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Biomonitoring of heavy metal pollution in the Brantas River using genotoxic and histopathological biomarkers in wild cyprinidae

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ABSTRACT

Heavy metal contamination in freshwater systems poses critical risks to aquatic organisms, particularly through bioaccumulation and sublethal cellular damage. This study evaluates the use of genotoxic and histopathological biomarkers – specifically micronucleus (MN) formation in erythrocytes and gill tissue alterations – in wild cyprinidae as indicators of heavy metal pollution along the Brantas River, Malang, Indonesia. Fish were sampled from three sites representing an upstream-to-downstream gradient: Batu (Site A), Dinoyo (Site B), and Kepanjen (Site C). Gill tissues from 180 individuals were analyzed for lead (Pb), cadmium (Cd), mercury (Hg), copper (Cu), and zinc (Zn) concentrations using atomic absorption spectrophotometry. Genotoxicity was assessed through MN assays on peripheral blood erythrocytes, while gill histopathological changes were evaluated and quantified using a gill histopathological index (GHI). Results demonstrated a clear spatial increase in metal accumulation, with Pb levels rising from 0.85 ± 0.22 mg/kg at Site A to 4.87 ± 0.61 mg/kg at Site C. Correspondingly, MN frequency increased from 1.34 ± 0.41‰ to 6.92 ± 0.88‰, and GHI scores rose from 3.2 ± 0.9 to 11.4 ± 2.2. The increases in micronucleus frequency ($p < 0.001$), gill histopathology scores ($p < 0.001$), and heavy metal concentrations ($p < 0.01$ to $p < 0.001$) across sites were statistically significant, confirming a strong correlation between pollution level and biological responses. Significant correlations were observed between heavy metal concentrations and both MN frequency and gill pathology severity. These findings validate the combined use of MN assay and gill histopathology as sensitive, complementary biomarkers for monitoring heavy metal pollution in riverine ecosystems. The study underscores the need for integrated biomonitoring strategies and strengthened pollution management in tropical freshwater systems.

Keywords: aquatic toxicology, bioindicator species, environmental stress, freshwater ecosystem, histological biomarker, metal contamination, micronucleus assay, riverine pollution.

INTRODUCTION

Freshwater ecosystems are increasingly subjected to contamination driven by anthropogenic activities, with heavy metals recognized as particularly hazardous due to their persistence, toxicity, and non-degradable nature. In contrast to organic pollutants, metals such as lead (Pb), cadmium (Cd), mercury (Hg), copper (Cu), and zinc (Zn) resist natural degradation processes and tend to bioaccumulate within aquatic organisms. This bioaccumulation contributes to chronic toxicity, posing serious threats not only to aquatic biodiversity but also to human communities dependent on freshwater resources (Afzaal et al., 2022; Suryanto Hertika et al., 2023). These contaminants originate from a range of diffuse and point sources, including industrial discharges, agricultural runoff, untreated urban wastewater, and atmospheric deposition, thereby facilitating their widespread presence in riverine systems.□□□

The Brantas River in Indonesia represents a critical freshwater resource that sustains diverse functions such as agriculture, aquaculture, domestic water supply, and inland fisheries. However, rapid urbanization coupled with inadequate waste management has severely compromised the river's water quality. Elevated concentrations of heavy metals have been detected in both sediments and aquatic biota, highlighting the urgent need for biologically relevant monitoring to assess long-term ecological risks (Lusiana et al., 2023). Among affected organs, fish gills – due to their constant contact with the aquatic environment – serve as sensitive targets for toxic metal exposure, often exhibiting sublethal damage such as epithelial lifting, lamellar fusion, hyperplasia, and necrosis (Addo-Bediako et al., 2021; Islamy et al., 2017).

Simultaneously, genotoxicity biomarkers such as the micronucleus (MN) assay provide robust and early-warning indicators of chromosomal instability and nuclear aberrations in fish erythrocytes. These biomarkers offer valuable insights into the cumulative genetic impacts of prolonged metal exposure in aquatic organisms (Sharma and Chadha, 2021). Wild cyprinidae, widely distributed in tropical freshwater ecosystems, are ecologically important benthic feeders and are particularly vulnerable to sediment-associated contaminants. Their ecological role and behavioral traits render them effective sentinel species for evaluating the bioavailability and biological effects of pollutants in freshwater systems (Astuti et al., 2024; Tiwari et al., 2024).

Despite the growing recognition of heavy metal threats to aquatic fauna, integrative biomonitoring studies employing a combination of metal bioaccumulation assessment, genotoxic evaluation, and histopathological analysis remain limited, especially within tropical river systems such as the Brantas. This study therefore aims to assess the extent of heavy metal bioaccumulation in the gill lamellae of wild cyprinidae and to examine the related genotoxic and histopathological responses along an upstream-to-downstream pollution gradient. The outcomes are expected to contribute essential baseline data for environmental risk evaluation and support policy development for more effective pollution control and public health safeguards.

MATERIALS AND METHODS

Study area and sampling sites

Fish samples were collected from three segments of the Brantas River in Malang, East Java, Indonesia. These sites were selected to represent a pollution gradient along the Brantas River, from relatively clean upstream areas (Site A) to increasingly impacted midstream (Site B) and downstream (Site C) regions, reflecting differences in land use, urbanization, and anthropogenic pressure (Table 1, Figure 1).

Fish collection and identification

A total of 180 wild cyprinidae (*Rasbora* sp.) specimens (n = 60 per site) were collected using gill nets and cast nets during the dry season (August–September 2024). Fish were transported alive to the laboratory in aerated containers and identified to the genus/species level using standard ichthyological taxonomic keys. Only healthy individuals were selected for analysis, defined as those with: (1) no visible external lesions, ulcers, or deformities; (2) normal swimming behavior and

Table 1. Sampling site coordinates

Site	GPS coordinate	Riparian description	Anthropogenic influence
A (Sidomulyo)	7°51'46.8"S, 112°31'28.4"E	Semi-natural vegetation with agricultural surroundings	Minimal; mostly agricultural runoff
B (Dinoyo)	7°51'46.8"S 112°31'28.4"E	Urban transition zone with reduced riparian vegetation	Moderate; domestic runoff and minor industrial activity
C (Kepanjen)	8°08'03.0"S 112°33'46.3"E	Highly urbanized with limited natural riparian cover	High; domestic sewage, solid waste, and possible industrial discharge

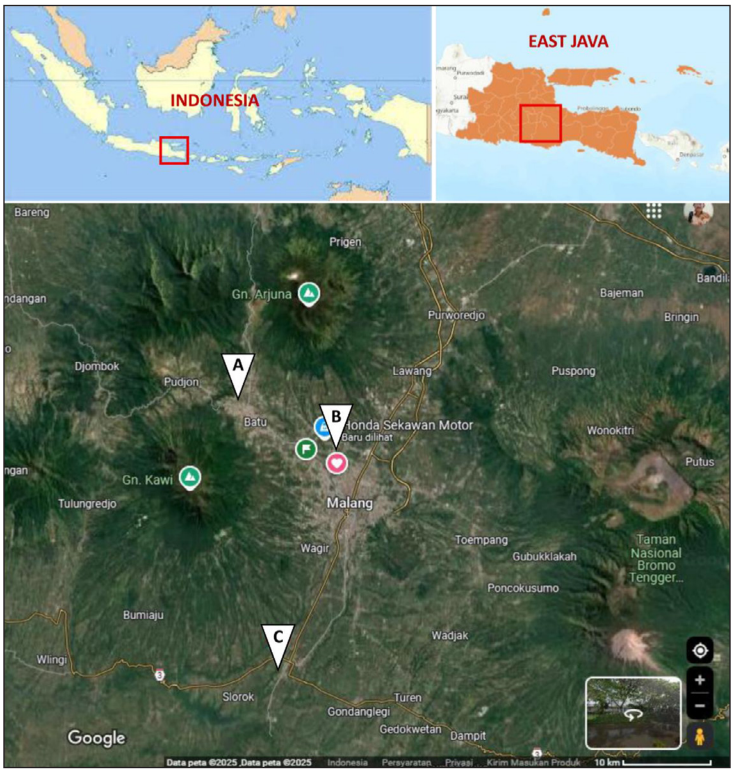

Figure 1. Map of sampling site

response to stimuli; (3) weight and length ranges within 10% of the population mean (e.g., 5.2 ± 0.5 g and 6.8 ± 0.7 cm) to minimize size-related physiological variability; and (4) absence of parasitic infestations upon macroscopic examination.

Heavy metal analysis (bioaccumulation assessment)

Gill lamellae were carefully dissected from each fish and rinsed with deionized water to remove surface contaminants. The tissues were then oven-dried at 60 °C for 48 hours, weighed to obtain dry weight, and homogenized using a ceramic mortar and pestle.

Approximately 0.5 grams of dried gill tissue was subjected to acid digestion using a mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 3:1 ratio. Samples were digested on a hotplate at 120 °C until the solution became clear, indicating complete digestion.

The digested solutions were cooled, filtered through Whatman No. 42 filter paper, and diluted to a fixed volume with deionized water. The concentrations of selected heavy metals (Pb, Cd, Hg, Cu, and Zn) were measured using atomic absorption spectrophotometry (AAS) (PerkinElmer AAnalyst 400), following standard procedures from the American Public Health Association (Islamy et al., 2017). Heavy metal concentrations were expressed as milligrams per kilogram of dry tissue weight (mg/kg dw). Quality control was ensured through the use of blank samples, certified reference materials (CRM), and triplicate measurements

Micronucleus assay in erythrocytes

Peripheral blood samples were obtained via caudal vein puncture using heparinized syringes (Kilawati and Islamy, 2019; Minhas et al., 2022). Thin blood smears were immediately prepared, air-dried, fixed in absolute methanol for 10 minutes, and stained with 10% Giemsa for 15 minutes. Micronuclei (MN) were identified under 1000× magnification (oil immersion) using the following criteria:

- Morphology – round or oval cytoplasmic bodies with smooth edges, distinct from irregularly shaped cellular debris or staining artifacts.
- Size – diameter ≤1/3 of the main nucleus, following standardized scoring protocols (Fenech et al., 2003).
- Staining intensity – similar chromatin texture and coloration to the main nucleus (i.e., Giemsa-positive), differentiating them from pale-staining cytoplasmic granules or residual bodies.

- Location – non-overlapping with the main nucleus and clearly within the cytoplasmic boundary.
- Absence of connections – no linkage to the main nucleus or other nuclear fragments (e.g., excluded blebbed or lobed nuclei).

For each fish, 2000 mature erythrocytes were examined under a light microscope at 1000× magnification (oil immersion). Micronuclei were identified as round or oval cytoplasmic bodies smaller than one-third the diameter of the main nucleus. Other nuclear abnormalities (binucleated, lobed, or notched nuclei) were also recorded according to standard criteria (Hernández-Cabanyero et al., 2023).

Histological analysis of gill lamellae

Eighteen fish per site were selected randomly from the total catch, ensuring similar size range (±10% total length variation) and excluding visibly injured or unhealthy individuals to maintain consistency in histopathological evaluation. Fish were anesthetized with clove oil (100 mg/L), and gill arches were excised. Fish were anesthetized with clove oil (100 mg/L), and gill arches were excised. The second gill filament from the left side was dissected for consistency. For each individual, one gill arch (specifically the second gill from the left side) was dissected and used for histological analysis to ensure consistency in tissue comparison across samples.

Histological analysis using published method (Islamy et al., 2024; Kilawati, Maimunah, et al., 2025). Tissues were fixed in 10% neutral-buffered formalin (NBF) for 48 hours, then processed through ethanol dehydration (70–100%), cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a rotary microtome, mounted on poly-L-lysine-coated slides, deparaffinized, and stained with Hematoxylin and Eosin (H&E). Slides were observed under a light microscope (Olympus CX23) at 100× and 400× magnification. Gill lesions were scored based on the gill histopathological index (GHI) system (Poleksic and Mitrovic-Tutundžić, 1994), evaluating:

- Epithelial lifting,
- Hyperplasia,
- Lamellar fusion,
- Chloride cell hypertrophy,
- Vascular congestion,
- Necrosis or degeneration.

Each lesion was scored semi-quantitatively using a modified GHI system (Table 2). Total histopathology scores were calculated for each fish by summing all lesion scores. The mean GHI per site was used to compare the severity of gill damage across the three stations.

Statistical analysis

All data were expressed as mean ± standard deviation. Normality and homogeneity were checked using the Shapiro-Wilk and Levene's tests, respectively. One-way ANOVA was used to detect differences among sites, followed by Tukey's HSD post hoc test ($p < 0.05$). Pearson's correlation was used to assess the relationship between tissue metal concentrations and both genotoxic and histopathological parameters. Statistical analysis was performed using SPSS version 25.0.

RESULTS AND DISCUSSION

The results (Table 3) of heavy metal analysis in gill lamellae of wild cyprinidae from the Brantas River revealed a clear spatial gradient in contamination levels, increasing from upstream (Site A) to downstream (Site C). The highest concentrations of all measured heavy metals – Pb, Cd, Hg, Cu, and Zn – were detected at Site C (Kepanjen), which is known for intensive agricultural and industrial activities.

Specifically, Pb levels ranged from 0.85 ± 0.22 mg/kg at Site A to 4.87 ± 0.61 mg/kg at Site C, while Cd increased from 0.09 ± 0.03 mg/kg to 0.58 ± 0.07 mg/kg over the same gradient. Mercury was detected at its lowest concentration at Site A (0.03 ± 0.01 mg/kg) and increased to 0.25 ± 0.04 mg/kg at Site C. Similarly, Cu and Zn showed substantial increases downstream, with Cu rising from 1.12 ± 0.30 mg/kg to 5.63 ± 0.77 mg/kg, and Zn from 3.95 ± 0.62 mg/kg to 9.23 ± 1.05 mg/kg.

Parallel to heavy metal accumulation, genotoxic markers also showed a significant rise.

Table 2. Modified gill histopathological index (GHI) system

Score	Description
0	No alteration (normal)
1	Mild alteration (focal, reversible)
2	Moderate alteration (multifocal)
3	Severe alteration (diffuse, possibly irreversible)

Table 3. Heavy metal concentrations in gill lamellae, micronucleus frequency, and gill histopathology in wild cyprinidae from the Brantas River, Malang

Parameter	Site A (Batu)	Site B (Dinoyo)	Site C (Kepanjen)
Lead (Pb, mg/kg dw)	0.85 ± 0.22	2.14 ± 0.34	4.87 ± 0.61
Cadmium (Cd, mg/kg dw)	0.09 ± 0.03	0.24 ± 0.05	0.58 ± 0.07
Mercury (Hg, mg/kg dw)	0.03 ± 0.01	0.10 ± 0.02	0.25 ± 0.04
Copper (Cu, mg/kg dw)	1.12 ± 0.30	2.76 ± 0.41	5.63 ± 0.77
Zinc (Zn, mg/kg dw)	3.95 ± 0.62	6.48 ± 0.89	9.23 ± 1.05
Micronucleus frequency (‰)	1.34 ± 0.41	3.78 ± 0.67	6.92 ± 0.88
Other nuclear abnormalities (%)	5.1 ± 1.3	8.6 ± 1.7	13.2 ± 2.1
Gill histopathology index (GHI)	3.2 ± 0.9	6.7 ± 1.5	11.4 ± 2.2

Micronucleus (MN) frequency in peripheral erythrocytes increased from 1.34 ± 0.41‰ at Site A to 6.92 ± 0.88‰ at Site C, indicating elevated chromosomal damage. The percentage of other nuclear abnormalities (ONAs), such as lobed or binucleated nuclei, followed a similar trend, rising from 5.1 ± 1.3% at Site A to 13.2 ± 2.1% at Site C.

Histopathological assessment of the gill lamellae reflected this trend, with the GHI averaging 3.2 ± 0.9 at Site A, 6.7 ± 1.5 at Site B, and peaking at 11.4 ± 2.2 at Site C. The progressive deterioration of gill tissue morphology – ranging from mild epithelial lifting to severe hyperplasia, lamellar fusion, and necrosis – corresponds with the increasing concentration of heavy metals across the three sites. These results strongly suggest a correlation between heavy metal exposure and both genotoxic and histopathological damage in wild cyprinidae inhabiting the Brantas River.

Micronucleus frequency and nuclear abnormalities in erythrocytes

The findings concerning the MN assay highlight significant genotoxic effects in fish collected from downstream locations of the Brantas River, specifically an increase in MN frequency correlating with levels of heavy metals, particularly Pb, Cd, and Hg. At Site A (Batu), the MN frequency averaged 1.34 ± 0.41‰, while at Site C (Kepanjen), it dramatically increased to 6.92 ± 0.88‰. This raises important concerns about the ecological health of river systems under anthropogenic stress. Similar trends were reported in other studies, where increased MN frequency correlated with pollution levels, demonstrating the effectiveness of the MN assay in monitoring genotoxic stress in aquatic environments (Göney and Gazeloğlu, 2020; Kontaş, 2022; Kontaş and Bostancı, 2020). The increase in observed nuclear abnormalities (ONAs) also follows the pattern of heavy metal exposure, with incidence escalating from 5.1 ± 1.3% at Site A to 13.2 ± 2.1% at Site C, paralleling findings from broader studies of genotoxicants associated with heavy metals (Aytekin et al., 2019; Quyet and Dung, 2023). These thresholds suggest that fish at Sites B and C may face compromised survival or reproductive success, though species-specific variability warrants further investigation.

Furthermore, the Pearson correlation analysis conducted demonstrated strong positive correlations between the concentration of heavy metals in gill tissues and both MN frequencies and ONAs (with Pb showing $r = 0.86$, $p < 0.01$). Such statistical relationships reinforce the hypothesis that heavy metals contribute significantly to genotoxic damage in fish (Drąg-Kozak et al., 2022; Kontaş, 2022). Prior research has consistently corroborated these findings, linking heavy metal exposure to chromosomal damage through mechanisms such as oxidative stress and disruption of DNA repair processes, highlighting metals like lead and cadmium for their particularly harmful effects (Chethanakumara et al., 2023; Vijayasree et al., 2023). Mercury's interaction with nuclear proteins, disrupting cell mitosis and causing nuclear anomalies, further supports the argument that even low concentrations of heavy metals can have profound biological impacts (Sharma and Chadha, 2021; Xian et al., 2021). The MN and ONA assays serve as sensitive biological indicators in determining the extent of genotoxic stress experienced by aquatic organisms due to heavy metal pollution. These findings are consistent with previous research, which emphasizes the utility of such biomonitoring tools in assessing both acute and chronic exposure to environmental contaminants, hence affirming their role in environmental health assessment (Mairaj et al., 2021).

Gill histopathological alterations

Histopathological assessments of gill lamellae across three sampling stations illustrate a clear pattern of progressive and cumulative tissue damage that correlates with rising levels of heavy metal bioaccumulation. The GHI showed an average of 3.2 ± 0.9 at Site A, 6.7 ± 1.5 at Site B, and a peak at 11.4 ± 2.2 at Site C, indicating a clear correlation between exposure and damage severity. Observed lesions included epithelial lifting, hyperplasia of epithelial cells, fusion of secondary lamellae, chloride cell hypertrophy, vascular congestion, and in the most severe instances, necrosis and lamellar degeneration. These alterations were most pronounced in fish from Site C, where concentrations of Pb (4.87 mg/kg) and Cu (5.63 mg/kg) were notably highest. Such findings align with the understanding that gill tissue, due to its extensive surface area and direct environmental contact, is particularly sensitive to waterborne contaminants (Al-Balawi et al., 2013; Bibi et al., 2021).

Heavy metals like copper and lead are known to disrupt gill ion regulation, inciting inflammation and spurring compensatory cellular proliferation that can lead to hyperplasia and lamellar fusion. The literature supports these findings, indicating that chronic lead exposure is directly associated with epithelial degeneration and necrosis of gill lamellae, severely impairing both oxygen uptake and ion balance in fish (Abdel-Mohsien and Mahmoud, 2015; Ayoola, 2019). Additionally, elevated levels of zinc and copper have been linked to chloride cell hypertrophy – an indicator of osmoregulatory dysfunction in fish that signals an adverse stress response (Bashir et al., 2012; Wulandari et al., 2024). These observations underline the significance of gill histology as a diagnostic tool for environmental toxicity assessments. Notably, the concentrations of Pb, Cd, and Hg in fish gill tissues at Site C exceeded permissible limits for edible fish tissues established by international guidelines such as the WHO (2008) and Indonesian National Standard (SNI 7387:2009), highlighting potential ecological and public health concerns. Not only do these histological changes serve as indicators of acute heavy metal exposure, but they also function as early warning signals for the overall health of aquatic ecosystems, proving crucial for monitoring environmental conditions and the potential impacts of heavy metal contamination (Kaymak et al., 2021; Sopon et al., 2021).

Integrated interpretation

The coherent integration of genotoxic, histopathological, and bioaccumulation endpoints in this study elucidates a significant pattern of heavy metal-induced stress in wild cyprinidae along the Brantas River gradient. This study reveals a marked increase in heavy metal concentrations – particularly Pb, Cd, Hg, Cu, and Zn – from upstream to downstream locations, coinciding with progressive rises in micronucleus frequency, nuclear abnormalities, and gill tissue damage. Such correlations strongly suggest a causal relationship between contaminant load and physiological impairment in fish. The increases in micronucleus frequency ($p < 0.001$), gill histopathology scores ($p < 0.001$), and heavy metal concentrations ($p < 0.01$ to $p < 0.001$) across sites were statistically significant, confirming a strong correlation between pollution level and biological responses. Aligning with findings in other freshwater systems where similar effects of heavy metal exposure on aquatic fauna have been documented (Chethanakumara et al., 2023; Kontaş and Bostancı, 2020; Simões et al., 2019).

Mechanistically, the impact of heavy metals as genotoxicants is well established. These metals can interfere with DNA replication and repair mechanisms, induce oxidative stress by generating reactive oxygen species (ROS), and bind to critical nucleophilic sites on DNA and associated nuclear proteins (Cano-Pérez et al., 2025; Minhas et al., 2022). Specifically, cadmium and lead have been shown to inhibit DNA repair enzymes and escalate chromosomal instability, while mercury disrupts mitotic spindle assembly, leading to the formation of micronuclei and abnormal nuclear morphologies. Prior studies reinforce this, highlighting the connection between heavy metal exposure and the disruption of cellular functions, ultimately culminating in broader tissue-level dysfunction and adverse physiological consequences (Drąg-Kozak et al., 2022; Shah et al., 2020).

Utilizing cyprinidae as bioindicator organisms significantly strengthens the credibility of observed patterns due to their ecological relevance and widespread distribution in southeast Asian freshwater systems. Their benthic-detritivorous feeding habits render them particularly susceptible to pollutants bound to sediments and organic detritus, where heavy metals accumulate due to their low solubility and high affinity for particulate matter (Hussain et al., 2018). Consequently, these fish species serve as ideal sentinel taxa for evaluating sublethal contaminant effects, with enhanced interactions with bottom substrates leading to elevated tissue concentrations and increased toxicological burden (Gbogbo et al., 2018; Mustapha and Patrick, 2023).

The histopathological changes observed in gill tissue, including epithelial lifting, hyperplasia, lamellar fusion, and necrosis, are classic indicators of respiratory and osmoregulatory stress in fish exposed to contaminants. These lesions not only compromise gas exchange efficiency and ion regulation, but they also reflect chronic exposure and compensatory tissue remodeling, contributing to a comprehensive understanding of physiological stress and declining fish health. The co-occurrence of high micronucleus frequencies alongside severe gill pathology underlines the biological effects driven by environmental contamination (Hussain et al., 2020; Nirchio et al., 2019).

Importantly, this research contributes to the growing body of evidence supporting multi-biomarker approaches in ecological risk assessment. Unlike traditional chemical analyses, which merely record environmental presence, biological markers like the micronucleus assay provide concrete evidence of biological impact, making them invaluable tools for early detection of pollution threats. They are critical for monitoring temporal trends in pollution, assessing remediation effectiveness, and informing evidence-based regulatory policies (Abass et al., 2019; Al-Barwary, 2020; Mansur et al., 2021).

Overall, this study underscores the importance of integrated biomonitoring strategies in evaluating and managing freshwater ecosystems facing anthropogenic pressure. The findings echo those reported in other severely affected river systems globally, necessitating coordinated actions to combat the transboundary challenges posed by freshwater pollution (Prayogo et al., 2024; Vishwakarma and Shukla, 2023).

The synergistic application of bioaccumulation data, genotoxic biomarkers, and histopathological indicators provides a robust framework for assessing ecological health in contaminated river systems. Such biomarker-based monitoring programs should be mainstreamed within national and local water management strategies to improve environmental health outcomes and protect public health, particularly in regions where fish consumption from polluted waters poses significant risks.

Building upon the findings of this study, future research should expand the biomonitoring framework by incorporating a broader range of sentinel organisms and treatment strategies to enhance ecological assessment and mitigation of heavy metal pollution in freshwater systems. Aquatic snails (Islamy and Hasan, 2020; Isroni et al., 2019), as benthic grazers with limited mobility and high site fidelity, offer excellent potential for evaluating localized contamination and bioaccumulation dynamics in sediments. In addition, future studies should include both native and non-native freshwater fish species (Hasan et al., 2022; Islamy et al., 2025; Islamy et al., 2025; Jatayu et al., 2023; Serdiati et al., 2022) found across Indonesia to understand interspecific differences in susceptibility to heavy metals and to evaluate broader ecosystem responses. The integration of freshwater periphytic diatoms (Masithah and Islamy, 2023) – known for their sensitivity to metal-induced oxidative stress – and aquatic macrophytes (Islamy et al., 2024) and indigenous bacteria (Pardamean et al., 2021) could further enhance the resolution of ecological assessments, as these primary producers play critical roles in nutrient cycling and habitat structure.

Beyond monitoring, future investigations should also explore remedial interventions that can mitigate the physiological damage induced by heavy metals in aquatic organisms. In particular, the application of immune-boosting treatments in fish using naturally derived herbal extracts has shown promising results in experimental studies (Kilawati et al., 2024). Potential candidates include seaweed-based antioxidants (Islamy et al., 2024; Islamy et al., 2025; Islamy et al., 2024; Kilawati et al., 2025), *Ipomoea pes-caprae* (Islamy et al., 2024b), Alligator weed (*Alternanthera philoxeroides*) (Serdiati et al., 2024), and Neem (*Azadirachta indica*) leaves (Islamy et al., 2024a), all of which possess bioactive compounds with anti-inflammatory, antioxidant, and detoxification properties. Evaluating the effectiveness of these phytotherapeutic agents under controlled and field conditions could offer sustainable, low-cost strategies to improve fish resilience against heavy metal stress while supporting aquaculture and conservation efforts in polluted freshwater systems.

CONCLUSIONS

This study provides compelling evidence of heavy metal-induced biological stress in wild cyprinidae inhabiting the Brantas River, Malang. By integrating data on gill tissue bioaccumulation, genotoxic biomarkers, and histopathological alterations, we demonstrate a clear downstream gradient of contamination and physiological damage – strongly associated with elevated concentrations of Pb, Cd, Hg, Cu, and Zn. The findings reveal a statistically significant increase in micronucleus frequency and other nuclear abnormalities in fish erythrocytes, alongside progressive gill tissue degradation, particularly at Site C (Kepanjen), a region heavily impacted by agricultural and industrial activity. These results not only confirm the vulnerability of cyprinidae as benthic-feeding bioindicators but also validate the effectiveness of combining genotoxic and histological biomarkers for early detection of sublethal toxicological impacts in freshwater systems.

The urgency of addressing heavy metal pollution in the Brantas River is underscored by the potential ecological and public health risks it poses. Continuous exposure to contaminated water and sediment threatens fish health, biodiversity, and the overall resilience of aquatic ecosystems, while also raising concerns regarding the safety of fish consumption by local communities. This study highlights the need for immediate intervention, including strengthened enforcement of industrial discharge regulations, improved wastewater treatment infrastructure, and systematic environmental monitoring.

In light of these findings, we recommend the adoption of integrated biomonitoring frameworks that include tissue bioaccumulation assessment, micronucleus assays, and gill histopathology as routine tools for freshwater quality evaluation. These biomarkers offer sensitive, cost-effective, and ecologically relevant metrics for detecting early signs of pollution and should be incorporated into both governmental and community-based water quality programs. Furthermore, long-term and seasonal studies are necessary to capture variations in pollutant dynamics and assess the effectiveness of future remediation efforts. Ultimately, this research emphasizes that safeguarding river health requires not only technical solutions but also cross-sector collaboration and public engagement to reduce pollution at its source and preserve the ecological integrity of vital freshwater systems like the Brantas River.

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