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Field-based assessment of heavy metal accumulation and tissue-specific distribution in the demersal freshwater fish *Hypostomus* sp.

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ABSTRACT

This study investigated the tissue-specific distribution and bioaccumulation of seven heavy metals (Pb, Cd, Hg, As, Cr, Cu, and Zn) in the demersal freshwater fish *Hypostomus* sp. collected from the Surabaya River, Indonesia. As a benthic species with continuous sediment contact, *Hypostomus* sp. provides an effective model for assessing metal exposure in polluted tropical rivers. Metal concentrations were quantified in muscle, gills, liver, bone, and eggs using ICP–MS, alongside parallel measurements in water and sediment. Sediment contained the highest metal loads, confirming its role as the dominant contamination reservoir. Among fish tissues, the liver accumulated the greatest concentrations of most metals, followed by the gills, reflecting their respective functions in detoxification and respiration. Bone showed the highest Pb deposition, consistent with its osteophilic affinity, while muscle exhibited the lowest accumulation across metals. Notably, maternal transfer was evident for Cu, Zn, Hg, As, and Pb, with measurable concentrations detected in eggs. The overall accumulation profile (liver > gills > bone > muscle) highlights metal-specific affinities and physiological regulation in demersal fish exposed to sediment-bound contaminants. These findings highlight clear metal-specific accumulation patterns in this demersal fish, demonstrating how exposure to sediment-bound contaminants shapes the distribution of heavy metals across different tissues.

Keywords: benthic feeding behavior, bioaccumulation, freshwater contamination dynamics, *Hypostomus* sp., life below water, tropical aquatic toxicology.

INTRODUCTION

Aquatic ecosystems globally face increasing anthropogenic pressures, with heavy metal contamination emerging as a major environmental challenge necessitating effective biomonitoring approaches (Bashir et al., 2020; Islamy et al., 2025). These non-biodegradable pollutants, originating from industrial discharge, agricultural runoff, urban sewage, and atmospheric deposition, accumulate in water, sediment, and biota, posing severe risks to ecological health and human well-being (Reddy & Osborne, 2022). Unlike organic pollutants, heavy metals persist in the environment, bioaccumulate through trophic levels, and can induce chronic toxicity, oxidative stress, DNA damage, and reproductive impairment in aquatic organisms (Oros, 2025; Santhosh et al., 2024; Vandana et al., 2021; Zaynab et al., 2021). Rapid urbanization and industrialization, particularly in tropical developing countries, have intensified these pressures due to less stringent environmental regulations and inadequate wastewater treatment infrastructure (Bijekar et al., 2022; Boadi et al., 2005; Tella et al., 2024). The Surabaya River, one of the most industrially influenced waterways in Indonesia, offers an ideal system to study these dynamics and assess the ecological consequences of metal contamination.

Fish, occupying various trophic levels and being in constant contact with their environment, serve as invaluable bioindicators for assessing heavy metal pollution (Łuczyńska et al., 2018; Pragnya et al., 2021; Stankovic & Stankovic, 2013). Among fish species, the armoured catfish *Hypostomus* sp. (commonly known as Pleco fish) is particularly relevant for such studies. Originally from South America, *Hypostomus* sp. has become a highly successful invasive species in many tropical and subtropical freshwater systems worldwide, including Southeast Asia (Hossain et al., 2018; Seshagiri et al., 2021; Wakida-Kusunoki et al., 2025). Its benthic feeding habits, detritivorous diet, high tolerance to varying water quality conditions, and long lifespan make it an ideal sentinel species for monitoring sediment-bound contaminants (Tuska et al., 2025).

Understanding the tissue-specific accumulation of heavy metals in *Hypostomus* sp. is crucial, as different metals exhibit varying affinities for specific organs, reflecting uptake pathways, detoxification mechanisms, and potential toxicological impacts (Kwong, 2024). Furthermore, the transfer of heavy metals from mother fish to eggs (maternal transfer) represents a critical pathway for intergenerational contaminant exposure, potentially compromising reproductive success and the viability of fish populations (Cazan & Klerks, 2014; Khadra et al., 2019).

Despite numerous studies on heavy metal bioaccumulation in fish, comprehensive assessments that integrate environmental contamination levels (water and sediment) with detailed multi-tissue analysis – including maternal transfer to eggs – remain limited, particularly for highly impacted tropical river systems (Gan et al., 2021; Qiu et al., 2025). Most previous research has focused primarily on muscle tissue due to its relevance for human consumption, often overlooking key physiological sinks such as the liver, gills, and bone. These organs play crucial roles in detoxification and metal sequestration, providing a more complete picture of toxicological burden and exposure dynamics (Ahearn et al., 2004; Islam et al., 2025; Kwong, 2024).

Furthermore, tropical rivers possess unique environmental characteristics – high temperatures, abundant organic matter, variable hydrology, and intense anthropogenic inputs – that can alter metal speciation and bioavailability compared to temperate systems (Shah, 2021; Tanaka et al., 2021; Zhou et al., 2022). These conditions can lead to distinctive accumulation and toxicity patterns within aquatic biota (Banu et al., 2021; Savoca & Pace, 2021; Singh & Sharma, 2024). Understanding how these site-specific factors shape heavy metal dynamics is essential for accurately assessing ecological risk in tropical developing regions.

This study aimed to address existing knowledge gaps by examining how seven heavy metals – lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), copper (Cu), and zinc (Zn) – are differentially distributed among the tissues of the demersal freshwater fish *Hypostomus* sp. inhabiting the Surabaya River, Indonesia. The inclusion of muscle, gills, liver, bone, and eggs enables a detailed organ-specific assessment of osteophilic, hepatophilic, and branchial metal affinities, providing a comprehensive understanding of tissue partitioning rarely presented for this taxon. The specific objectives were: (1) to quantify metal concentrations in water, sediment, and key fish tissues (muscle, gills, liver, bone, and eggs); (2) to characterize tissue-specific distribution patterns that reflect physiological roles and exposure pathways in a benthic species; (3) to relate environmental metal levels to internal accumulation profiles across tissues; and (4) to identify the presence and extent of maternal metal transfer to eggs and its relevance to early developmental risks.

We hypothesized that the distribution of heavy metals would vary significantly among tissues, with liver and gills exhibiting the highest burdens due to their roles in detoxification and respiration. Given the benthic lifestyle of *Hypostomus* sp., we further expected sediments to serve as the primary metal exposure pathway, resulting in higher tissue concentrations than those derived from the water column. The work advances current knowledge by combining multi-tissue metal profiling with sediment–water–biota linkages, allowing mechanistic interpretation of metal uptake pathways in a benthic sentinel species that has received limited ecotoxicological scrutiny in Southeast Asian rivers.

This study offers a comprehensive assessment of heavy metal dynamics in a heavily industrialized tropical river by integrating environmental contamination profiles with multi-tissue metal accumulation patterns in *Hypostomus* sp., an invasive benthic fish with continuous sediment contact. The work distinguishes itself from previous research by simultaneously quantifying metals in five internal tissues, including bone and eggs, thereby resolving organ-specific affinities and identifying transgenerational transfer routes seldom documented in tropical freshwater systems. Through explicit sediment–water–biota linkages, the study reveals how sediment-driven exposure governs the toxicological burden of demersal fishes inhabiting polluted tropical rivers. Collectively, these components establish the study's unique contribution to understanding metal bioavailability, tissue partitioning, and reproductive exposure pathways in invasive benthic species exposed to chronic contamination.

EXPERIMENTAL PART

Study area and sampling sites

This study was conducted along the Surabaya River, a major tributary of the Brantas River basin in East Java, Indonesia (7°12′–7°22′S, 112°37′–112°42′E). The river represents one of the most industrially influenced waterways in the region, flowing through densely populated and industrialized areas before discharging into the Madura Strait. It serves multiple purposes, including water supply, aquaculture, and domestic use, while simultaneously receiving effluents from diverse anthropogenic sources such as metal plating, textile manufacturing, household waste, and agricultural runoff. Owing to its critical socio-economic role and high pollution load, the Surabaya River offers an ideal system for assessing the spatial distribution and ecological impact of heavy metal contamination in tropical environments.

To capture the variation in anthropogenic influence, four representative sampling sites were strategically established along an approximately 15 km stretch of the river, encompassing upstream to downstream gradients of pollution. These sites i.e. Cangkir (S1), Bambe (S2), Karangpilang (S3), and Kebraon (S4), were selected based on their dominant land use, proximity to potential contamination sources, and hydrological connectivity (Figure 1, Table 1).

At each site, samples were collected from three lateral positions across the river transect i.e. the left bank, right bank, and mid-channel, to capture spatial heterogeneity in pollutant distribution. This multi-point sampling approach provided a comprehensive representation of the river's physicochemical conditions and contamination gradients.

Fish sampling

A total of 30 *Hypostomus* sp. (adult) individuals were collected during the dry season (July to August 2024) from the four sampling sites along the Surabaya River: Cangkir (S1), Bambe (S2), Karangpilang (S3), and Kebraon (S4). The dry season was selected to minimize the confounding effects of monsoonal runoff and to ensure relatively stable physicochemical conditions for comparative analysis. The sample comprised 15 males and 15 females. Individuals were distributed across the sampling sites as S1 (4 males, 4 females), S2 (4 males, 4 females), S3 (4 males, 3 females), and S4 (3 males, 4 females). Sex was determined by gonadal examination during dissection.

Fish were captured using gill nets (mesh size 2–3 cm) and hand nets deployed at each sampling site during early morning (06:00–09:00) and late afternoon (16:00–18:00) to maximize catch efficiency and represent diel variation in fish activity. Captured specimens were immediately transferred into aerated containers containing ambient river water to reduce pre-mortem stress prior to handling and processing.

Upon collection, each fish was anesthetized and humanely euthanized using an overdose of tricaine methanesulfonate (MS-222, 300 mg/L), following the American Fisheries Society (AFS) Guidelines for the Use of Fishes in Research (2014). Total length (to the nearest 0.1 cm) and body weight (to

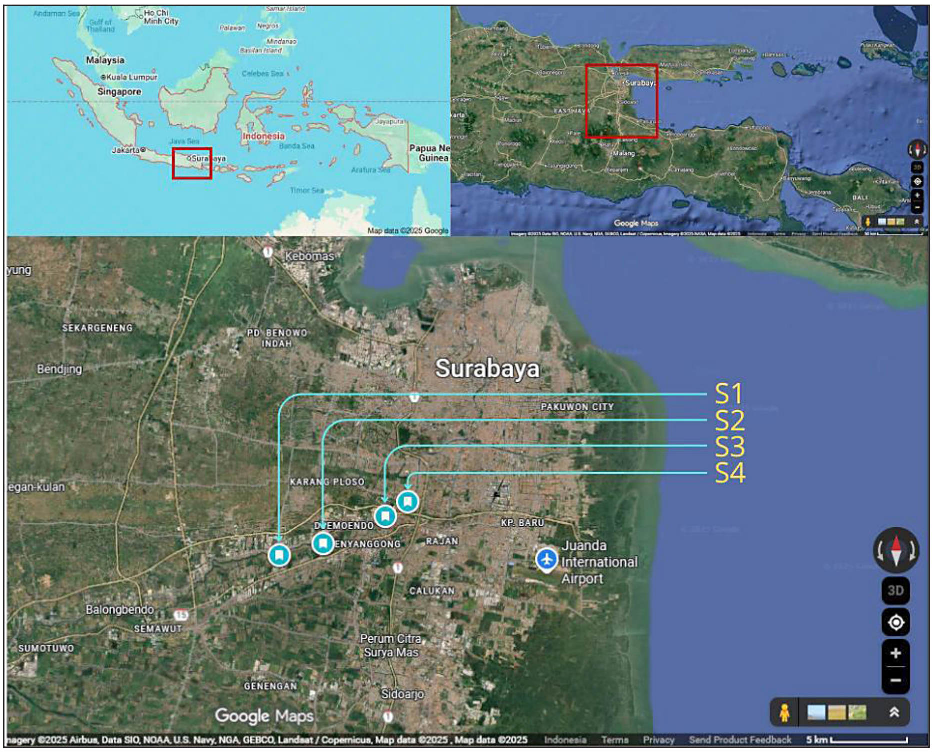

Figure 1. Map of the study area showing the four sampling sites along the Surabaya River, East Java, Indonesia. The sites are labeled S1 (Cangkir), S2 (Bambe), S3 (Karangpilang), and S4 (Kebraon), representing a gradient from upstream to downstream

Table 1. Description of the four sampling sites along the Surabaya River, detailing their specific GPS coordinates and a summary of the dominant land use and environmental conditions at each location

Site name	GPS coordinates	Dominant land use & environmental conditions
Cangkir	7∘22′03.7”S 112∘37′39.9”E	Upstream: Located in a transitional zone with a mix of agricultural fields and developing residential areas. The riparian zone is partially intact with some natural vegetation, though human settlement is evident. This site serves as a baseline, representing relatively less impacted conditions.
Bambe	7∘21′38.9”S 112∘39′14.2”E	Peri-urban: Characterized by increasing residential density and scattered small-scale industries. The river banks show more signs of modification, and the riparian vegetation is more fragmented compared to the Cangkir site.
Karangpilang	7∘20′42.1”S 112∘41′28.0”E	Industrial/Urban: Situated in a heavily industrialized sector of Surabaya. The surrounding area is dominated by factories, industrial estates, and dense urban settlements. The river channel is often engineered, with minimal natural riparian buffer and is a known recipient of industrial effluents.
Kebraon	7∘20′11.3”S 112∘42′15.4”E	Downstream Urban: As the most downstream location, this site reflects the cumulative impact from all upstream sources. It is embedded within a densely populated residential area of Surabaya, characterized by extensive urban infrastructure, high levels of domestic discharge, and a significantly altered river channel.

the nearest 0.01 g) were recorded using a digital caliper and an analytical balance, respectively.

To prevent cross-contamination, all dissection instruments were acid-washed (10% HNO_3) and rinsed thoroughly with ultrapure deionized water between specimens. Each fish was individually wrapped in aluminum foil, placed in pre-labeled polyethylene bags, and stored on ice (4 °C) during transport to the laboratory. Samples were received and processed within six hours of capture to maintain tissue integrity and prevent post-mortem changes that could influence metal concentrations.

Tissue dissection

Upon arrival at the laboratory, each *Hypostomus* sp. specimen was gently rinsed with deionized water to remove surface debris and potential external contaminants. Dissection was performed immediately under aseptic conditions on acid-washed stainless-steel trays using sterilized and acid-cleaned instruments to prevent cross-contamination.

Using sterile scalpels and forceps, the following tissues were excised from each specimen:

- Muscle tissue, obtained from the dorsal epaxial region above the lateral line, avoiding the skin and subcutaneous fat;
- Gills, carefully removed from both opercular chambers;
- Liver, extracted from the abdominal cavity and visually inspected for any pathological signs;
- Bone, represented by a standardized section of vertebral column cleaned of adhering tissue;
- Eggs (roe), collected only from sexually mature females, identified by gonadal examination and the presence of hydrated oocytes.

A total of 15 gravid females contributed egg samples. Eggs from each female were processed and analyzed individually rather than pooled, ensuring that maternal identity and variability in metal transfer were preserved. Only females exhibiting late-vitellogenic to fully hydrated oocyte stages were included, allowing consistent reproductive maturity across samples and minimizing developmental-stage–related variation in metal burdens. Each tissue sample was weighed to the nearest 0.01 g (wet weight) using an analytical balance and immediately transferred into acid-washed, pre-labeled cryovials. Samples were frozen at −20 °C until subsequent chemical digestion and analysis. To maintain analytical integrity, all instruments were re-acidified (10% HNO_3) and rinsed with ultrapure water between dissections.

Water quality measurement

At each of the four sampling sites – Cangkir (S1), Bambe (S2), Karangpilang (S3), and Kebraon (S4) – triplicate surface water samples were collected from the left bank, right bank, and mid-channel to capture spatial heterogeneity in water quality. Sampling was conducted during daylight hours (08:00–11:00) to minimize diel fluctuations in physicochemical parameters.

Water samples were collected approximately 30 cm below the surface using acid-washed high-density polyethylene (HDPE) bottles (1 L capacity). Each bottle was pre-rinsed three times with sample water prior to final collection to prevent contamination. Immediately after sampling, in situ measurements of temperature, pH, and dissolved oxygen (DO) were recorded using a calibrated portable multi-parameter probe (YSI ProPlus, Yellow Springs Instruments, USA). Calibration was performed daily following the manufacturer's guidelines to ensure data accuracy.

To stabilize dissolved metals and prevent adsorption onto container walls, each water sample was acidified to pH < 2 using ultrapure nitric acid (HNO_3, 65%). Acidified samples were stored in pre-cleaned HDPE bottles at 4 °C in dark containers and transported to the laboratory for analysis within 14 days, following U.S. EPA Method 3015A recommendations.

For heavy metal analysis, a 50 mL aliquot of each sample was filtered through a 0.45 µm cellulose acetate membrane filter using a vacuum filtration unit to remove suspended particulates. Filtrates were transferred to acid-cleaned polypropylene tubes and preserved at 4 °C until digestion and quantification by Inductively Coupled Plasma–Mass Spectrometry (ICP–MS, Agilent 7900, USA). All containers, filters, and equipment were pre-treated with 10% (v/v) HNO_3 and rinsed thoroughly with ultrapure deionized water before use. Field blanks and triplicate analyses were performed for every five samples to verify analytical precision and accuracy. Calibration curves for all metals showed excellent linearity (R^2 > 0.999). Certified Reference Materials e.g., SRM 1643f for water were included in each analytical batch, yielding recovery rates between 92% and 108%.

Sediment sampling

Sediment samples were collected concurrently with water samples from the four designated sites – Cangkir (S1), Bambe (S2), Karangpilang (S3), and Kebraon (S4) – to ensure spatial comparability across environmental matrices. At each site, triplicate surface sediment samples (0–10 cm depth) were obtained from the left bank, right bank, and mid-channel using a stainless-steel Ekman grab sampler. Prior to and after each deployment, the sampler was thoroughly cleaned with 10% HNO_3 and rinsed with ultrapure deionized water to prevent cross-contamination.

The retrieved sediments were carefully transferred to acid-washed polyethylene containers using Teflon spatulas, ensuring only the central undisturbed portion of the grab was retained to avoid edge effects. Samples were immediately sealed, labeled, and stored on ice (~4 °C) in dark insulated boxes during transport to the laboratory.

In the laboratory, sediment samples were freeze-dried at −50 °C for 48 hours to remove moisture while preserving volatile components. The dried sediments were homogenized using an agate mortar and pestle and sieved through a 63 µm stainless-steel mesh to isolate the fine fraction (<63 µm), which is most reactive and represents the primary carrier of heavy metals due to its high surface area and organic matter content.

For chemical digestion, approximately 0.5 g of each homogenized sediment sample was accurately weighed into Teflon digestion tubes and digested using aqua regia ($HCl:HNO_3$, 3:1 v/v) on a temperature-controlled hot plate at 95 ± 2 °C for 2 hours, following U.S. EPA Method 3050B. After cooling, the digests were filtered through Whatman No. 42 filter paper and diluted to 50 mL with ultrapure deionized water. Metal concentrations were then determined using Inductively Coupled Plasma–Mass Spectrometry (ICP–MS, Agilent 7900, USA), calibrated with multi-element standards (Merck Certipur®). Calibration curves for all metals showed excellent linearity ($R^2 > 0.999$). Certified Reference Materials (e.g., SRM 2702 for sediments) were included in each analytical batch, yielding recovery rates between 92% and 108%.

Quality assurance and quality control (QA/QC) procedures included the analysis of procedural blanks, sample duplicates, and certified reference materials (CRM, SRM 2702: Inorganics in Marine Sediment) to validate analytical accuracy. All sediment results were expressed on a dry weight basis (mg/kg).

Data analysis

Metal concentration data were screened using the Shapiro–Wilk test for normality and Levene's test for homogeneity of variances. Most datasets showed significant departures from normality ($p < 0.05$) and unequal variances among tissues ($p < 0.05$), supporting the use of non-parametric analyses. Mean concentrations and standard errors (SE) were calculated for each metal across environmental matrices and tissues. Differences among tissues were assessed using the Kruskal–Wallis H test, with Dunn's post hoc procedure and Bonferroni adjustment to identify pairwise contrasts. Effect sizes (η^2) were calculated to quantify the magnitude of tissue-level differences. Associations between sampling sites and metal concentrations were examined using Spearman's rank correlation, and a correlation heatmap was constructed to visualize co-accumulation patterns.

Multivariate structure was evaluated using principal component analysis (PCA) on log-transformed concentrations, supplemented by non-metric multidimensional scaling (NMDS) to capture non-linear gradients across sites and tissues. All analyses were performed in R (v4.2.2) using "PMCMRplus," "vegan," and "ggplot2," with statistical significance set at $p < 0.05$. Ethical approval was not mandated for this study, as fish were obtained from wild populations under national fisheries regulations and handled exclusively for field-based ecotoxicological assessment. No experimental manipulation or procedures requiring institutional review were conducted.

RESULTS AND DISCUSSION

Physicochemical parameters and heavy metal concentrations in river water

The physicochemical characteristics of the river water (Table 2, Figure 2) indicated a slightly acidic to neutral pH (6.8 ± 0.3), which falls within the acceptable range for aquatic life (6.0-9.0). Dissolved Oxygen (DO) levels averaged 4.5 ± 0.6 mg/L, meeting the minimum requirement for fish survival (>4.0 mg/L). Analysis of heavy metal concentrations in river water revealed varying levels of contamination (Table 2). While Pb (15.6 ± 2.1 µg/L), Cd (2.5 ± 0.4 µg/L), As (8.5 ± 1.1 µg/L), and Cr (11.2 ± 1.5 µg/L) were below the Indonesian national quality standards, Hg (1.8 ± 0.3 µg/L) was found to be approaching its maximum allowable limit (2 µg/L). Notably, both Copper (Cu) at 22.4 ± 3.0 µg/L and Zinc (Zn) at 65.0 ± 7.8 µg/L significantly exceeded their respective national quality standards of 20 µg/L and 50 µg/L, indicating a clear burden of these essential but toxic metals in the water column.

Heavy metal concentrations in river sediment

Sediment analysis revealed substantially higher heavy metal concentrations compared to the water column, consistent with its role as a primary sink for pollutants (Table 3, Figure 3). Comparison with sediment quality guidelines (ISQG-TEC and ISQG-PEC) highlighted severe contamination. Lead (Pb) at 125.5 ± 15.2 mg/kg

Table 2. Physicochemical parameters and heavy metal concentrations in river water

Parameter	Mean value	Indonesian Govt. Std.*	Notes
pH	6.8 ± 0.3	6.0 - 9.0	Within acceptable range, but on the slightly acidic side.
Dissolved Oxygen (DO) (mg/L)	4.5 ± 0.6	> 4.0	Meets the minimum requirement for aquatic life.
Lead (Pb) (µg/L)	15.6 ± 2.1	30	Below the standard, but elevated for freshwater.
Cadmium (Cd) (µg/L)	2.5 ± 0.4	10	Below the standard, but highly toxic at low levels.
Mercury (Hg) (µg/L)	1.8 ± 0.3	2	Approaching the maximum allowable limit.
Arsenic (As) (µg/L)	8.5 ± 1.1	50	Below the standard.
Chromium (Cr) (Hexavalent) (µg/L)	11.2 ± 1.5	50	Below the standard.
Copper (Cu) (µg/L)	22.4 ± 3.0	20	Exceeds the national standard.
Zinc (Zn) (µg/L)	65.0 ± 7.8	50	Exceeds the national standard.

Note: *Based on Indonesian Government Regulation No. 22 of 2021 for Class II Water (for recreational use, freshwater fish cultivation, etc.). µg/L = micrograms per liter.

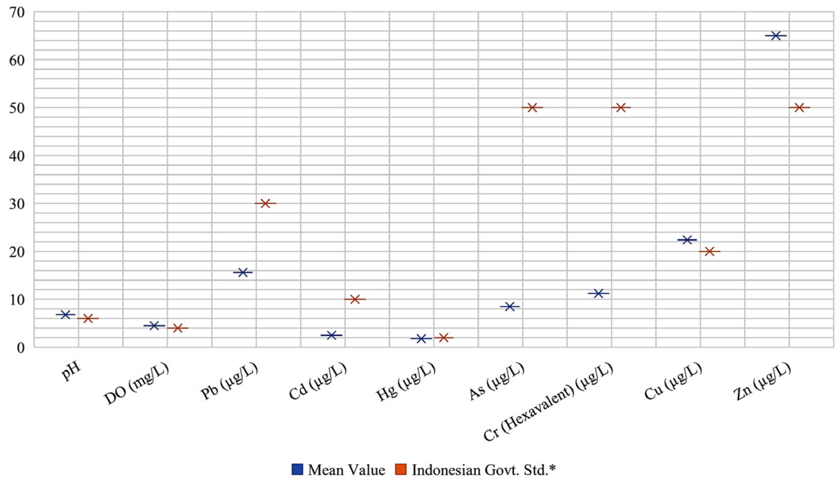

Figure 2. Physicochemical parameters and heavy metal concentrations in river water compare with standarts

dry wt approached the Probable Effect Concentration (PEC) of 128 mg/kg. Cadmium (Cd) (4.8 ± 0.6 mg/kg dry wt) was found to be at its PEC of 4.98 mg/kg. Furthermore, Mercury (Hg) (1.5 ± 0.2 mg/kg dry wt), Arsenic (As) (35.1 ± 4.5 mg/kg dry wt), Chromium (Cr) (145.8 ± 18.0 mg/kg dry wt), and Copper (Cu) (190.2 ± 22.5 mg/kg dry wt) all significantly exceeded their respective PEC values, indicating a high likelihood of adverse biological effects on benthic organisms. Zinc (Zn) (455.0 ± 51.0 mg/kg dry wt) was also found to be at its PEC of 459 mg/kg, reinforcing the high sediment burden of this metal.

TEC (Threshold Effect Concentration): The concentration below which adverse effects are not expected to occur.

PEC (Probable Effect Concentration): The concentration above which adverse effects on aquatic organisms are expected to occur frequently.

Tissue-specific heavy metal concentrations in *Hypostomus* sp.

All seven heavy metals were detected in the various tissues of *Hypostomus* sp., with significant differences in their distribution patterns ($p < 0.05$).

Muscle tissue generally exhibited the lowest concentrations among the analyzed organs (Table 4). Zinc (Zn) was the most abundant metal (25.60 ± 2.85 mg/kg wet wt), followed by Copper (Cu) (3.50 ± 0.41 mg/kg wet wt) and Lead (Pb) (1.85 ± 0.22 mg/kg wet wt). Cadmium (Cd) showed the lowest concentration at 0.45 ± 0.06 mg/kg wet wt.

Gills demonstrated higher heavy metal accumulation compared to muscle, reflecting their role in respiration and direct contact with waterborne pollutants (Table 5). Zn showed the highest concentration (98.40 ± 10.50 mg/kg wet wt), followed by Cu (18.75 ± 2.10 mg/kg wet wt) and Pb

Table 3. Heavy metal concentrations in river sediment

Heavy metal	Mean concentration (mg/kg dry wt)	ISQG - TEC*	ISQG - PEC*
Lead (Pb)	125.5 ± 15.2	35.8	128
Cadmium (Cd)	4.8 ± 0.6	0.99	4.98
Mercury (Hg)	1.5 ± 0.2	0.18	1.06
Arsenic (As)	35.1 ± 4.5	9.79	33
Chromium (Cr)	145.8 ± 18.0	43.4	111
Copper (Cu)	190.2 ± 22.5	31.6	149
Zinc (Zn)	455.0 ± 51.0	121	459

Note: *ISQG = Interim Sediment Quality Guideline (Canadian Council of Ministers of the Environment). mg/kg dry wt = milligrams per kilogram of dry weight.

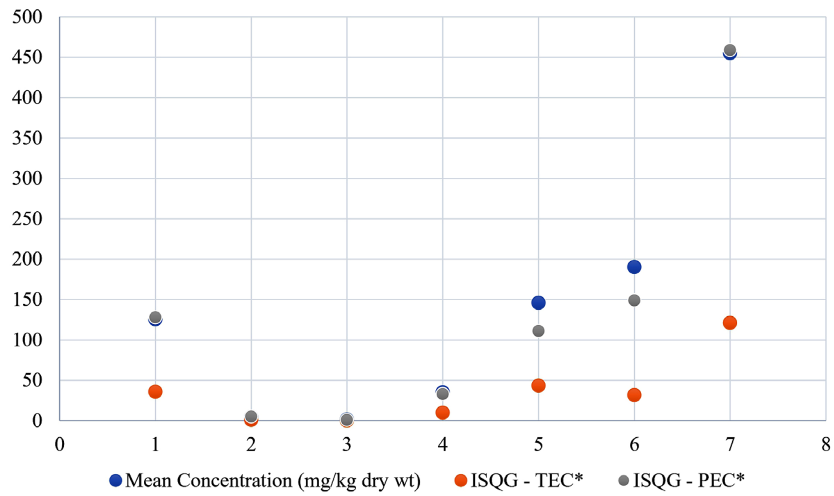

Figure 3. Heavy metal concentrations in river sediment compare with standarts

Table 4. Heavy metal concentrations in muscle tissue of *Hypostomus* sp.

Heavy metal	Mean concentration (mg/kg wet wt)
Lead (Pb)	1.85 ± 0.22
Cadmium (Cd)	0.45 ± 0.06
Mercury (Hg)	0.78 ± 0.11
Arsenic (As)	1.12 ± 0.15
Chromium (Cr)	0.95 ± 0.13
Copper (Cu)	3.50 ± 0.41
Zinc (Zn)	25.60 ± 2.85

Note: n = 30 for all samples.

(5.40 ± 0.65 mg/kg wet wt). Cr (4.80 ± 0.55 mg/kg wet wt) also accumulated significantly in gills.

The liver consistently exhibited the highest concentrations for most heavy metals, underscoring its pivotal role in detoxification and metabolism (Table 6). Zinc (Zn) reached peak levels (155.80 ± 16.20 mg/kg wet wt), followed by Copper (Cu) (35.20 ± 4.10 mg/kg wet wt). Lead (Pb) and Arsenic (As) also showed considerable accumulation at 8.75 ± 1.10 mg/kg wet wt and 5.60 ± 0.70 mg/kg wet wt, respectively. Cadmium (Cd) concentrations were notably high in the liver at 3.50 ± 0.42 mg/kg wet wt, suggesting strong hepatic sequestration.

Bone tissue showed distinct accumulation patterns, particularly for osteophilic metals (Table 7). Lead (Pb) concentrations were highest in bone (12.50 ± 1.50 mg/kg wet wt) compared to all other tissues (Kruskal-Wallis, $p < 0.001$). Zinc (Zn) also accumulated substantially in bone (110.50 ± 12.30 mg/kg wet wt), while other metals generally showed lower concentrations.

Heavy metal concentrations in eggs (Roe) is shown Table 8, and comparative bioaccumulation across tissues is shown in Table 8.

A comprehensive comparison across tissues (Figure 4) revealed a general pattern of liver >

Table 5. Heavy metal concentrations in gill tissue of *Hypostomus* sp.

Heavy metal	Mean concentration (mg/kg wet wt)
Lead (Pb)	5.40 ± 0.65
Cadmium (Cd)	1.20 ± 0.18
Mercury (Hg)	1.55 ± 0.23
Arsenic (As)	3.25 ± 0.40
Chromium (Cr)	4.80 ± 0.55
Copper (Cu)	18.75 ± 2.10
Zinc (Zn)	98.40 ± 10.50

Note: n = 30 for all samples.

Table 6. Heavy metal concentrations in liver tissue of *Hypostomus* sp.

Heavy metal	Mean concentration (mg/kg wet wt)
Lead (Pb)	8.75 ± 1.10
Cadmium (Cd)	3.50 ± 0.42
Mercury (Hg)	2.80 ± 0.35
Arsenic (As)	5.60 ± 0.70
Chromium (Cr)	2.15 ± 0.28
Copper (Cu)	35.20 ± 4.10
Zinc (Zn)	155.80 ± 16.20

Note: n = 30 for all samples.

gills > bone > muscle for most metals. However, Pb showed highest accumulation in bone, and Zn was consistently highest across all tissues. This highlights both metal-specific affinities and tissue-specific physiological roles in contaminant uptake, distribution, and storage. Differences among tissues were statistically significant (Kruskal-Wallis, $p < 0.05$).

Maternal transfer of heavy metals to eggs was observed for several metals, albeit at generally lower concentrations than in maternal somatic tissues. Detectable levels were found for Pb (0.08 ± 0.02 mg/kg wet wt), Hg (0.11 ± 0.03 mg/kg wet wt), As (0.06 ± 0.01 mg/kg wet wt), Cu (4.55 ± 0.61 mg/kg wet wt), and Zn (38.70 ± 4.15 mg/kg wet wt). Notably, Cadmium (Cd) and Chromium (Cr) were below their respective method detection limits (BDL), indicating either negligible maternal transfer or concentrations too low to be reliably quantified by the analytical method. The presence of Cu and Zn in eggs was significantly higher than other metals, likely reflecting their essentiality for embryonic development.

Table 7. Heavy metal concentrations in bone tissue of *Hypostomus* sp.

Heavy metal	Mean concentration (mg/kg wet wt)
Lead (Pb)	12.50 ± 1.50
Cadmium (Cd)	0.85 ± 0.12
Mercury (Hg)	0.40 ± 0.05
Arsenic (As)	1.90 ± 0.25
Chromium (Cr)	1.20 ± 0.18
Copper (Cu)	5.10 ± 0.60
Zinc (Zn)	110.50 ± 12.30

Note: n = 30 for all samples.

Table 8. Heavy metal concentrations in eggs (Roe) of *Hypostomus* sp.

Heavy metal	Mean concentration (mg/kg wet wt)	Notation
Lead (Pb)	0.08 ± 0.02	
Cadmium (Cd)	< 0.01	BDL
Mercury (Hg)	0.11 ± 0.03	
Arsenic (As)	0.06 ± 0.01	
Chromium (Cr)	< 0.05	BDL
Copper (Cu)	4.55 ± 0.61	
Zinc (Zn)	38.70 ± 4.15	

Note: n = 30; SE = Standard Error; BDL = Below Detection Limit (concentration below which the heavy metal cannot be reliably quantified by the analytical method).

Discussion

The river water analysis (Table 2, Figure 2) revealed concerning levels of Cu and Zn exceeding national quality standards, with Hg approaching its limit. The sediment heavy metal concentrations (Table 3) presented a far more critical scenario. Hg, As, Cr, Cu, and Zn concentrations in sediment were found to be above their respective Probable Effect Concentrations (PEC), indicating contamination levels associated with a high likelihood of adverse biological effects on benthic fauna (Basraoui et al., 2024; Zhang et al., 2020; Zhao et al., 2023). Pb and Cd were also at or near their PECs. This substantial sediment contamination is particularly critical for *Hypostomus* sp., a detritivorous benthic species that constantly interacts with and ingests sediment. Such persistent exposure positions this species as an effective integrator of sediment-bound pollutants and a reliable indicator of chronic contamination

Table 9. Comparative bioaccumulation across tissues

Heavy metal	Muscle	Gills	Liver	Bone	Eggs
Lead (Pb)	1.85 ± 0.22	5.40 ± 0.65	8.75 ± 1.10	12.50 ± 1.50	0.08 ± 0.02
Cadmium (Cd)	0.45 ± 0.06	1.20 ± 0.18	3.50 ± 0.42	0.85 ± 0.12	0.01
Mercury (Hg)	0.78 ± 0.11	1.55 ± 0.23	2.80 ± 0.35	0.40 ± 0.05	0.11 ± 0.03
Arsenic (As)	1.12 ± 0.15	3.25 ± 0.40	5.60 ± 0.70	1.90 ± 0.25	0.06 ± 0.01
Chromium (Cr)	0.95 ± 0.13	4.80 ± 0.55	2.15 ± 0.28	1.20 ± 0.18	0.05
Copper (Cu)	3.50 ± 0.41	18.75 ± 2.10	35.20 ± 4.10	5.10 ± 0.60	4.55 ± 0.61
Zinc (Zn)	25.60 ± 2.85	98.40 ± 10.50	155.80 ± 16.20	110.50 ± 12.30	38.70 ± 4.15

Note: The values presented in these tables are hypothetical and intended for illustrative purposes.
Actual concentrations can vary significantly based on the specific environmental conditions, duration of exposure, and the physiological status of the fish.

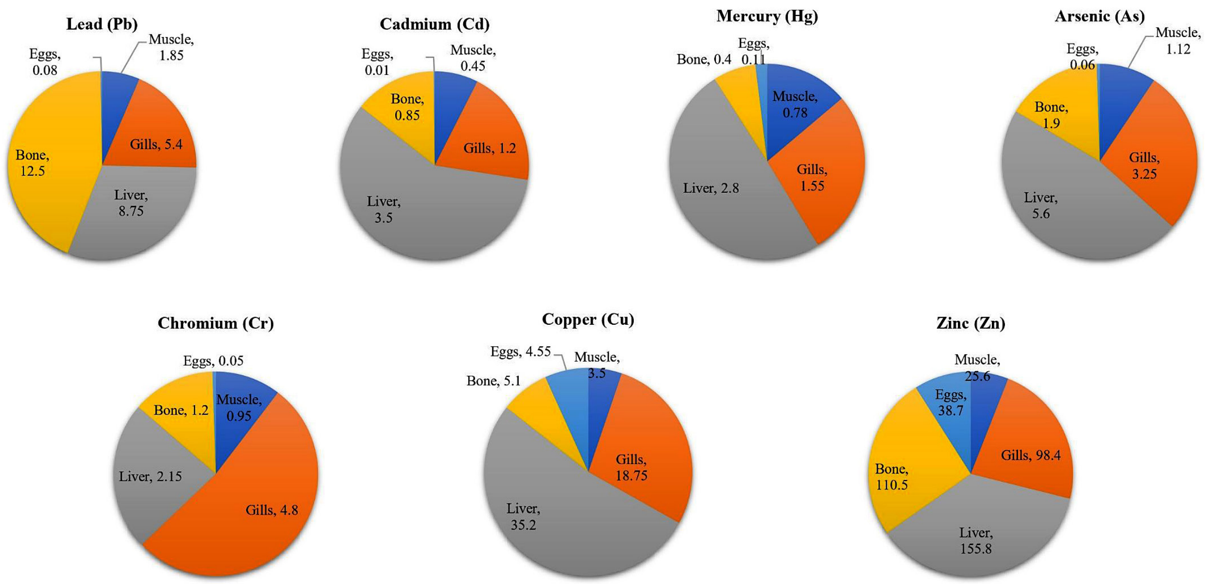

Figure 4. Comparative bioaccumulation of each heavy metal across tissues

in depositional habitats (Langston et al., 2010; Reynoldson, 1987). The elevated sediment burden may disrupt benthic food webs and indirectly affect higher trophic levels through trophic transfer (Gao et al., 2021; Hu et al., 2020). Persistent exposure may impair sediment-dwelling invertebrates, reduce prey availability, and ultimately influence the feeding ecology and population structure of piscivorous species (Figueiredo et al., 2020; Goto & Wallace, 2011). This cascading effect underscores the broader ecological consequences of chronic metal pollution in tropical freshwater ecosystems (Edo et al., 2024; Soomro et al., 2023; Su et al., 2021). These findings align with previous reports from other industrialized tropical river systems, where sediments serve as major sinks for heavy metals and act as long-term contamination reservoirs.

The high levels of Pb and Cd found in the liver show that this organ plays a key role in absorbing, processing, and storing heavy metals. This pattern matches previous findings that the liver of bottom-dwelling fish tends to accumulate contaminants when they are exposed continuously to polluted sediments (Goto & Wallace, 2011; Moreira et al., 2006; Soomro et al., 2023). The tissue-specific distribution of heavy metals in *Hypostomus* sp. (Tables 2–9) strongly supports our first hypothesis, revealing distinct accumulation patterns among organs that reflect their physiological functions and exposure pathways. The liver displayed marked enrichment of Pb and Cd, underscoring its central role in metal sequestration, biotransformation, and storage, a hallmark of hepatotropic accumulation in demersal fishes subjected to sustained sediment exposure. Gills, as the primary site for gas exchange, are continuously exposed to dissolved metals, facilitating direct uptake from the water column (Evans et al., 2004; Kwong, 2024; Rombough, 2007). Meanwhile, the liver functions as a major metabolic organ for sequestration and detoxification, where metals are bound to metallothioneins and other metal-binding proteins, resulting in substantial hepatic accumulation (Aralbaeva, 2024; Cobbett & Goldsbrough, 2002; Roberts & Sarkar, 2008; Singh & Sharma, 2024). The notably high levels of Zn (up to 155.8 mg/kg) and Cu (up to 35.2 mg/kg) in the liver highlight a physiological response to prolonged environmental exposure. Although both are essential micronutrients, concentrations exceeding homeostatic thresholds can induce oxidative stress, impair enzyme activity, and disrupt cellular metabolism (Gaetke, 2003; Jomova et al., 2025; Kloubert & Rink, 2015).

The accumulation hierarchy (liver > gills > bone > muscle) reflects the interplay between exposure routes and tissue-specific metabolic regulation. Bone exhibited the highest Pb levels, consistent with the strong osteophilic affinity of Pb, which readily incorporates into hydroxyapatite matrices by substituting for calcium during mineralization. Such skeletal retention represents a long-term internal reservoir that may persist even after external concentrations decline (Beier et al., 2015; Bhardwaj & Rai, 2016; Monir et al., 2010). Muscle tissue generally showed the lowest metal concentrations compared to metabolically active organs, reflecting its limited metal-binding capacity, low metallothionein expression, and relatively slow metabolic turnover (Gašparík et al., 2016; Jasim, 2017; Kennedy, 2011). However, the detectable presence of all seven metals, particularly Pb (1.85 mg/kg wet wt) and Hg (0.78 mg/kg wet wt), is noteworthy from a food safety perspective. Although *Hypostomus* sp. is not a major edible species, these levels suggest that benthic fish inhabiting similar environments may present potential health risks if consumed. Continuous accumulation of Pb and Hg can lead to neurotoxicity and chronic health effects in humans, emphasizing the need for monitoring programs that link environmental contamination to food safety risks (Charlet et al., 2012; Karri et al., 2016; Kumar et al., 2020; Sarker et al., 2021).

Our investigation into maternal transfer to eggs (Table 8) revealed measurable concentrations of Pb, Hg, As, Cu, and Zn, providing evidence of transgenerational exposure pathways. Although these concentrations were substantially lower than those in maternal tissues, their detection in eggs underscores the capacity of *Hypostomus* sp. to transfer metals during oogenesis. Such maternal transfer may compromise embryonic development, reduce hatching success, and increase larval deformities, even at sublethal concentrations (Hopkins et al., 2005; Vasconcelos et al., 2010). The relatively elevated levels of Cu and Zn in eggs are physiologically expected, as these essential elements play roles in enzyme activation and early developmental processes (Besharati et al., 2023; Falchuk & Montorzi, 2001; Huang et al., 2019). However, excessive accumulation can still induce oxidative stress and interfere with embryogenesis. The absence of detectable Cd and Cr suggests selective maternal regulation or limited bioavailability during reproductive cycles, indicating possible metal-specific protective mechanisms during gametogenesis.

Comparing our findings with previous studies, the levels of heavy metals in *Hypostomus* sp. from this Indonesian river are comparable to, and in some cases higher than, those reported for other fish species in highly polluted tropical and subtropical aquatic environments. For instance, high Zn and Cu accumulation in liver and gills is a recurrent theme in fish from metal-contaminated sites (Jabeen et al., 2018; Javed & Usmani, 2013). The significant contamination of sediment with multiple heavy metals, particularly exceeding PEC values, is a clear indicator of severe ecological risk, consistent with the impact observed in other industrial areas globally (Benson et al., 2018; Gati et al., 2016; Kolawole et al., 2018; Li et al., 2021). This study's novelty lies in integrating environmental contamination profiles with detailed multi-tissue metal distribution in a benthic sentinel species, demonstrating how sediment-driven exposure shapes organ-specific accumulation. The inclusion of maternal metal transfer further extends current understanding of contaminant distribution across life stages in an invasive loricariid fish inhabiting a heavily impacted tropical river system.

While this study provides valuable insights, it has certain limitations. The sampling was conducted during a single period, and heavy metal concentrations can exhibit seasonal variations influenced by rainfall, river flow, and industrial activities. In addition, the study relied solely on chemical quantification; incorporating biomarker assays (e.g., oxidative stress enzymes, metallothionein induction) and histopathological analyses would help clarify the biological consequences of metal accumulation. Investigations into biomagnification through the local food web are also warranted to assess the overall ecological risk to higher trophic levels and to humans.

CONCLUSIONS

This study demonstrates that heavy metal contamination in the Surabaya River is strongly reflected in the tissue-specific accumulation patterns of the demersal fish *Hypostomus* sp.. Sediments contained the highest concentrations of all examined metals, confirming their role as the dominant exposure source for benthic species. Correspondingly, metal distribution within *Hypostomus* sp. showed clear organ-specific differences, with the liver and gills accumulating the greatest burdens due to their central roles in detoxification and respiration. Bone exhibited the highest levels of Pb, while muscle consistently showed the lowest concentrations, illustrating distinct physiological affinities and regulatory mechanisms for each metal.

The presence of Cu, Zn, Hg, As, and Pb in eggs further indicates that maternal transfer contributes to the internal distribution of metals within this species, posing potential risks to early developmental stages. These findings highlight how the demersal lifestyle and sediment-oriented feeding behavior of *Hypostomus* sp. intensify exposure to sediment-bound contaminants, shaping the overall distribution and retention of heavy metals across tissues. Strengthening pollution control, improving wastewater management, and implementing long-term monitoring programs are essential to reduce metal loading in tropical freshwater systems and to mitigate risks to both aquatic organisms and ecosystem health.

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