Journal of Environmental Biology
p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

JEB®

Original Research Paper
Volume 47 Issue 2 March 2026 pp. 430-439
DOI: http://doi.org/10.22438/jeb/47/2(SI)/SI-16

J. Environ. Biol., 2026
© Triveni Enterprises, Lucknow (India)
Journal website : www.jeb.co.in ★ E-mail : editor@jeb.co.in

16S rRNA gene sequence-based metagenomics assessment of microbial community structure and hospital wastewater chemical impacts on urban river

R.S. Thakur[1], A.K. Singh[1], R.S. Dhanwar[1], T.D. Lodha[3], B.S. Lopes[4,5], S.S. Donde[2] and O. Prakash[1]*

[1]Symbiosis Centre for Climate Change and Sustainability, Symbiosis International (Deemed University), Pune-412 115, India
[2]Department of Chemistry, Earth Science and Environmental Science, BCC, City University of New York, 10017, New York
[3]Department of Bioenergy, Agharkar Research Institute, Pune-411 004, India
[4]School of Health and Life Sciences, Teesside University, Middlesbrough, TS1 3BX, UK
[5]National Horizons Centre, Teesside University, Darlington, DL1 1HG, UK

Received: 16 January 2026 **Revised:** 11 March 2026 **Accepted:** 19 March 2026

***Corresponding Author Email:** prakas1974@gmail.com ***ORCiD:** https://orcid.org/0000-0002-6568-0567

Abstract

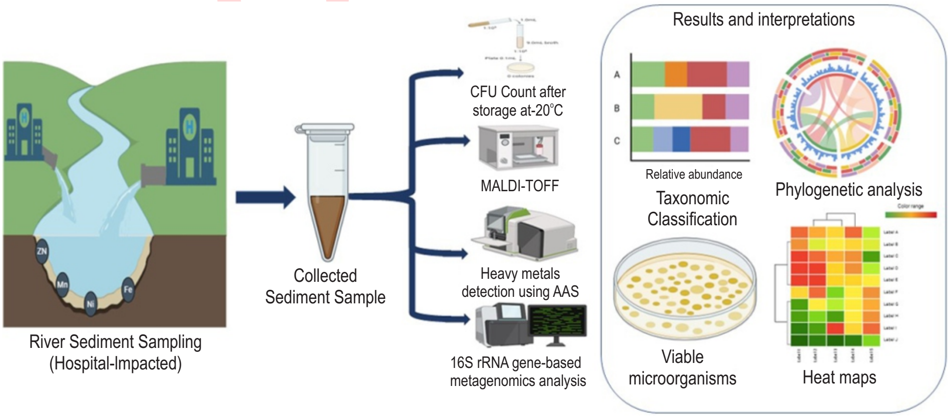
Aim: To assess the impact of hospital wastewater–associated contamination on the microbial diversity and community structure of sediments in urban river systems.

Methodology: Sediment samples were collected from hospital-contaminated urban river sites. Physico-chemical characteristics of sediments, including pH, total organic carbon, and heavy metal concentrations, were determined using standard protocols. The Composition and diversity microbial community were analysed by 16S rRNA gene–based metagenomics.

Results: The results showed substantial variation in sediment microbial diversity, which is correlated with the sediment chemistry and geographical locations of the sampling sites. A significant variations were also reported in pH, heavy metals, total viable bacterial count, and total organic carbon in the test sediments.

Interpretation: The results demonstrate that hospital-associated contamination plays a critical role in shaping microbial communities in urban river sediments. Alterations in the microbial structure may influence key ecosystem processes, such as nutrient cycling and organic matter degradation, and pose risks to the ecosystem and public health. The findings highlight the importance of continuous monitoring and improved management of hospital wastewater discharges to protect the urban aquatic ecosystems.

Key words: Hospital wastewater, Heavy metals, Microbial community, River sediments



How to cite: Thakur, R.S., A. K. Singh, R. S. Dhanwar, T. D. Lodha, B.S. Lopes, S. S. Donde and O. Prakash: 16S rRNA gene–based metagenomics assessment of microbial community structure and hospital wastewater chemical impacts on urban river sediments. *J. Environ. Biol.*, **47**, 430-439 (2026).

Introduction

River ecosystems in urban areas are heavily contaminated by domestic, municipal, pharmaceutical, and hospital wastewater (Nimonkar *et al.,* 2019). Anthropogenic contaminants enter these rivers and accumulate in the sediments, which serve as a sink for residual antibiotics, heavy metals, and pathogenic microorganisms (Rastmanesh *et al.,* 2018). River sites in urban areas surrounded by hospitals are hotspots for pollutants due to discharge of partially treated or untreated wastewater from hospitals into nearby drainage systems (Woodward *et al.,* 2021). The level of the contaminants in flowing river water may be lower, and sometimes below detection limit; however, prolonged discharge leads to the accumulation of contaminants in the sediment matrices, exerting selective pressure on microbial populations, affecting ecosystem functionality (Carles *et al.,* 2022). Hospital wastewater is known to contain diverse microbial populations and elevated levels of heavy metals, resulting from the use of pharmaceuticals and medical equipments (Ajala *et al.,* 2022). Wastewater entering urban rivers alters the sediment quality and chemistry, influencing sediment microbial diversity and chemical composition (Carles *et al.,* 2022). Sediment microbes play an important role in nutrient cycling and organic matter degradation, but long-term exposure to pollutants, such as heavy metals from hospitals, can cause structural disruptions and reduce microbial diversity (Wang *et al.,* 2021; Silvester *et al.,* 2025). Such changes may alter crucial ecosystem functions and increase the abundance of potentially pathogenic or resistant microorganisms. Microbial diversity from contaminated sediment samples remain understudied and an area of interest (Silvester *et al.,* 2025). The changes in the microbial diversity not only affect public and environmental health, but also influence nutrient cycling in the ecosystems (Wang *et al.,* 2021).

Therefore, understanding the impact of hospital wastewater on sediment microbial community structure is crucial for evaluating environmental health risks and ecosystem resilience (Carles *et al.,* 2022). This study provides insights into how sediments from hospital wastewater-contaminated rivers influence microbial communities, and the types of heavy metal contamination they acquire. The research also explores the survival of microbes in contaminated sediments after storage at low temperatures. Diversity analysis using 16S rRNA gene sequencing provides detailed information on the structure of taxa and their variation across contaminants and geographic regions. The study highlights the need for further investigation into hospital wastewater-contaminated sediments, which remain understudied compared to contaminated soil and water matrices.

Materials and Methods

Study sites and sample collection: Sediment samples were collected from seven river sites across five cities: Mula–Mutha, Pune, Maharashtra (18.53303°N, 73.85883°E); Mithi, Mumbai, Maharashtra (19.20228°N, 72.98736°E); Tapi, Nandurbar, Maharashtra (21.51417°N, 74.34227°E); Kamavari, Mumbai, Maharashtra (19.30263°N, 73.04556°E); Mula, Pune, Maharashtra (18.57497°N, 73.74239°E); Narmada, Indore, Madhya Pradesh (22.14816°N, 75.45433°E); and Sina, Ahilyanagar, Maharashtra (19.17979°N, 74.71664°E). Sampling was performed in accordance with protocol standard to ensure consistency and minimize contamination (ISO 5667-12:2017) (Zhang and Liu, 2002). Metadata, including time, location, depth, and environmental conditions, were recorded to ensure integrity, in accordance with ISO 5667-15:2009. The grab method was employed for sediment sampling, with samples collected from a depth of 10-15 cm following established USGS protocols (Shelton and Capel, 1994). Samples were collected in pre-sterilized 15 ml^{-1} capacity Falcon tubes and transported to the laboratory on ice. For metagenomics and chemical analyses, the samples were stored at -20 °C in airtight, sterile containers to avoid contamination.

Physico-chemical analyses of samples: The sediment samples were analysed for TOC, pH, and heavy metals. Heavy metals in the sediment samples were analysed by Atomic Absorption Spectroscopy (AAS). The samples were prepared and analyzed as per the standard guidelines of the (APHA, 2023). AAS analysis was performed at distinct wavelengths specified for each metal instructed in the manufacturer's manual. The metal and analysis wavelengths are as follows: Mn (@279 nm), Fe (@248.06 nm), Zn (@213.60 nm), Co (@240.44 nm), Ni (@231.73 nm), and Cd (@228.51 nm). Total organic carbon in the contaminated sediment samples was analysed by Walkey and Black method (Gerenfes *et al.,* 2022; FAO, 2019). The carbon content was estimated by the formula of Gerenfes *et al.* (2022). The pH of sediment samples was measured using a calibrated pH meter (Labman Scientific Instruments, Pvt. Ltd.).

Bacterial viable count and community analysis

Enumeration and identification of cultivated bacteria: A total viable count approach was used. The samples were thawed overnight at 4°C. One gram of sediment was suspended in normal saline, and a dilution series was prepared to assess microbial survival in sediments stored at-20°C without cryoprotectants for three months in accordance with ISO 6887-1:2017 and (APHA, 2023). Therefore, 100 µl aliquots from 10^{-3}, 10^{-4}, and 10^{-5} dilutions were spread on Reasoner's 2A Agar (R2A) medium plates, and the plates were incubated at 30°C for 72 hr to observe the visible growth of microorganisms that survived the frozen storage conditions. After incubation, the bacterial colonies were counted to determine the total viable count (TVC) and expressed as colony-forming units (CFUs) per gram of sample. Morphotypically distinct colonies were selected for further purification and analysis by Matrix-Assisted Laser Desorption/ Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) as described by Karas and Hillenkamp (1988) and Rahi *et al.* (2016).

Bacterial community analysis by 16S rRNA gene sequence-based metagenomics

DNA extraction and 16S rRNA gene sequencing: 16S rRNA gene sequencing and metagenomics analysis of the sediment samples were outsourced from Advait Thera Diagnostics Pvt. Ltd., Ahmedabad. Total genomic DNA was extracted from 0.25 g of sediment using the Dneasy Power Soil Pro Kit (QIAGEN, Hilden, Germany) following the manufacturer's standard protocol to ensure high yield and purity. The eluted genomic DNA was quantified using the Qubit dsDNA High Sensitivity assay (Thermo Fisher Scientific) according to established fluorometric standards and stored at -20 °C until library preparation.

Library preparation: Amplicon libraries targeting the bacterial 16S rRNA gene V3–V4 region were prepared using a two-step PCR workflow. A ten ng of DNA was amplified with high-fidelity polymerase and region-specific primers, then purified using magnetic beads to remove primer dimers. In a second step, dual indices and sequencing adapters were incorporated via limited-cycle PCR, followed by another bead-based purification. Final libraries were eluted in low-salt buffer and quantified before quality control and sequencing according to 16S metagenomic sequencing library preparation (Nimonkar *et al.,* 2022).

Library quality control (QC) and sequencing: Final indexed libraries were quantified using a Qubit fluorimeter and assessed for fragment size (expected 550–600 bp) via microfluidics. Libraries meeting quality and concentration criteria were normalized to equimolar concentrations before pooling for sequencing (Illumina, 2013). To denature, a 5 µl of the 4 nM pooled library was mixed with 5 µl of 0.2N NaOH for 5 min. The library was then diluted in pre-cooled hybridization buffer, including a 20% PhiX control for base diversity (Illumina, 2013). Sequencing was performed on an Illumina MiSeq (2 x 300 bp), generating 0.5 to 1 million paired-end reads (0.3–0.6 GB) per sample. Demultiplexed FASTQ files were produced for bioinformatics analysis.

Bioinformatics and data analysis: Raw sequencing reads underwent quality checks, including adapter removal and trimming of low-quality bases. Bioinformatics was performed using the QIIME2 pipeline with the SILVA database, and DADA2 was used for denoising and sequence processing (Estaki *et al.,* 2020). Raw reads were demultiplexed, and paired-end sequences were joined to generate error-free feature tables and representative sequences (Bolyen *et al.,* 2019).

Diversity analysis was performed to determine about the microbial community complexity (Estaki *et al.,* 2020). Beta diversity was evaluated using Unweighted UniFrac and Bray-Curtis metrics to capture phylogenetic and abundance-based variations. Taxonomic assignment from phylum to genus level utilized curated databases for precise classification. Relative abundance revealed community structure, while differential abundance analysis identified key taxa and potential biomarkers linked to environmental gradients (Estaki *et al.,* 2020).

Statistical Analyses: Statistical analyses were performed using R Studio software (version 4. x). TVC was log 10 transformed to normalize the distribution of values. Basic descriptive statistics were calculated for the analysed physico-chemical properties. Pearson correlation analysis was performed to study the relationship between environmental variables and microbial abundance. Alpha diversity indices were calculated in QIIME2. Principal Coordinate Analysis (PCoA) was performed to assess the differences among the studied samples.

Results and Discussion

All samples were analysed for six heavy metals, including manganese (Mn), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), and zinc (Zn). Manganese, Nickel, Iron, and Zinc were detected across all sites, with concentrations ranging from 1.08 ± 0.39 to 367.53 ± 11.47mg kg^{-1} (Table 2). Iron was found in abundance (359.55 ± 2.82 to 367.53 ± 11.47 mg kg^{-1}), followed by manganese and zinc. Manganese was found in the highest concentration in the Mula-Mutha river (122.23 ± 1.10 mg kg^{-1}), whereas it was lowest in the Narmada river (18.95 ± 3.56 mg kg^{-1}). Nickel concentration peaked in the Tapi river (13.99 ± 2.46 mg kg^{-1}); however, it was lowest in the Mula-Mutha river (1.08 ± 0.39 mg kg^{-1}). Zinc concentration ranged from 82.30 ± 3.86 mg kg^{-1} (Mithi) to 17.44 ± 0.18 mg kg^{-1} (Sina). Cadmium and cobalt were below the detection limits (Dadebo and Gelaw, 2024).

Manganese was reported at elevated concentrations in the Mula-Mutha and Sina Rivers, suggesting that these river sediments may be contaminated by urban runoff, sewage discharge, and hospital or industrial effluents (Nishmitha *et al.,* 2025). A lower manganese concentration in the Narmada River sediments indicate reduced anthropogenic influence and diluted contamination from hospital and pharmaceutical effluents (Nishmitha *et al.,* 2025). High concentrations of nickel indicate greater anthropogenic influence, amplified by specific hospital effluents from surgical instruments and pharmaceuticals, while lower concentrations might be due to geological differences and hydrodynamic conditions (Khan *et al.,* 2025). The highest iron abundance was observed across all samples, distributed relatively uniformly, suggesting its natural abundance in the riverine sediment system (Lenstra *et al.,* 2022). A higher-to-lower concentration of zinc suggested greater contamination from urban and hospital discharges driven by anthropogenic factors (Khan *et al.,* 2025). These observations provide a baseline for sediment quality assessment and highlight the need for further monitoring and comparative assessments of contamination in urban rivers in India.

TOC of the samples ranged from 10.55% (Kamavari) to 0.2% (Tapi). Descriptive statistical analysis showed that pH across the sampling sites ranged from 5.0 to 8.0, with a mean value of 7.03 ± 0.82.Total organic carbon (TOC) varied considerably among locations, ranging from 0.20% to 10.55% with a mean value of 3.37 ± 4.60% (Table 1). The Mithi and Kamavari Rivers, located in the same geographic location (Mumbai, MH), showed the highest TOC and slightly acidic pH, suggesting high organic loads from urban wastewater and

Table. 1: Microbial and physico-chemical analysis of the sediment samples

Sampling sites	Sample ID	pH	TOC (%)	Heavy metals	TVC (CFU g^{-1})	Log_{10} (TVC)	Bacterial identification (MALDI-TOF/MS)
Mula–Mutha River, Pune, India	Sed-1-SWA	7.5–8.0	0.56	Mn, Ni, Fe, Zn	2.53×10^{6}	6.40	*Robertmurrayaberingensis;* *Bacillus pseudomycoides*
Mithi River, Mumbai, India	Sed-2-MT	5.0–6.0	9.58	Mn, Ni, Fe, Zn	1.86×10^{9}	9.27	*Bacillus cereus;* *Exiguobacteriumartemiae;* *Robertmurrayaberingensis*
Tapi River, Nandurbar, India	Sed-3-TP	7.0–8.0	0.20	Mn, Ni, Fe, Zn	1.05×10^{6}	6.02	NRI
Kamavari River, Mumbai, India	Sed-4-KM	6.0–7.0	10.55	Mn, Ni, Fe, Zn	2.95×10^{10}	10.47	*Bacillus cereus;* *Exiguobacteriumartemiae;* *Robertmurrayaberingensis*
Mula River, Pune, India	Sed-5-ML	7.0–8.0	1.33	Mn, Ni, Fe, Zn	1.54×10^{7}	7.19	*Exiguobacterium artemiae;* *Robertmurraya beringensis*
Narmada River, Indore, India	Sed-6-NM	6.0–7.0	0.86	Mn, Ni, Fe, Zn	2.80×10^{6}	6.45	*Bacillus pseudomycoides;* *Exiguobacterium artemiae*
Sina River,	Sed-7-SN	7.0–8.0	0.49	Mn, Ni, Fe, Zn	1.52×10^{6}	6.18	*Fictibacillus arsenicus*

Note. NRI- No reliable identification, TOC- Total Organic Carbon, TVC- Total Viable Count.TVC values were log_{10} transformed for statistical analysis to normalize microbial count distribution.

Table 2: Heavy metal content (mg kg^{-1}) in contaminated sediment samples

Sample Location		Mean ± SD		
		Mn	Ni	FeZn
Mula-Mutha River, Pune, India	122.23 ± 1.10	1.08 ± 0.39	367.53 ± 11.47	66.11 ± 7.93
Mithi River, Mumbai, India	67.09 ± 1.63	2.42 ± 0.57	366.20 ± 9.59	82.30 ± 3.86
Tapi River, Nandurbar, India	81.20 ± 0.35	13.99 ± 2.46	359.55 ± 2.82	21.77 ± 6.48
Kamavari River, Mumbai, India	80.33 ± 0.30	10.63 ± 1.35	361.94 ± 6.21	76.11 ± 2.49
Mula River, Pune, India	87.84 ± 3.08	6.36 ± 1.02	364.07 ± 4.33	19.76 ± 2.22
Narmada River, Indore, India	18.95 ± 3.56	4.00 ± 1.04	366.60 ± 6.02	35.48 ± 3.09
Sina River, Ahilyanagar, India	114.81 ± 1.18	3.28 ± 0.62	360.48 ± 3.01	17.44 ± 0.18

decomposition-driven acidification (Xiao *et al.,* 2023; Jayalath *et al.,* 2016). A mildly acidic pH was observed in the sediments of the Mithi River and Kamavari River, with high TOC, suggesting that acidic conditions are linked to organic matter decomposition (Jayalath *et al.,* 2016). Furthermore, a lower pH may indicate that the sediments are highly contaminated with untreated hospital wastewater, which elevate biological activity and oxygen consumption (Jayalath *et al.,* 2016).

Neutral pH suggests agricultural runoff in the sediments, whereas the alkaline pH of the Tapi and Sina Rivers indicate isolation from urban areas, well-oxygenated conditions, and greater hydrodynamic energy with a limited organic carbon load (Jayalath *et al.,* 2016). High organic load in the sediment may be due to water discharge from forest areas or agricultural runoff, which contains detritus and residues from biomass degradation. It is generally considered that samples stored at sub-zero temperatures without cryoprotectants are unfit for cultivation (Prakash *et al.,* 2020). In the present study, after storing the sediment samples in frozen conditions, the count of viable organisms revealed that pigmented colonies predominated in all samples following 72 hr of incubation at 30 °C, an optimal growth temperature on R2A medium. (Fig. 1). The dominance of pigmented colonies in all samples indicate that under colder conditions, pigmented microbes showed better potential to survive cold stress (Sajjad *et al.,* 2020). This study substantiates that pigments produced by bacteria play a crucial role in protecting microbial cells by preventing damage to cell membranes and stabilizing reactive oxygen species generated during crystallization of sediment samples, thereby protecting against oxidative damage (Agarwal *et al.,* 2023).

The number of viable colonies was maximum in the Kamavari River sediments (2.95 x 10^{-7}) and lowest in the Tapi River sediments (1.05 x 10^{-3}) (Fig. 1). Microbial abundance expressed as log_{10}-transformed total viable count (log TVC) showed a mean value of 7.43 ± 1.74.Overall, the TVC of sediment microorganisms varied from 10^{6} to 10^{10} CFU g^{-1} and showed a clear correlation with TOC and pH. As discussed earlier in the total organic carbon load and pH analysis result section, the organic

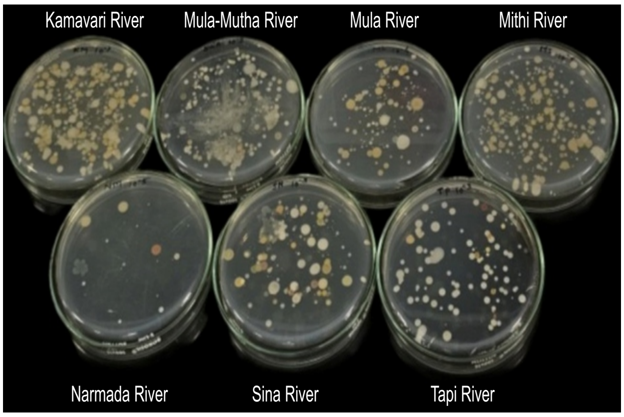


Fig.1: Isolation of viable microorganisms from sediment samples after storage in frozen conditions (-20°C) on R2A medium showed domination of pigmented colonies.

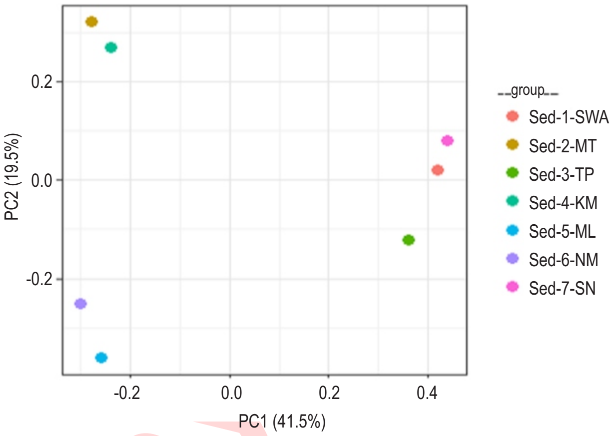


Fig. 2: Bray_curtis_emperor.qzvPCoA plot illustrates microbial community variation by location. Each dot represents a site, with color-coded groups showing partial separation that indicates distinct differences in community composition.

Table. 3: Sequencing QC

Sample-ID	Input Sequences	Filtered Input Sequences Passed Filter (%)	Passed Filter Sequences (%)	Denoised Non-chimeric Sequences	Input Non-chimeric Sequences (%)	Non-chimeric Sequences (%)
Sed-1-SWA	151424	151308	99.92	146742	140538	92.81
Sed-2-MT	192169	192118	99.97	188182	178952	93.12
Sed-3-TP	80719	80709	99.99	77418	74881	92.77
Sed-4-KM	157410	157365	99.97	153127	146193	92.87
Sed-5-ML	222692	222583	99.95	218284	205633	92.34
Sed-6-NM	166288	166244	99.97	161957	151637	91.19
Sed-7-SN	115194	115169	99.98	111315	106512	92.46

load in the Mithi River and Kamavari River sediments was highest among all the text sediment samples, with pH in the range of 6 to 7 showing the highest TVC, indicating favourable conditions for survival and growth of the microorganisms (Leong *et al.*, 2018). In contrast, sediments with low TOC content and neutral to alkaline pH (7–8), such as those collected from the Tapi, Mula-Mutha, and Sina Rivers, showed comparatively lower TVC values.

The observations indicated that the organic matter in the sample can protect cells from freezing damage compared with samples with lower organic matter content, resulting in lower TCV (Jahed *et al.,* 2023). A strong positive correlation was observed between TOC and $\log_{10}$ TVC (r = 0.98), indicating that sediments with higher organic carbon content supported greater microbial populations. A moderate relationship was observed between pH and TVC (r = -0.69). Bacterial isolates were identified using MALDI-TOF mass spectrometry (Bruker MALDI Biotyper). A total of 14 isolates were analysed from all the cultivated sediment samples. Based on the Biotyper score criteria, isolates were classified into high-confidence (score≥2.0), low-confidence (1.70–1.99), and no reliable identification (<1.70). Of the analysed isolates, five were successfully identified at the species level with high confidence, while others showed low confidence or no identification. Some of the species, *Bacillus cereus, Bacillus pseudomycoides, Robertmurraya beringensis, Exiquobacterium artemiae* and *Fictibacillus arsenicus,* were observed with a detectable confidence score. Sample-wise the presence of bacteria is shown in Table 1. Several other isolates could not be identified due to insufficient spectral peaks, which may be attributed to the absence of closely related reference spectra in the database (Rahi *et al.,* 2016). High-throughput sequencing generated raw reads ranging from 80,719 (Sed-3-TP) to 222,692 (Sed-5-M) per sample (Table 3). Quality filtering retained over 99.9% of reads for each sample. After denoising and removing chimeras, a substantial portion of reads remained as high-quality, non-chimeric sequences. The percentage of non-chimeric reads compared to the initial input ranged from 91.19% for Sed-6-NM to 93.12% for Sed-2-MT. Final non-chimeric counts ranged from 74,881 to 205,633, confirming the robustness of the data for taxonomic and diversity analyses. Rarefaction curves reached a plateau for all samples, indicating that almost all communities achieved sufficient sampling depth during sequencing (Vasar *et al.,* 2017). This study showed clear saturation for all the test sediment samples collected. Highest Amplicon Sequence Variants (ASV) richness in the Sed-1-SWA sample (~830 ASVs) while sample Sed-2-MT (~450 ASVs) showed the lowest richness. The variation in richness in samples could be due to a number of factors including levels of contamination, TOC load, physical factors and sediment chemistry or high residual antibiotics concentration (Thakur *et al.,* 2025). Alpha diversity measures the richness and evenness of microbial communities (Li *et al.,* 2017). Observed species, Shannon Index, and Simpson Index help assess microbial balance and ecosystem health (Li *et al.,* 2017). Higher alpha diversity indicates a more stable and diverse microbiome, whereas lower diversity signals imbalance or dysbiosis (Evans *et al.,* 2023). Alpha diversity analysis revealed clear variation in richness and evenness with observed ASVs ranging from 455 to 830. The Mula-Mutha River showed the highest ASVs (830), followed by the Mula River (683) and Kamavari River (657), suggesting more complex communities (Beaudry *et al.,* 2021), whereas sediments from the Mithi River showed the lowest observed ASVs and Shannon diversity (4.30), likely due to high toxicity (Li *et al.,* 2017). Simpson index values were consistently high across all samples (0.95–1.00), suggesting overall high community evenness and low dominance despite differences in richness (Li *et al.,* 2017). Sediments from the Narmada and Sina rivers showed slightly lower Simpson values than other sediment samples, indicating slightly higher dominance (Evans *et al.,* 2023). The study of alpha diversity matrices showed site-specific variation in microbial community structure, with differences in richness and evenness across samples.

Beta diversity measures the differences in the microbial communities between samples (Mori *et al.,* 2018). Principal Coordinate Analysis (PCoA) (Fig. 2) showed distinct separation along Principal Coordinates 1 (PC1) (41.5% variation), likely driven by hospital wastewater contamination and antibiotic selection. Principal Coordinates 2 (PC2) (19.5% variation) represents secondary factors such as geochemistry and local hydrology (Mori *et al.,* 2018). Combined, these first two axes accounted approximately 61% of the total community variation, highlighting significant ecological restructuring driven by hospital-induced selective pressure. According to Fig. 2, the sediment samples from the Mithi and Kamavari Rivers in Mumbai showed similar microbial composition. Sediments of the Sina River, Ahilyanagar, and the Mula-Mutha River in Pune have nearly similar microbial populations, whereas the Mula River, Pune, and the Narmada River, Indore, showed closely related populations, which might be due to similar levels of pollutants, anthropogenic factors, and physicochemical conditions (Mori *et al.,* 2018). Although the amplicon was conducted using bacterial universal primers, archaeal taxa were also detected in each sample. The highest archaeal abundance (1.74%) was observed in Sed-4-KM (Kamavari River, Mumbai), 0.88% in Sed-6-NM (Narmada River, Indore) and 0.67% in Sed-5-ML (Mula River, Pune). In this study, the sediment samples were dominated by the Phyla *Proteobacteria, Firmicutes, Chloroflexi, Bacteroidota, Actinobacteriota* and *Acidobacteriota,* all known for their roles in organic matter degradation (Table 4) (Zhang *et al.,* 2024). *Proteobacteria,* especially *Gammaproteobacteria* and *Deltaproteobacteria,* were highly abundant, indicating significant involvement in sulphur and nitrogen cycling (Zhang *et al.,* 2024).

In all sediment samples, the phylum-level community structures reflected environmental and contamination gradients across sites. As shown in Fig. 3, Sed-1-SWA was mainly dominated by *Proteobacteria, with Myxococcota, Acidobacteriota, Chloroflexi* and *Actinobacteriota* also present, indicating a mixture of heterotrophicand predatorymicrobial groups. Sed-2-MT showed a prevalence of *Firmicutes* and *Bacteroidota,* suggesting the dominance of fermentative and anaerobic taxa typical of organic-rich environments (Tufail *et al.,* 2025). The community in Sed-3-TP was led by *Proteobacteria* and *Bacteroidota,* reflecting a combination of diverse metabolic strategies, including predation and complex carbon degradation. In Sed-4-KM, *Bacteroidota* and *Chloroflexi* were the most abundant phyla, followed by *Firmicutes, Proteobacteria* and *Actinobacteriota,* indicating strong anaerobic and phototrophic influences (Zhang *et al.,* 2024). Sed-5-ML was dominated by *Proteobacteria* and *Bacteroidota,* consistent with environments that support sulfur cycling and organic matter breakdown. In Sed-6-NM, *Firmicutes* and *Bacteroidota* were the dominant phyla, indicating that the communities were adapted to fluctuating redox and nutrient conditions. Lastly, Sed-7-SN displayed a unique composition dominated by *Actinobacteria* and *Proteobacteria,* followed by *Firmicutes, Chloroflexi* and *Acidobacteria,* reflecting a blend of aerobic and facultative anaerobic populations (Zhang *et al.,* 2024).

16S rRNA gene sequence based metagenomic analysis of contaminated sediments in near-hospital zones revealed a dominance of uncultured microorganisms. *Macellibacteroides* was the most abundant genus overall, followed by *Azospira, Exiguobacterium,* and *Bacteroidetes_vadin HA17* (Table 4, Fig. 4). Dominant bacterial genera showed site-specific characteristics. Sed-1-SWA was composed largely of *Haliangium, MND1* and *Cellvibrio,* indicating mixed *Myxobacterial* and soil lineages. Sed-2-MT was dominated by *Macellibacteroides, Bacteroidetes_vadinHA17,* and *Sporacetigenium,* highlighting anaerobic fermentative groups (Mei *et al.,* 2020). Sed-3-TP featured *Bdellovibrio, Thermomonas,* and *Nitrospira,* suggesting predatory and nitrifying microbes (Kop *et al.,* 2025). Sed-4-KM was characterized by *Macellibacteroides, Anaerolinea*, and *Petrimonas*, typically linked to organic-rich anaerobic settings (Maus *et al.,* 2020). In Sed-5-ML, *Azospira* and *Dechloromonas* were the most prevalent genera, along with *Bacteroidetes_ vadin HA17* and *Fonticella,* indicating active denitrifying and organotrophic populations (Mei *et al.,* 2020). Sed-6-NM displayed a notably different profile, with *Exiguobacterium, Azospira, Macellibacteroides, Proteiniclasticum* and *Erysipelothrix* dominating, reflecting communities adapted to varying nutrient

Table. 4: Comparative abundance of top phyla and genera in contaminated sediment samples

Abundance	**Sample ID**						
	Sed-1-SWA	**Sed-2-MT**	**Sed-3-TP**	**Sed-4-KM**	**Sed-5-ML**	**Sed-6-NM**	**Sed-7-SN**
Abundant Phyla	*Proteobacteria*	*Firmicutes*	*Proteobacteria*	*Bacteroidota*	*Proteobacteria*	*Firmicutes*	*Actinobacteriota*
	Myxococcota	*Bacteroidota*	*Bacteroidota*	*Chloroflexi*	*Bacteroidota*	*Bacteroidota*	*Proteobacteria*
	Acidobacteriota	*Proteobacteria*	*Verrucomicrobiota*	*Firmicutes*	*Firmicutes*	*Proteobacteria*	*Firmicutes*
	Chloroflexi	*Chloroflexi*	*Acidobacteriota*	*Proteobacteria*	*Chloroflexi*	*Chloroflexi*	*Chloroflexi*
	Actinobacteriota	*Actinobacteriota*	*Bdellovibrionota*	*Actinobacteriota*	*Desulfobacterota*	*Acidobacteriota*	*Acidobacteriota*
Abundant Genera	*Haliangium*	*Macellibacteroides*	*Bdellovibrio*	*Macellibacteroides*	*Azospira*	*Exiguobacterium*	*Hydrogenophaga*
	MND1	*Bacteroidetes_vadinHA17*	*uncultured*	*Anaerolinea*	*Dechloromonas*	*Azospirau*	*uncultured*
	uncultured	*uncultured*	*Thermomonas*	*Petrimonas*	*Bacteroidetes_vadinHA17*	*Macellibacteroides*	*Nocardioides*
	Cellvibrio	*uncultured*	*Vicinamibacteraceae*	*Candidatus_Nomurabacteria*	*Fonticella*	*Proteiniclasticum*	*Vicinamibacteraceae*
	Subgroup_7	*Sporacetigenium*	*Nitrospira*	*Bacteroidetes_vadinHA17*	*uncultured*	*Erysipelothrix*	*Nitrospira*

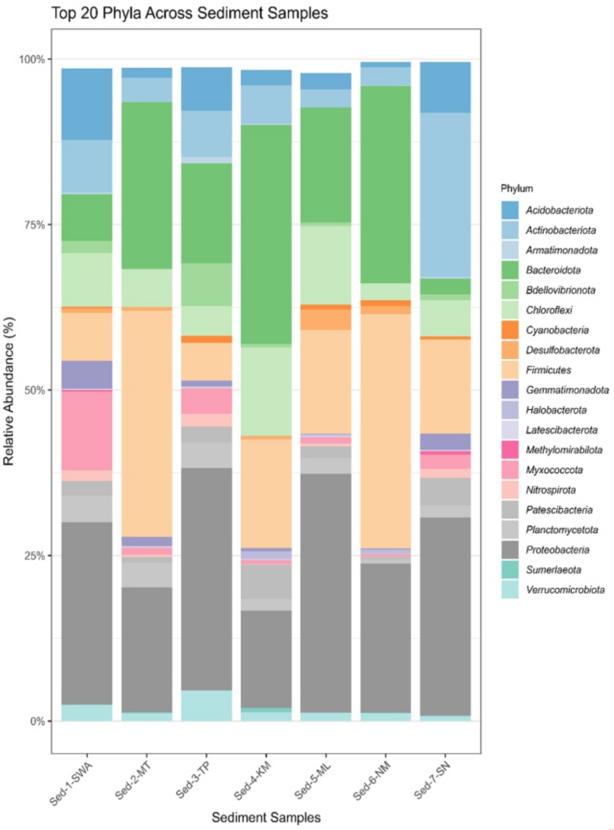


Fig. 3: Stacked bar graph showing top 20 phyla per sediment sample

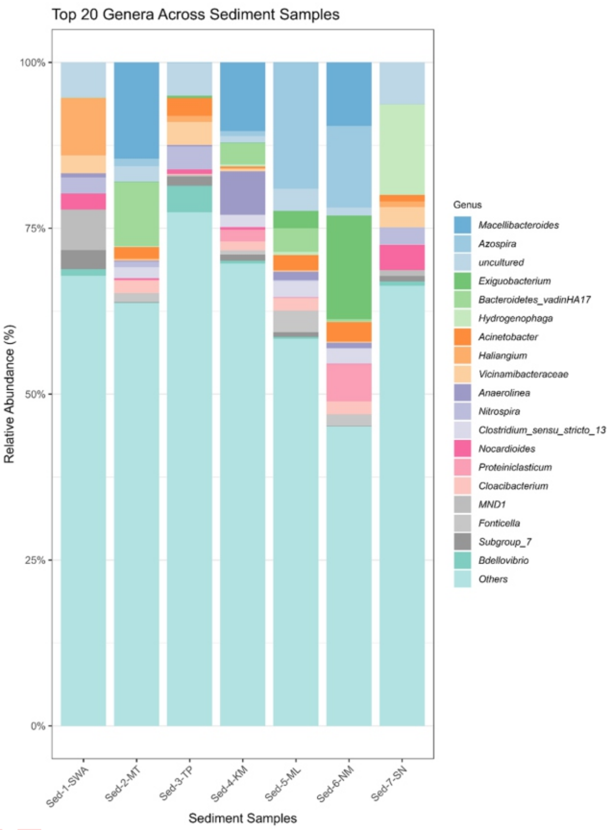


Fig. 4: Stacked bar graph showing top 20 genera per sediment sample.

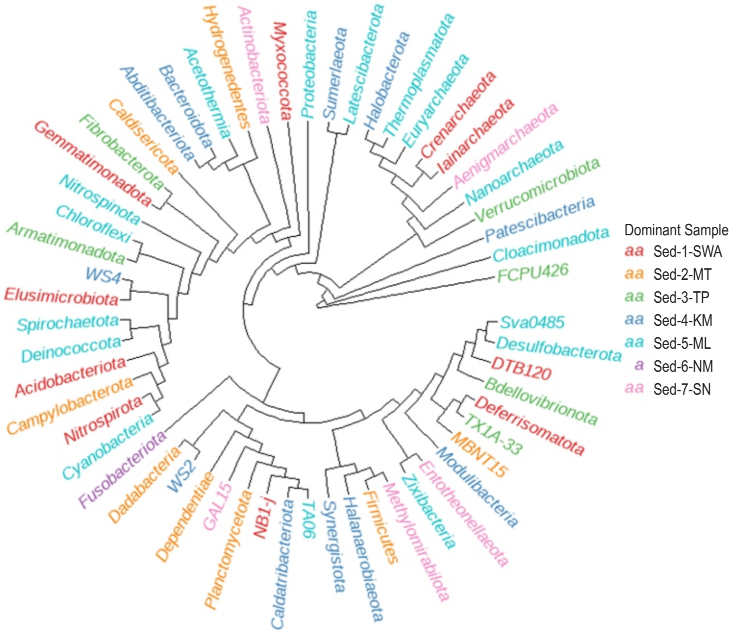


Fig. 5: Phylum-level phylogenetic tree from 16S rRNA gene metagenomics assessment of the contaminated sediment samples is color-coded by sample ID.

and redox conditions. Lastly, Sed-7-SN was led by *Hydrogenophaga,* followed by *Nocardioides, Vicinamibacteraceae* and *Nitrospira,* indicating the coexistence of hydrogen-oxidizing, nitrifying, and soil-derived taxa (Li *et al.,* 2025).

The phylogenetic tree (Fig. 5) illustrates evolutionary relationships among dominant phyla, with color-coded bars indicating prevalence across samples. Major bacterial groups, such as *Proteobacteria, Bacteroidota* and *Firmicutes,* formed distinct clades, highlighting their ecological significance in the contaminated sediments (Zhang *et al.,* 2024). Samples heavily influenced by hospital wastewater (Sed-2-MT, Sed-4-KM, and Sed-6-NM) clustered around phyla linked to anaerobic degradation and high organic loads, such as *Firmicutes* and *Chloroflexi.* In contrast, Sed-1-SWA and Sed-7-SN aligned with soil-associated phyla, including *Actinobacteriota, Acidobacteriota* and *Verrucomicrobiota,* highlighting the impact of natural runoff alongside anthropogenic inputs (Zhang *et al.,* 2024). Specialized, less abundant phyla like *Bdellovibrionota* and *Nitrospirota* occupied outer branches, indicating niche specialization. Overall, the tree demonstrates how wastewater exposure and environmental conditions drive the phylogenetic structure and composition of sediment microbial communities.

In conclusion, this study demonstrates that site geochemistry and levels of sediment contamination and organic load shape the sediment microbial community. In this study, only a limited number of samples, were studied, however, a large scale study with more samples from different geographical locations and varying levels of contamination would provides a better insight. Given that polluted sediments may pose risks to public, animal and environmental health, continued research is important for managing and restoring these ecosystems.

Acknowledgments

RT and RD are obliged to Symbiosis International (Deemed University) for providing the doctoral Fellowship to support this work. AKS acknowledges the financial support in the form of a JRF from DST-PURSE.

Authors' contribution: R.S. Thakur: Conducted the experimental work, prepared the draft, and took primary responsibility; all other authors, including; **A.K. Singh**, **R.S. Dhanwar**, **T.D. Lodha** and **B.S. Lopes**: Helped with editing, writing, and critical analysis and discussion during the preparatory stages of the manuscript; **S.S. Donde** Supported sample collection; **O. Prakash:** Designed the work and supervised the study. All authors have agreed to the final version of the manuscript before its submission.

Funding: This study was supported by the Research Support Fund and Junior Research Fellowships from Symbiosis International (Deemed University).

Research content: The research content of the manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: The author declare no conflict of interest.

Data availability: Data will be made available from the first or Corresponding author upon reasonable request.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

References

American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF): Standard Methods for the Examination of Water and Wastewater. 23rd Edn., American Public Health Association, Washington, DC (2023).

Agarwal, H., S. Bajpai, A. Mishra, I. Kohli, A. Varma, M. Fouillaud, L. Dufossé and N.C. Joshi: Bacterial pigments and their multifaceted roles in contemporary biotechnology and pharmacological applications. *Microorganisms,* **11**, 614 (2023).

Ajala, O.J., J.O. Tijani, R.B. Salau, A.S. Abdulkareem and O.S. Aremu: A review of emerging micro-pollutants in hospital wastewater: Environmental fate and remediation options. *Results Eng.,* **16**, 100671 (2022).

Beaudry, M.S., J. Wang, T.J. Kieran, J. Thomas, N.J. Bayona-Vásquez, B. Gao, A. Devault, B. Brunelle, K. Lu, J.-S. Wang, O.E. Rhodes and T.C. Glenn: Improved microbial community characterization of 16S rRNA via metagenome hybridization capture enrichment. *Front. Microbiol.,* **12**, 644662 (2021).

Bolyen, E., J.R. Rideout, M.R. Dillon, N.A. Bokulich, C.C. Abnet, G.A. Al-Ghalith, H. Alexander, E.J. Alm, M. Arumugam and F. Asnicar *et al.*: Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.*, **37**, 852–857 (2019).

Carles, L., S. Wullschleger, A. Joss, R.I.L. Eggen, K. Schirmer, N. Schuwirth, C. Stamm and A. Tlili: Wastewater microorganisms impact microbial diversity and important ecological functions of stream periphyton. *Water Res.,* **225**, 119119 (2022).

Dadebo, T.T. and G.T. Gelaw: Determination of metals in water samples within the irrigation area in Telo District, Kaffa Zone, South Western Ethiopia. *Heliyon,* **10**, e29003 (2024).

Estaki, M., L. Jiang, N.A. Bokulich, D. McDonald, A. González, T. Kosciolek, C. Martino, Q. Zhu, A. Birmingham, Y. Vázquez-Baeza, M.R. Dillon, E. Bolyen, J.G. Caporaso and R. Knight: QIIME 2 enables comprehensive end-to-end analysis of diverse microbiome data and comparative studies with publicly available data. *Curr. Protoc. Bioinform.,* **70**, 100 (2020).

Evans, T., U. Ali, R. Anderton, E. Raby, L. Manning and E. Litton: Lower gut dysbiosis and mortality in acute critical illness: A systematic review and meta-analysis. *Intensive Care Med. Exp.,* **11**, 6 (2023).

Food and Agriculture Organization (FAO): Standard operating procedure for soil organic carbon (Walkley–Black method). FAO, Rome (2019).

Gerenfes, D., A.G. Giorgis and G. Negasa: Comparison of organic matter determination methods in soil by loss on ignition and potassium dichromate method. *Int. J. Hortic. Food Sci.,* **4**, 49–53 (2022).

Illumina: 16S Metagenomic Sequencing Library Preparation. Illumina Inc., San Diego, USA (2013).

Jahed, K.R., A.K. Saini and S.M. Sherif: Coping with the cold: Unveiling cryoprotectants, molecular signaling pathways, and strategies for cold stress resilience. *Front. Plant Sci.,* **14**, 1246093 (2023).

Jayalath, N., L.M. Mosley, R.W. Fitzpatrick and P. Marschner: Addition of organic matter influences pH changes in reduced and oxidised acid sulfate soils. *Geoderma,* **262**, 125–132 (2016).

Karas M. and F. Hillenkamp: Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Analytic. Chem*., **60**, 2299–2301 (1988).

Khan, K., M. Younas, M. Yaseen, H. Sher, A. Maryam, S.M. Ibrahim, A. Adnan, A. Ali, M. Fawad, A.Z. Khan, N. Khan and I.A. Shah: Heavy metals pollution in riverine sediments: Distribution, source, and environmental implications. *Environ. Monit. Assess.,* **197**, 225 (2025).

Kop, L.F.M., H. Koch, P. Dalcin Martins, C. Suarez, S. Karačić, F. Persson, B.-M. Wilén, P. Hagelia, M.S.M. Jetten and S. Lücker: High diversity of nitrifying bacteria and archaea in biofilms from a subsea tunnel. *FEMS Microbiol. Ecol.,* **101**, fiaf032 (2025).

Lenstra, W.K., N.A.G.M. van Helmond, O.M. Żygadłowska, R. van Zummeren, R. Witbaard and C.P. Slomp: Sediments as a source of iron, manganese, cobalt and nickel to continental shelf waters (Louisiana, Gulf of Mexico). *Front. Mar. Sci.*, **9**, 811953 (2022).

Leong, S.S., J. Ismail, N.A. Denil, S.R. Sarbini, W. Wasli and A. Debbie: Microbiological and physicochemical water quality assessments of river water in an industrial region of the northwest coast of Borneo. *Water,* **10**, 1648 (2018).

Li, H., Z. Xu, Z. Wang, Q. Yan, Y. Deng, Z. He, J.D. Van Nostrand, S. Yang, J. Zhou and Y. Jiang: Soil microbial beta-diversity is linked with compositional variation in aboveground plant biomass in a semi-arid grassland. *Plant Soil,* **423**, 465–480 (2017).

Li, R., Y. Jiang, K. Luo, B. Chen, H. Wang, X. Fan, J. Pan, Y.V. Litti, S. Fu and R. Guo: Hydrogen-oxidizing bacteria: A promising contributor to environmental sustainability and resource recycling. *Green Carbon,* **3**, 398–406 (2025).

Maus, I., T. Tubbesing, D. Wibberg, R. Heyer, J. Hassa, G. Tomazetto, L. Huang, B. Bunk, C. Spröer, D. Benndorf, V. Zverlov, A. Pühler, M. Klocke, A. Sczyrba and A. Schlüter: The role of *Petrimonas mucosa* ING2-E5AT in mesophilic biogas reactor systems as deduced from multiomics analyses. *Microorganisms,* **8**, 2024 (2020).

Mei, R., M.K. Nobu, T. Narihiro and W.-T. Liu: Metagenomic and metatranscriptomic analyses revealed uncultured Bacteroidales populations as the dominant proteolytic amino acid degraders in anaerobic digesters. *Front. Microbiol.,* **11**, 593006 (2020).

Mori, A.S., F. Isbell and R. Seidl: β-diversity, community assembly, and ecosystem functioning. *Trends Ecol. Evol.,* **33**, 549–564 (2018).

Nimonkar, Y.S., B. Yadav, P. Talreja, R. Kumar, S. Sharma and A. Singh: Assessment of the role of wastewater treatment plant in spread of antibiotic resistance and bacterial pathogens. *Ind. J. Microbiol*., **59**, 261–265 (2019).

Nimonkar, Y.S., S. Kajale, M. Dake, D.R. Ranade, K.K. Yadav, R. Kumar and O. Prakash: A culture-based and culture-independent approach to the study of landfill leachate bacterial and archaeal communities. *Anaerobe*., **77**, 102626 (2022).

Nishmitha, P.S., K.A. Akhilghosh, V.P. Aiswriya, A. Ramesh, M. Muthuchamy and A. Muthukumar: Understanding emerging contaminants in water and wastewater: A comprehensive review on detection, impacts, and solutions. *J. Hazard. Mater. Adv.,* **18**, 100755 (2025).

Prakash, O., Y. Nimonkar and D. Desai: A recent overview of microbes and microbiome preservation. *Indian J. Microbiol.,* **60**, 297–309 (2020).

Rahi, P., O. Prakash and Y.S. Shouche: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) based microbial identifications: Challenges and scopes for microbial ecologists. *Front. Microbiol.,* **7**, 1359 (2016).

Rastmanesh, F., A.R. Zarasvandi, S. Safaie and M. Edraki: Heavy metal enrichment and ecological risk assessment of surface sediments in Khorramabad River, West Iran. *Environ. Monit. Assess.,* **190**, 273 (2018).

Sajjad, W., G. Din, M. Rafiq, A. Iqbal, S. Khan, S. Zada, B. Ali and S. Kang: Pigment production by cold-adapted bacteria and fungi: Colorful tale of cryosphere with wide range applications. *Extremophiles,* **24**, 447–473 (2020).

Shelton, L.R. and P.D. Capel: Guidelines for collecting and processing samples of stream bed sediment for analysis of trace elements and organic contaminants for the National Water-Quality Assessment Program. *U.S. Geol. Surv. Open-File Rep.,* **94-458,** 1-20 (1994).

Silvester, R., W.B. Perry, G. Webster, L. Rushton, A. Baldwin, D.A. Pass, N.A. Byrnes, K. Farkas, M. Heginbothom, N. Craine, G. Cross, P. Kille, B. Kasprzyk-Hordern, A.J. Weightman and D.L. Jones: Metagenomic profiling of hospital wastewater: A comprehensive national scale analysis of antimicrobial resistance genes and opportunistic pathogens. *J. Infect.,* **90**, 106503 (2025).

Thakur, R., A. Singh, R. Dhanwar, S. Kadam, U. Waghmare, T. Lodha, B.S. Lopes and O. Prakash: Global perspectives on residual antibiotics: Environmental challenges and trends. *Discover Sustain.,* **6**, 232 (2025).

Tufail, A., T. Bo, N. Zhao, S. Willows-Munro, B.N. Khan, J. Duan, J. Jin, Y. Qu, F. Lei and G. Song: Exploring microbiome shifts across taxonomic and ecological groups of birds at a key stopover site in Punjab, Pakistan. *Curr. Res. Microb. Sci.,* **9**, 100492 (2025).

Vasar, M., R. Andreson, J. Davison, T. Jairus, M. Moora, M. Remm, J.P.W. Young, M. Zobel and M. Öpik: Increased sequencing depth does not increase captured diversity of arbuscular mycorrhizal fungi. *Mycorrhiza,* **27**, 761–773 (2017).

Wang, C., X. Wang, J. Ma, S. Liu, Q. Yuan, P. Wang and J. Chen: How sediment bacterial community shifts along the urban river located in a mining city. *Environ. Sci. Pollut. Res.,* **28**, 42300–42312 (2021).

Woodward, J., J. Rothwell, J. Li and R. Hurley: Acute riverine microplastic contamination due to avoidable releases of untreated wastewater. *Nat. Sustain.,* **4**, 793–802 (2021).

Xiao, T., F. Ran, Z. Li, S. Wang, X. Nie, Y. Liu, C. Yang, M. Tan and S. Feng: Sediment organic carbon dynamics response to land use change in diverse watershed anthropogenic activities. *Environ. Int.,* **172**, 107788 (2023).

Zhang, Z., J. Lu, S. Zhang, Z. Tian, C. Feng and Y. Liu: Analysis of bacterial community structure, functional variation, and assembly mechanisms in multi-media habitats of lakes during the frozen period. *Ecotoxicol. Environ. Saf.,* **284**, 116903 (2024).