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Research Article

Bioaccumulation of Heavy Metals in Freshwater Invertebrates of the Kallada River, India: Implications for Ecological Risk and Food Safety

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ABSTRACT

Background: Rivers are crucial freshwater ecosystems that sustain biodiversity, livelihoods, and essential ecosystem services, but face increasing contamination from industrial, domestic, agricultural, and mining activities. Heavy metals, being non-biodegradable, persist in sediments and bioaccumulate in aquatic organisms, posing ecological and public health concerns. **Materials and Methods:** This study assessed heavy metal contamination in the Kallada River and connected sites in Kollam district by analyzing water, sediment, and tissues of freshwater invertebrates (crabs, shrimps, prawns, bivalves, and aquatic insects). Invertebrates were selected as bioindicators because of their sediment association and metal accumulation potential, metals, thereby providing time-integrated evidence of contamination. Trace metals (Ni, Cu, Zn, As, Cd, and Pb) were quantified using ICP MS. **Results and Discussion:** While water and sediment metal levels were within WHO and ISQG guideline values, bioaccumulation was evident, particularly at downstream sites, with species-specific uptake influencing the Metal Pollution Index (MPI). Spatial variations in MPI showed peak contamination in Site A, crabs and shrimps at Site B, and prawns at Site C, with prawns at Site C exhibiting the highest MPI (15.4). **Conclusion:** These findings highlight the critical role of species-specific uptake in shaping ecological risk and underscore the importance of combining MPI with tissue-level metal analysis to provide a more comprehensive assessment of ecological and public health implications in aquatic environments.

Keywords: Ecotoxicology, Sediment, Trophic Transfer.

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INTRODUCTION

Rivers are vital freshwater ecosystems supporting biodiversity, nutrient cycling and ecosystem services, like water purification, irrigation, and recreation.[1] However, anthropogenic pressures, especially chemical pollution, are degrading these systems. Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), copper (Cu), and zinc (Zn) enter rivers through industrial effluents, agricultural runoff and sewage or mining and smelting[2] and persist due to their toxicity, non-biodegradability, and bioaccumulation potential across food webs.[3] Elevated concentrations of nickel, chromium, and lead are reported in Narmada and Mahi rivers of India, with fish species such as *Labeo rohita* showing significant accumulation alongside microplastic contamination.[4]

Heavy metals adsorb particulates and sediments, forming long-term contamination reservoirs.[5] Climate warming mobilises metals, as observed in the Colorado Rockies, where Cu and Zn levels doubled in three decades.[6] Conversely, reduced atmospheric metal inputs in England improved freshwater biodiversity.[7]

At the organism level, fish exhibit oxidative stress, tissue damage, and bioaccumulation of Cd, Hg, and Arsenic (As), with implications for ecosystem and human health.[8] Benthic invertebrates, including molluscs, crustaceans, and insect larvae, are particularly vulnerable due to absorbing metals through gills, integument, and ingestion from sediments, acting as vectors of trophic transfer and biomagnification[9] and effective bioindicators of chronic contamination[10] due to their sedentary nature and relatively long life span. Tissue analysis reveals bioavailable fractions, spatial patterns and ecological risks.[11] In Kollam district of Kerala, rivers like Kallada, Ithikkara, and Pallichal face heavy metal pollution from industries (Chavara and Kundara areas), and agriculture. Ashtamudi Lake in Kollam showed high Iron (Fe) and Cr,[12] while Paravoor mangroves showed bioaccumulation of Fe, Cu, Zn, and Pb in water, sediment,

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and fish tissues.[13] Current study used freshwater invertebrates from multiple sites as bioindicators to 1. Quantify Ni, Cu, Zn, As, Cd, and Pb in tissues 2. Compare metal loads across functional feeding groups to assess trophic transfer 3. Correlate tissue metal concentrations with water and sediment parameters. 4. Contextualize findings within toxicological benchmarks to evaluate ecological risks. This study provides a robust assessment of the Kallada River's ecological status to inform conservation and pollution control in Kerala's freshwater ecosystems.

MATERIALS AND METHODS

Study Area

The study was conducted along the Kallada River, Kollam district, Kerala, India. To analyse spatial variations in heavy metal contamination, three distinct sites were selected along the course of the river (Figure 1). Site A (8.8723° N, 77.0484° E), located near Dally Bridge, Kulathupuzha, served as the upstream reference with minimal anthropogenic influence. Site B (9.0024° N, 76.5931° E) at Arinalloor, Thevalakkara, represented the midstream stretch with moderate impacts from agriculture and tourism. Site C (8.8961° N, 76.5849° E), near the river's confluence with Ashtamudi Lake at Kollam city, was the downstream site, heavily affected by urban discharge, industrial effluents, and agricultural waste.

Sample collection and preparation

Samples of water, sediment, and benthic invertebrates were collected from each site. Surface water was collected in pre-cleaned, acid-washed polyethylene bottles, filtered, and acid-preserved for analysis of dissolved metals. For sediments, benthic samples were obtained using a grab or core sampler, stored in airtight containers, and prepared for metal analysis. For tissue analysis, invertebrates with high bioaccumulation potential (molluscs, crustaceans, and aquatic insects) were collected from each site. Collections from site A include *Barytelphusa cunicularis* (crab), *Macrobranchium Idella* (prawn), *Gerris lacustris, Nepa cinerea, Aquarius remingis* (aquatic insects); site B includes *Scylla serrata* (crab), *Macrobradium gurudeve* (shrimp) and *Geloina expansa* (clam); from site C include *Scylla transquebarica* (crab), *Fenneropenaeus indicus* (prawn) and *Corbicula fluminea* (clam). Tissues were rinsed with deionised water and homogenised (whole body for small specimens, muscle tissue for larger ones), with sufficient specimens collected to capture intraspecific variability.

Heavy Metal Quantification

Concentrations of Pb, Cd, As, Cu, Zn, and Ni in all samples were determined using Inductively Coupled Mass Spectrometry (ICP-MS) and evaluated against WHO and FAO standards for toxicity and human consumption (Table 1).[14-17]

Water and Sediments

Water samples were used for analysis using ICP-MS after filtering to remove particulates, followed by acidifying with nitric acid to preserve dissolved metal content. Sediment samples were oven-dried at 105ºC for 24 hr to remove moisture and then homogenized into fine powder using a mortar and pestle. Approximately 0.25 g of homogenized sediment was accurately weighed and transferred into a clean, dry Teflon crucible. Samples were digested using a mixture of concentrated Nitric Acid (HNO_3) and Perchloric Acid ($HClO_4$) in a 5:1 ratio, following established protocols.[18,19] Digestion was performed on a hot plate at ≤50ºC to minimise volatilisation losses, followed by dilution of extracts with distilled water to 25 mL and filtered into 100 mL volumetric flasks. Heavy metal concentrations in the digested solutions were determined using a Microwave Plasma - Atomic Emission Spectrometer (MP-AES; Thermo Scientific iCAP RQ) operated in helium Kinetic Energy Discrimination (KED) mode.

Invertebrate tissues

Invertebrate tissues were dissected using stainless steel instruments to prevent contamination. Fresh tissue samples (5 g each) were oven-dried at 105ºC for 24 hr, homogenised, and ground into fine powder using a mortar and pestle. For digestion, 10 mL of concentrated nitric acid (65% HNO_3) was added to ~0.5 g of powdered tissue in a glass beaker. The mixture was heated on a hotplate under a fume hood until dense white fumes appeared, indicating completion of digestion.[20] The digested material was cooled, diluted to 100 mL with ultrapure water, and filtered to remove particulates. Final filtrates were diluted as required and analysed for trace metals using ICP-MS, PerkinElmer NexION series.

Data Analysis

We performed Principal Component Analysis (PCA) to identify the major contributing metals influencing variation among the samples.[21] The Metal Pollution Index (MPI) was calculated for each sample using the geometric mean of concentrations of all measured metals.[22] MPI values were compared across different sites to identify sites with higher pollution loads and to assess spatial variation in overall metal contamination levels. The formula was as follows:

$$MPI= (C1 \times C2 \times \ldots \times Cn)1/n$$

Where C1, C2… Cn are the concentrations of n different metals in the sample.

To assess significant differences in heavy metal concentrations across sites, PERMANOVA (Permutational Multivariate Analysis of Variance) was employed using the adonis2() function from the vegan package in R. The analysis was based on Euclidean distance with 999 permutations. A stratified permutation design was used to account for sample-level variability in water, sediment, and invertebrate tissues.[23] Statistical analyses were performed using R version 4.4.1.

RESULTS

Analysis of heavy metal concentrations across three sites revealed distinct accumulation patterns from upstream, to downstream sites.

Arsenic was highest in prawns from Site C (22.01 ppb), followed by shrimp (5.77 ppb) and crab (3.34 ppb) from Site B. Cadmium peaked in site A sediments (0.98 ppb) and site C prawns (0.79 ppb). Copper was highest in site B shrimp (56.91 ppb) and site C prawn (51.19 ppb). Nickel peaked in crab (32.66 ppb) and sediment (27.60 ppb) from site C. Lead was the highest in site B crab (16.94 ppb) and site C prawn (9.22 ppb). Zinc showed maximum concentration in shrimp from site B (269.30 ppb), prawn from site C (242.18 ppb), and prawn from Site A (239.59 ppb). Clam from both site A and site B had consistently lower concentrations of all metals (Figure 2).

In contrast, water and aquatic insect samples generally showed lower concentrations across all metals. These results were further substantiated by PCA analysis. The first two principal components (Dim1- 43.5%, Dim2- 31.6%) together explain 75.1% of the total variation in the data (Table 2). Pb and Ni were major contributors to the variation and were particularly high in sediment (site A) and crab (site C). Shrimp showed higher concentrations of Zn and Cu. As and Cd indicate moderate influence on prawn and sediments from site B. Water and insects reflect relatively lower concentrations and not a specific association with any metals (Figure 3).

PERMANOVA results showed no significant difference in metal concentration profiles among sites ($F=0.076$, $R^2=0.0125$, $p=0.3125$). The low R^2 value showed that site-wise variation explains only 1.25% of the total variance, indicating that variation in metal levels was likely due to within-site variability or other factors.

The MPI values showed distinct site- and sample-specific variations. At site A, sediment recorded the highest MPI (5.25), followed by water (2.84), crab (2.72), prawn (2.55), and aquatic insects (1.33), indicating moderate contamination, especially in sediment. In Site B crab (6.98) and shrimp (5.3) exhibited the highest MPI values, while sediment (2.02) and water (0.63) had lower MPI. In site C, prawns had an exceptionally high MPI (15.4), far exceeding all other sample types, followed by crab (5.45). Sediment, water, and clam had relatively low MPI values (Figure 4).

DISCUSSION

Heavy metal concentrations across samples from the three sites revealed distinct spatial patterns, although measured values remained within international safety limits.[16,17]

Water quality

Surface waters metals were consistently below WHO guidelines, indicating no acute contamination risk. However, site-specific variation was evident: the upstream site (Site A) exhibited relatively elevated levels of Ni, Cd, and Pb, suggesting localised anthropogenic inputs, possibly from agricultural activities. In contrast, the midstream site (Site B) exhibited the lowest concentrations across most metals, marking it as the least impacted zone. Downstream (Site C), Zn and As were comparatively higher, leading to pollutant accumulation or secondary inputs along the river course. Similar downstream enrichment trends have been reported in Asian river systems subjected to mixed land-use pressures.[24]

Sediment Quality

Sediment analysis showed metal concentrations well below ISQG thresholds, indicating no immediate ecological risk, though site-specific contamination was evident. Site A sediments contained higher Zn, Cd, and Pb, aligning with the elevated metal signals observed in upstream water samples. Site B was distinguished by higher Cu, while Site C sediments carried greater Ni and As loads. Such site-specific heterogeneity mirrors patterns documented in earlier global assessments[25] and more recently in Chinese river systems, where hydrological and geochemical controls shaped sedimentary metal accumulation.[26]

Bioaccumulation in invertebrates

Crustacean and molluscan tissues revealed stronger bioaccumulation compared to water or sediment samples. Crabs

Table 1: Various global standards of heavy metals (ppb/ppm) in water, sediment, crabs, shrimps, prawns, and aquatic insects.

Heavy Metal	Water (ppb)[14]	sediment (ppb)[15]	Crabs (ppb)	Human consumption of shrimps, prawns (ppb)[16,17]	Bivalves and aquatic insects (ppm)[16,17]
Ni	70	16000	70	20-70	70
Cu	2000	35700	120	10000	30
Zn	3000	123000	1000	50000	100
As	10	5900	10	1000	5
Cd	3	676	2	100-200	2
Pb	10	35000	100	300	1.5

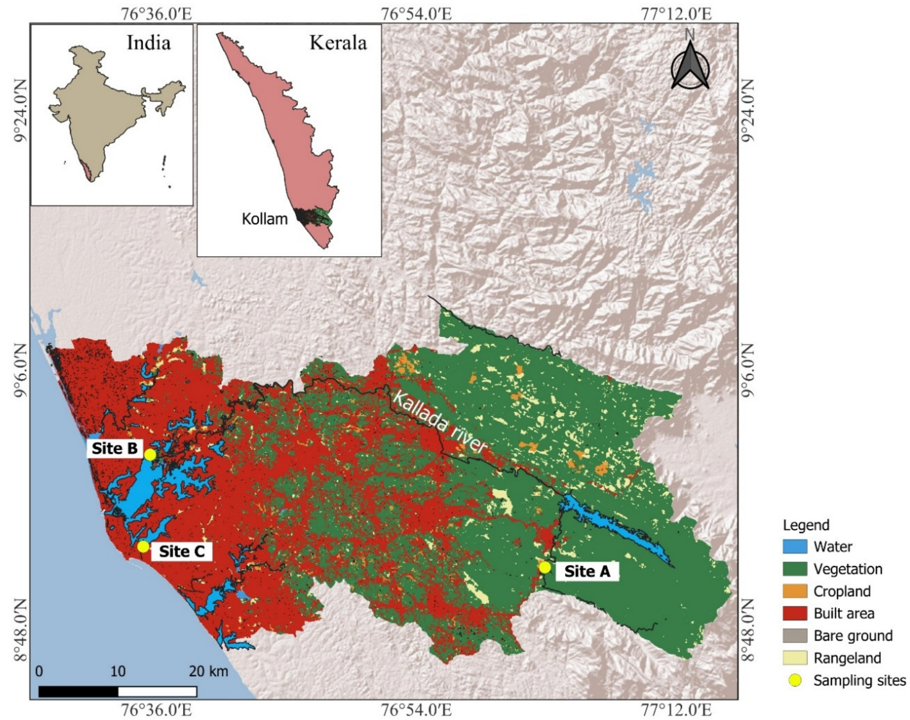

Figure1: Map showing the sampling sites along Kallada river in Kollam district.

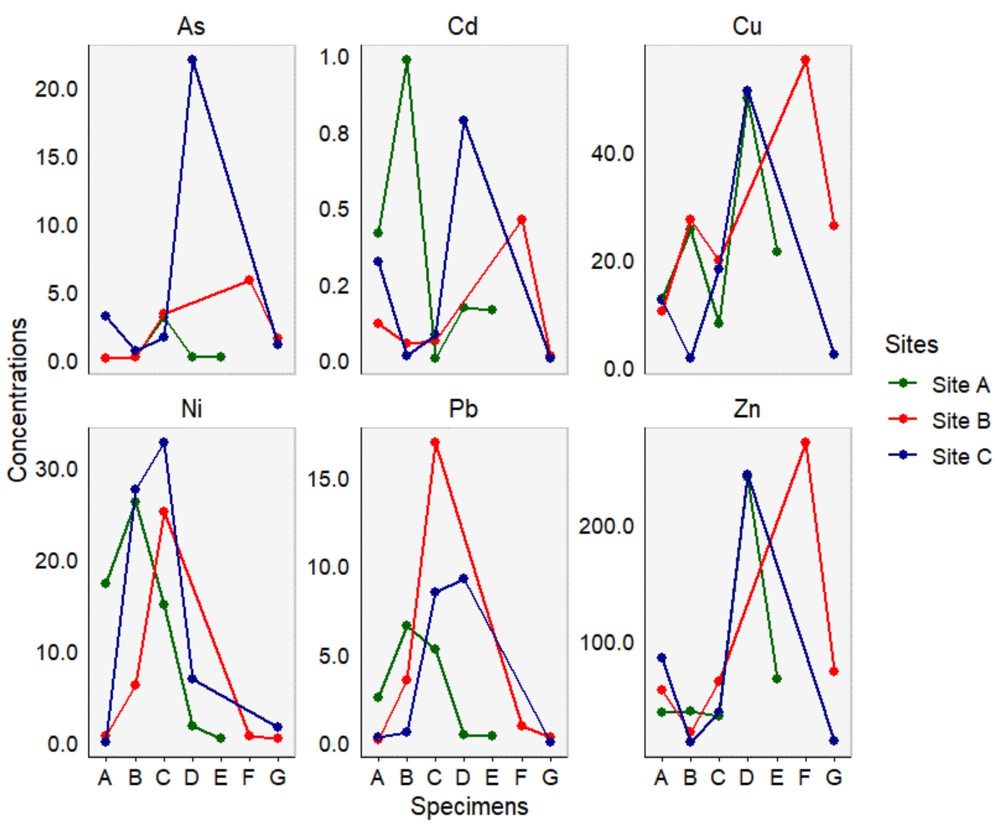

Figure 2: A- Concentrations of the heavy elements across various samples in the study sites Water; B- Sediment; C- Crab; D- Prawn; E- Aquatic insect; F- Shrimp; G- Clam, As-Arsenic, Cd-Cadmium, Cu-Copper, Ni-Nickel, Pb-Lead, Zn-Zinc.

showed elevated Ni and Pb at the upstream and midstream sites, respectively, suggesting their benthic feeding habits promoted the transfer of sediment-bound metals. Shrimps and prawns accumulated higher Zn and Cu, particularly at midstream and downstream sites, where prawns also reflected elevated as content. Bivalves and aquatic insects, while generally low in contamination, displayed site-specific differences-highest Zn and Cu at midstream, while higher Ni in downstream. These patterns confirm species-specific accumulation linked to habitat and feeding behaviour, consistent with findings from Indian coastal systems.[27] These outcomes align with international studies demonstrating that benthic invertebrates integrate contamination signals more effectively than water or sediment alone.[28]

Patterns of bioaccumulation and ecological implications

Multivariate analyses underlined the site- and species-specific associations. Benthic crustaceans with Zn, Cu, and Ni, along with sediment-bound Pb and Cd and insect samples clustered centrally due to uniformly low concentrations in PCA, indicating water as a short-term carrier, while sediments and benthic organisms act as longer-term reservoirs.[29] MPI values highlighted the sample specific bioaccumulation potential, with Site A sediments, Site B crabs and shrimps, and Site C prawns which displayed disproportionately high MPI, underscoring species specific bioaccumulation patterns. Comparable conclusions were drawn in ecological risk assessments of Asian freshwater prawns.[30]

Although no concentration exceeded international thresholds for aquatic life or human consumption reflecting resilience of the Kallada River system, the patterns suggest emerging risks since localized pressures could shift this balance. Crustaceans and bivalves proved to be effective bioindicators of metal transfer, reinforcing their use in ecological assessments and food safety monitoring.

Table 2: Principal Components, the eigenvalues and percentage of variance explained by each dimension.

Dimension	Eigenvalue	Variance Explained (%)
1	2.612	43.54
2	1.898	31.63
3	0.78	13.00
4	0.533	8.89
5	0.137	2.29
6	0.039	0.66

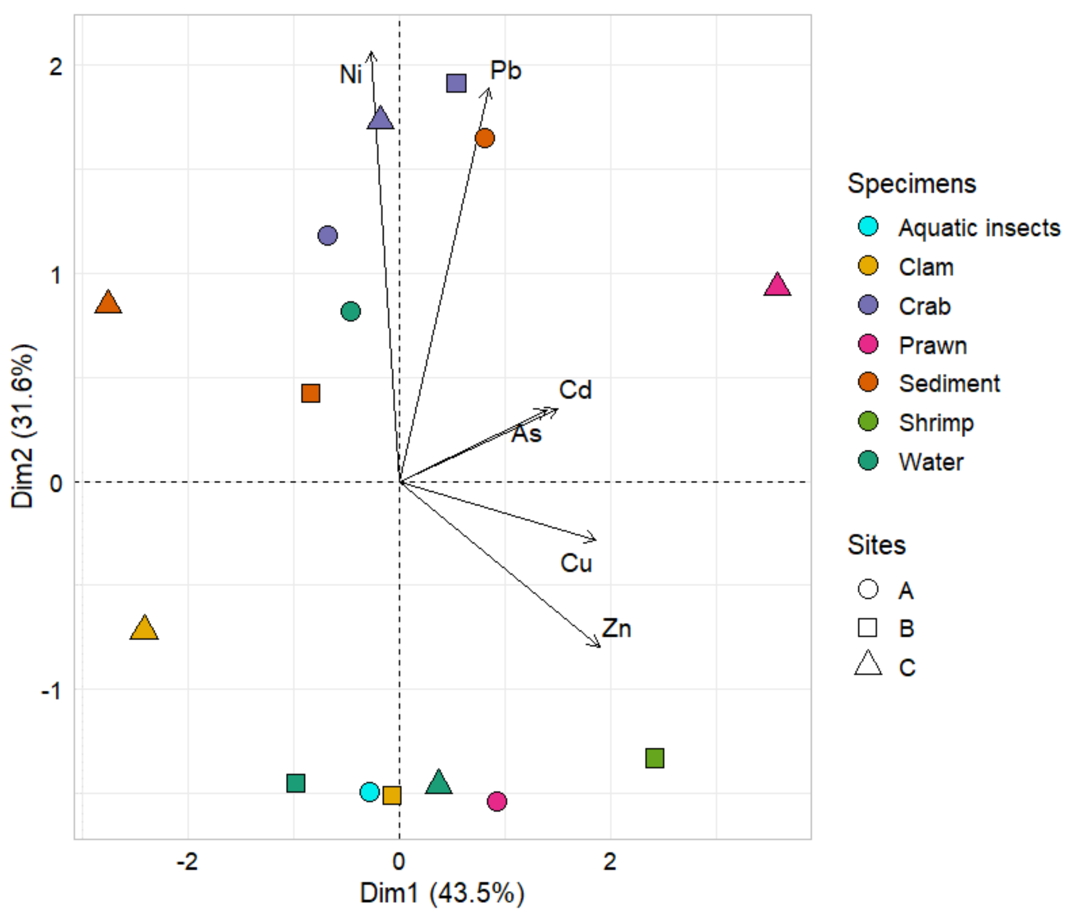

Figure 3: PCA analysis of the association of different elements with samples across study sites.

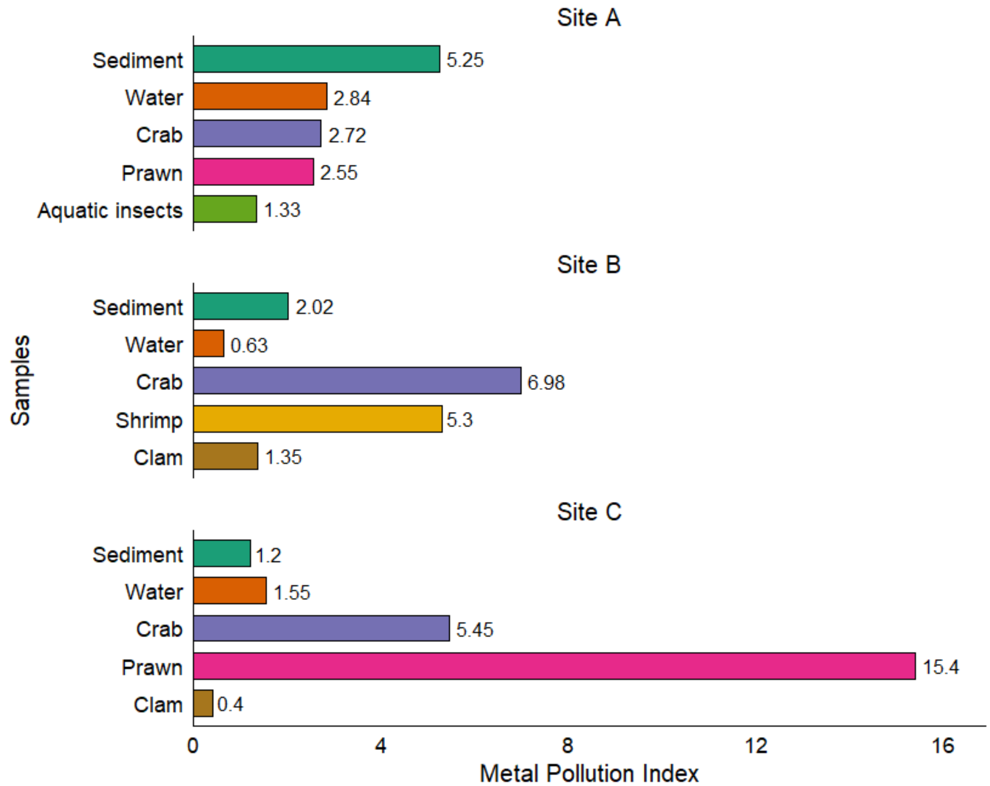

Figure 4: MPI values of various samples analysed at the three study sites.

CONCLUSION

While the Kallada River remains within safe ecological and toxicological limits, distinct spatial and organism-specific bioaccumulation patterns of heavy metals exist. Such biomonitoring approaches are critical for informing river management and safeguarding freshwater biodiversity under intensifying anthropogenic pressures.

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ABBREVIATIONS

ICP-MS: Inductively Coupled Mass Spectrometry; **PCA:** Principal Component Analysis; **MPI:** Metal Pollution Index; **PERMANOVA:** Permutational Multivariate Analysis of Variance; **WHO:** World Health Organization; **FAO:** Food and Agriculture Organization.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Tarunpal CS- experimental studies, data acquisition; Reshmi V- manuscript editing and manuscript review; Byju H- manuscript preparation, manuscript editing and manuscript review; Maitreyi H- data analysis, statistical analysis, manuscript editing.

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