 6.6 μg/L to 15 μg/L in the Vrishabhavathi River, Bellandur Lake and Byramangala Reservoir, and were not detected in the four rejuvenated tanks Allalasandra, Sankey, Lalbagh and Kaikondrahalli.

3.2. *Prevalence of intI1 and potential risk of AMR development*

The prevalence of the AMR-indicating gene *intI1* was found to be variable across sampling site type, with the highest measurements found in the Vrishabhavathi River, Bellandur Lake and Byramangala Reservoir (prevalences of 2.8499, 3.0225 and 4.3604 gene copies/16S rRNA copies, respectively), and lowest in the rejuvenated tanks (range of 0.0004–0.0025 gene copies/16S rRNA copies). Groundwater and tap water samples generally had lower *intI1* prevalences (prevalence ranges of 0.0001–1.4229 and 0.0185–0.7244 gene copies/16S rRNA copies, respectively) than the river, tank and reservoir samples. The prevalence of *intI1* had a significant positive relationship with all EOC metrics analysed, including the total concentration of all antibiotics detected ($R^2 = 0.72$, $p \leq 0.001$), total concentrations of all antimicrobials (including antibiotics) ($R^2 = 0.88$, $p \leq 0.001$), total concentration of all measured compounds ($R^2 = 0.27$, $p = 0.005$), and total number of compounds detected (including qualitative detections) ($R^2 = 0.78$, $p \leq 0.001$; see also Fig. S1). It should be noted, however, that the samples with highest total number of compounds detected (Vrishabhavathi River, Bellandur Lake and Byramangala Reservoir) have a strong influence on the dataset and the above relationships would not be significant if considering the samples with lower detection EOCs on their own. This is likely due to the lower levels of *intI1* being within what can be considered as "normal background levels" (Abramova et al., 2023), which might be less indicative of a strong pollutant-based response. Ten of the 29 antimicrobials detected in this study were found in the AMR Industry Alliance list of PNECs (AMR Industry Alliance, 2023) or in the list of $PNEC_R$s created by Bengtsson-Palme and Larsson (2016): azithromycin, clarithromycin, fluconazole, lincomycin, ofloxacin, rifaximin, roxithromycin, sulfamethoxazole, sulfanilamide and trimethoprim. However, only nine were included in further risk analysis, as ofloxacin was only detected on a qualitative basis. From this, the lowest PNEC was used and RQs for resistance selection were calculated as the MEC/PNEC ratio ($RQ = MEC/PNEC_R$) (Table 2).

RQs ≥1 indicate the MEC poses a significant environmental risk of AMR development. Of the nine antimicrobials analysed here, three (azithromycin, fluconazole and sulfanilamide) were found at

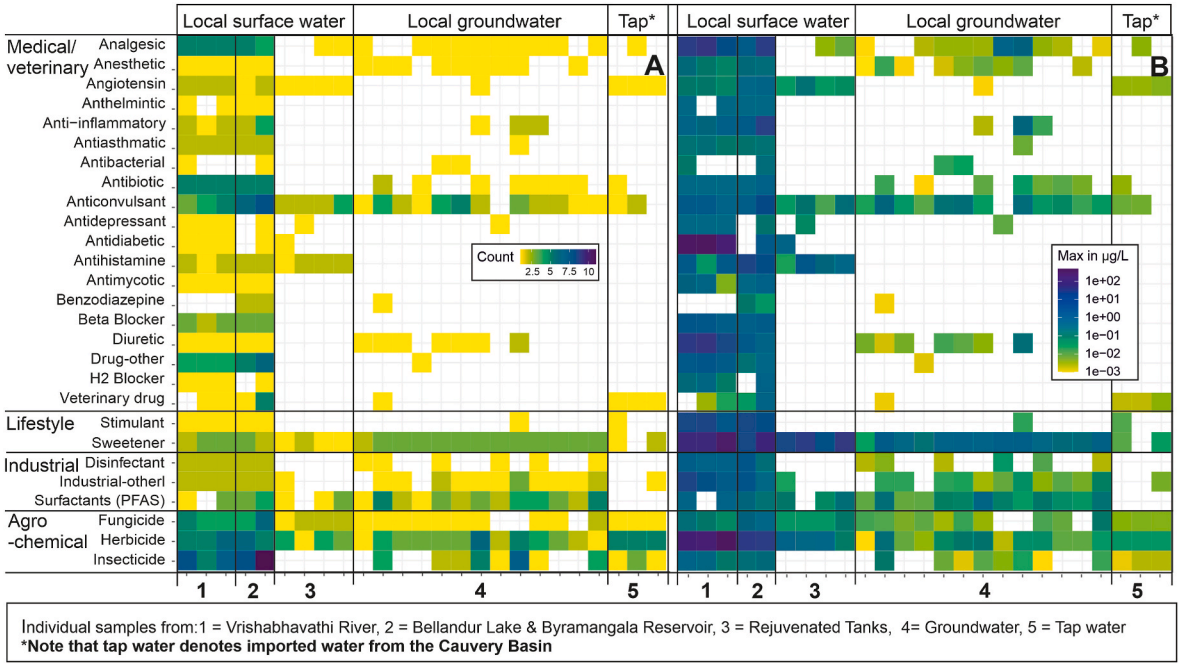

Fig. 3. Heatplot of A) number of detections for subgroups of EOCs by sampled site, and B) maximum concentration within subgroup be sampled site.

Table 2
Percentage of all sample types with antimicrobial measured environmental concentrations (MECs) above the AMR Industry Alliance suggested PNECs for use in environmental risk assessments of antibiotics (AMR Industry Alliance, 2023) or PNECRs for resistance (PNEC$_R$s) created by Bengtsson-Palme and Larsson (2016). Risk quotients (RQs) of concentrations of antimicrobials measured in this study were calculated as follows: RQ = MEC/PNEC$_R$. Anse RQ ≥ 1 indicates the MEC of this compound poses a significant environmental risk of AMR development. (Bell. Lake = Bellandur Lake, Byra. Reservoir = Byramangala Reservoir).

	PNEC$_R$ (µg/L)	Azithromycin	Clarithromycin	Fluconazole	Lincomycin	Rifaximin	Roxithromycin	Sulfamethoxazole	Sulfanilamide	Trimethoprim
		0.03	0.25	0.25	0.81	0.11	1	6.6	230	0.5
Vrishabhavathi River (n = 3)	% above PNEC$_R$	0%	0%	67%	0%	67%	0%	0%	0%	0%
	Minimum RQ	0	0.11	3.48	0	0	0	0.03	0	0.13
	Maximum RQ	0	0.24	3.76	0.009	4.91	0	0.06	0	0.2
	Mean RQ	0	0.18	3.62	0.003	2.91	0	0.05	0	0.17
Bell. Lake & Byra. Reservoir (n = 2)	% above PNEC$_R$	50%	0%	100%	0%	0%	0%	0%	0%	0%
	Minimum RQ	0	0.20	4	0	0	0	0.05	0	0
	Maximum RQ	2.9	0.88	8.8	0	0	0.06	0.35	0	0.52
	Mean RQ	1.45	0.54	6.4	0	0	0.03	0.20	0	0.26
Rejuvenated tanks (n = 4)	% above PNEC$_R$	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Minimum RQ	0	0	0	0	0	0	0	0	0
	Maximum RQ	0	0	0	0	0	0	0	0	0
	Mean RQ	0	0	0	0	0	0	0	0	0
Groundwater (n = 13)	% above PNEC$_R$	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Minimum RQ	0	0	0	0	0	0	0.002	0	0
	Maximum RQ	0	0	0	0	0	0	0.003	<0.001	0
	Mean RQ	0	0	0	0	0	0	0.<0.001	<0.001	0
Tap water (n = 3)	% above PNEC$_R$	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Minimum RQ	0	0	0	0	0	0	0	0	0
	Maximum RQ	0	0	0	0	0	0	0	0	0
	Mean RQ	0	0	0	0	0	0	0	0	0

concentrations above the PNEC$_R$, indicating a significant risk of AMR development. These above-PNEC$_R$ concentrations were only found in the Vrishabhavathi River, Bellandur Lake and Byramangala Reservoir (i.e., not in rejuvenated tanks, groundwater or tap water). Most concerning were the concentrations of fluconazole, an antifungal, resulting in RQs ranging from 3.48 to 8.8. Of the three antimicrobials at concentrations above PNEC$_R$, fluconazole was found to exceed the PNEC$_R$ the most (Table 2).

4. Discussion

4.1. Groundwater-surface water connectivity

4.1.1. Groundwater-surface water connectivity

Out of 125 EOCs detected in this study, 62 were present in groundwaters and a number of these compounds could be linked directly to distinct recharge sources. For example, PFAS detected in groundwaters are likely recharged from surface waters influenced by sewage, in which PFAS were detected at highest concentrations of up to 1.8 µg/L (Table S4). PFAS (n = 3) were also detected in rejuvenated tanks, so recharge from these tanks is another potential source for PFAS in groundwater. However, very shallow groundwater levels have previously been reported particularly in the central parts of the city, and seasonal gradient changes indicated that some of the tanks could also be recharged by groundwater (Brauns et al., 2022); hence the there is some uncertainty around this. There was no PFAS detection in tap waters, ruling it out as PFAS source for groundwater. Conversely, some agricultural products such as atrazine and its metabolite desethyl-atrazine were detected in groundwater and piped mains, i.e. tap water imported from the Cauvery Basin, only. Atrazine is a pre-emerging herbicide that is widely in the Cauvery Basin for production of both sugarcane and wheat (Nageswari et al., 2022; Sharma et al., 2016b) and could potentially be entering the river, such as observed in the agricultural areas of the Indian Gangetic Plain (Richards et al., 2023) or the Chinese North China Plain (Brauns et al., 2018). Thus, the detection of atrazine and its metabolite desethyl-atrazine in all tap water samples, and some of the groundwater samples may indicate groundwater recharge from mains water leakage (see also Brauns et al., 2022). This observations supports qualitatively the finding of previous studies that discussed Bengaluru's water cycle (or *urban water metabolism*) on a more quantitative basis and showed complex urban groundwater recharge processes that are anthropogenically dominated by surface water storage, water imports and pipe leakage, overlaying the more natural rainfall-driven recharge (Mehta et al., 2014; Tomer et al., 2020).

Moreover, the detection of recently (post-2000) introduced artificial sweeteners in all groundwaters indicates a high degree of system-connectivity since all groundwater must have received recharge in the last twenty years. The connectivity of the groundwater-surface water system, likely increased by heavy abstractions, and the presence of contaminants linked to a variety of recharge sources has critical implications for the quality of the groundwater resources which sustain domestic, agricultural and industrial water demand in those parts of the study area where piped water is limited. As such, monitoring and limiting contamination occurring in surface waters and imported (less-contaminated) Cauvery River water is crucial to protect groundwater resources in Bengaluru.

4.2. Comparison of EOCs and PFAS with previous studies in Bengaluru and India

A seasonal surface water study (Iyanee et al., 2013), conducted in August and November 2012 at eight locations along the Vrishabhavathi and Cauvery River analysed for and detected the antibiotics sulfamethoxazole, trimethoprim, erythromycin and chloramphenicol, with the highest concentrations detected at Byramangala Reservoir. All of these analytes were included in our study, but only sulfamethoxazole

and trimethoprim were detected in our study (in all samples from Vrishabhavathi River and at Byramangala Reservoir, but not Bellandur Lake), with maximum concentrations that were about 2-3-times higher than those in the previous study. This suggests that EOC contamination in Bengaluru's surface waters is highly variable or may have increased over the past decade. A more recent study by Gopal et al. (2021) investigated the occurrence of 11 selected EOCs in urban surface waters at 35 locations within Bengaluru as well as up- and downstream along the Arkavathi river and its tributaries in October 2018 and February 2019. Five of the compounds investigated by Gopal were included and detected in our study, namely diclofenac, ibuprofen, sulfamethoxazole, triclocarban and triclosan (detected in the non-rejuvenated surface waters and in groundwaters). With the exception of only one compound (chloramphenicol), peak concentrations were detected in Gopal et al. (2021) at similar locations to those sampled in our field campaign, which confirms Bengaluru as a local hotspot of EOC contamination in the Arkavathi river basin.

To our knowledge, no previous studies on EOCs in Bengaluru's groundwaters have been undertaken, but recent studies across India using a similar broad band screening approach to this study detected EOCs in groundwater at concentrations in sub-microgram concentrations (with one exception of maximum concentration of 1.2 μg/L for sucralose) in the Indian cities Varanasi and Patna (Lapworth et al., 2018; Richards et al., 2021). In our study, EOC concentrations in Bengaluru groundwater ranged up to 314 μg/L and 22% of concentrations were in the μg/L-range (all of these for the compounds sucralose, acesulfame K, and ibuprofen). Similarly, PFAS concentrations both in surface waters and groundwaters (up to 1.8 and 0.9 μg/L, respectively, Table S 4) were broadly one order of magnitude higher in our study than in previous studies on Indian surface waters and groundwaters (Lapworth et al., 2018; Richards et al., 2021; Richards et al., 2023; Sharma et al., 2016a; Sunantha and Vasudevan, 2016; Yeung et al., 2009). The higher frequency of PFAS substances detected in groundwaters (n = 7, versus n = 3–4 in surface waters) may be an indication of legacy PFAS contamination, i.e., of substances that are currently not in use anymore and hence are not detected in the surface waters. The lack of detected PFAS in tap water is notable, however, the detection limits of our broadband screening (for PFAS 0.005–0.1 μg/L) are higher than those from other studies. For example, Sunantha and Vasudevan (2016) detected PFOA at all sampling sites at the Cauvery River at a concentration of 0.005 μg/L, which was the detection limit in this study. This indicates that PFAS in the Bengaluru tap water (which is predominantly imported water from the Cauvery River) may have been present, but at concentrations below the detection limit of our study. PFAS are currently unregulated in India but based on the EU Drinking Water Directive (European Union, 2020), the recommended threshold for the sum of selected PFAS (0.1 μg/L) was exceeded for all water types except tap water (Table S 4).

4.3. Effects of tank rejuvenation on presence of EOCs and AMR indicators

Protection from sewage dumping and wastewater inflows has been a key component in Bengaluru's tank rejuvenation efforts and the lower number of detected EOCs in rejuvenated tank samples in this study is a good indication that the protection from sewage inflows is limiting the occurrence of EOCs in comparison to waters that receive higher inflow. E.g., the highest numbers of EOCs per sample were detected at Byramangala reservoir and Bellandur Lake (n = 84 and n = 67, respectively) which contrasted with the much lower number of detections in the rejuvenated tanks (n = 11–17, see Fig. 2A). There was also a strong difference in prevalence of the AMR-related gene *intI1* between the rejuvenated tanks (*intI1* prevalences <0.003 gene copies/16S rRNA copies) and the other local surface water sources (Vrishabhavathi River, Bellandur Lake, Byramangala Reservoir, *intI1* prevalences >1.358 gene copies/16S rRNA copies). The *intI1* prevalences found here in the rejuvenated tanks (<0.003 gene copies/16S rRNA copies) are very similar to the mean *intI1* prevalence found by Abramova et al. (2023) (0.004 gene copies/16S rRNA copies (presented in their study as −2.366 $\log_{10}$ gene copies/16S rRNA copies). Their review compiled environmental baseline environmental levels of AMR genes quantified by qPCR from global literature, collating results from studies on a range of samples, including air, biofilms, wastewater, waters and soil, and a range of polluted states, from impacted to likely unimpacted environments (Abramova et al., 2023). However, the higher *intI1* prevalences found in the current study (i.e., those >2 gene copies/16S rRNA copies) found in the Vrishabhavathi River, Bellandur Lake, Byramangala Reservoir, are closer in magnitude to the maximum value of 39.8 gene copies/16S rRNA (presented in their study as 1.6 $\log_{10}$ gene copies/16S rRNA copies) from the global literature (Abramova et al., 2023). Comparing the results of the current study to a similar review collating global *intI1* qPCR data (Gillings, 2018), indicates that the highest prevalences found here (i.e., those >2 gene copies/16S rRNA copies) were in the same order of magnitude as more polluted samples, such as wastewater effluent from China (Ma et al., 2017) and freshwater sediment near a drug formulation facility in Pakistan (Khan et al., 2013). Also, Skariyachan et al. (2013) also recorded high levels of bacterial resistance in the Byramangala reservoir using a culture-based approach. Thus, the differences in *intI1* prevalences found in the current study indicate that rejuvenated tanks have a lower risk of containing high levels of AMR, and largely represent average environmental levels, whereas other sites such as Byramangala Reservoir and Bellandur Lake, are likely to have higher levels of AMR, similar to other polluted sites globally. This is also supported by the finding here that AMR RQ values > 1 were only identified for antimicrobial concentrations measured in the Vrishabhavathi River, Bellandur Lake and Byramangala Reservoir, thus no other sites posed a risk of development of AMR based on the antimicrobials analysed in this study using this assessment approach. Results such as these are important in terms of risks from water usage, particularly in India, where few studies have investigated resistance genes in such aquatic environments (as highlighted by Das et al., 2023).

4.4. Risk of AMR development

The concentrations of antibiotics measured in this study varied across sample sites, with the highest being 2.3 μg/L of sulfamethoxazole, detected in the Byramangala Reservoir. The highest concentrations measured when including other antimicrobials were 5.8 μg/L of triclocarban (Bellandur Lake) and 2.2 μg/L for fluconazole (Byramangala Reservoir). The highest prevalences of the AMR-indicator, *intI1*, were also found in the Byramangala Reservoir (prevalence of 4.3604 gene copies/16S rRNA copies) and Bellandur Lake (prevalence of 3.0225 gene copies/16S rRNA copies), indicating that the high antimicrobial concentrations are a likely driver of increased AMR prevalences. Further, Kristiansson et al. (2011) found similar trends in AMR and antimicrobials in polluted waters, reporting high abundances of both class 1 integrases and pharmaceuticals (notably, fluoroquinolones) downstream of a wastewater treatment plant in Hyderabad that received effluent from many drug manufacturers. The positive relationships between prevalence of *intI1* and the total concentration of antibiotics/antimicrobials/total concentration of all measured compounds/total number of compounds detected, support the narrative that increased concentrations and diversity of resistance-driving chemicals in aquatic environments are likely to result in higher prevalences of AMR (Taylor et al., 2011; Alderton et al., 2021; Stanton et al., 2022). Philip et al. (2018) found that very few studies have correlated the presence of AMR genes with antibiotics, thus the results found in the current study add to a small body of research requiring further investigation.

Of the nine detected antimicrobials that could have RQs calculated, three were found at concentrations posing a risk of AMR development (i.e., having RQs ≥1), azithromycin, fluconazole and sulfanilamide. Of these, fluconazole had the highest RQ (8.8, Table 2). RQs ≥1 in this study were only determined from concentrations in the Vrishabhavathi

River, Bellandur Lake and Byramangala Reservoir, thus no risk of AMR development was found in tap water, groundwater and rejuvenated tanks using this risk assessment method (Table 2). A review by Sengar & Vijayanandan (2022), which compiled data on concentrations of antimicrobials in Indian waters (including wastewater, surface water and groundwater samples) from multiple studies, found higher RQ values than this study. For example, in our study the highest RQ for AMR for clarithromycin was 0.88, whereas Sengar & Vijayanandan (2022) calculated an RQ of 50.8 based on detected concentrations from surface water downstream of a WWTP in the Musi River in Hyderabad City in Lübbert et al. (2017). The same was seen for azithromycin, lincomycin, sulfamethoxazole and trimethoprim (highest RQs of 2.9, 0.009, 0.35 and 0.52 respectively) which resulted in higher RQs than found in the literature on Indian water samples (highest literature RQs 3.96, 0.255, 0.564 and 15.1 respectively, Sengar & Vijayanandan (2022). However, Sengar & Vijayanandan (2022) chose the highest antimicrobial measurements from the studies they identified to create their RQ values. Also, the risk assessment method used here only considers compounds in a singular nature, which limits the interpretation of risk from multiple antimicrobials, where a mixture might have additive or synergistic effects on the selection of AMR (Mitosch and Bollenbach, 2014; Singer et al., 2016; Singer et al., 2019). Therefore, these RQ values are a likely best-case scenario, with the real risk of AMR development possibly being much greater.

5. Conclusions

In this study, an EOC broad screening approach was used in combination with AMR assessment in a large Indian city to identify the quality of different water source types and their interlinkages. Our main conclusions are.

- Having detected a high number of EOCs in our study (n = 125), this data can be used to improve understanding of groundwater recharge processes. For example, it was possible to link some compounds to different recharge sources, and the ubiquitous detection of the sweetener sucralose in groundwater (in use since 2000) indicated recent groundwater recharge at all sampled sites; hence providing information about groundwater age.
- High PFAS concentrations of up to 0.9 μg/L and the detection of 3 PFAS compounds in groundwater that were not detected in any of the other waters demonstrated the vulnerability of urban groundwater systems and the persistence of PFAS in groundwater found by other studies.
- Medical/veterinary compounds, including 27 antimicrobials were the dominant pollutant class in the urban surface waters (60% of all detected compounds) and, to a lesser extent, in groundwater (31%). The number of antimicrobial compounds had a significant correlation ($R^2 = 0.88$, with $p \leq 0.001$) with the prevalence of the AMR indicator *intI1*.
- Surface water bodies with recently implemented protection measures such as prevention of sewage inflows had fewer EOCs detected than other surface waters and were found to have much lower risk of AMR development; thus indicating how relatively simply urban protection measures can protect freshwater quality.
- The comparison of our findings with other urban groundwater studies in India highlights the importance of local differences in the occurrence and concentration of EOCs, and thus demonstrating the need to develop bespoke urban groundwater quality monitoring approaches, ideally based on initial broad-screening techniques.

In summary, this study demonstrated how our approach helps to identify priority contaminants of concern and their links to AMR development, and to better understand the complexity of groundwater recharge mechanisms in the urban environment. The approach can be equally applicable in other cities to inform the development of tailored monitoring and protection efforts.

CRediT authorship contribution statement

Bentje Brauns: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Subhash Chandra:** Writing – review & editing, Investigation. **Wayne Civil:** Writing – review & editing. **Dan J. Lapworth:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Alan M. MacDonald:** Writing – review & editing, Funding acquisition, Conceptualization. **Andrew A. McKenzie:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Daniel S. Read:** Writing – review & editing, Funding acquisition, Formal analysis. **Muddu Sekhar:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Andrew C. Singer:** Writing – review & editing, Formal analysis. **Amritha Thankachan:** Writing – review & editing, Investigation. **Holly J. Tipper:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Muddu Sekhar reports financial support was provided by Indian Ministry of Earth Sciences (MoES). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124983.

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