JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(11), 1–16
https://doi.org/10.12911/22998993/205979
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Received: 2025.06.02
Accepted: 2025.08.01
Published: 2025.08.15

Assessment of microplastic accumulation, genotoxicity and gill histopathological alterations in wild herbivorous fishes from the Brantas River, Malang, Indonesia

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ABSTRACT

The presence of microplastics in freshwater ecosystems poses emerging ecological and public health challenges, yet integrative studies in tropical river systems remain limited. This study assessed microplastic accumulation, genotoxicity, and gill histopathological alterations in wild herbivorous fishes across three segments of the Brantas River in Malang, Indonesia, representing increasing gradients of anthropogenic pressure. A total of 54 fish from six species were analyzed using a multi-biomarker approach involving microplastic quantification from digestive tracts, micronucleus assays on peripheral erythrocytes, and histopathological scoring of gill tissues. Results revealed significantly higher microplastic loads ($p < 0.001$), micronucleus frequencies ($p < 0.01$), and gill damage scores ($p < 0.001$) in fish from downstream sites, indicating cumulative pollutant exposure. Fiber-type microplastics were most prevalent, with evidence of bioaccumulation in larger, benthic-feeding species. These findings suggest localized ecological risks and early biological impacts in species critical to riverine food webs. Notably, given that these fish species are consumed by local communities, the study highlights potential human health concerns related to microplastic exposure and associated toxicants. Recommendations include enhancing wastewater treatment, regulating plastic-based product discharge, and implementing biomonitoring programs that integrate genotoxic and histopathological markers. This work contributes to the growing understanding of microplastic impacts in tropical freshwater systems and informs both environmental risk assessment and public health protection efforts in densely populated river basins.

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

INTRODUCTION

Freshwater ecosystems are increasingly threatened by a wide range of anthropogenic pollutants, and among these, microplastics are emerging as a particularly pervasive and insidious contaminant. Microplastics, defined as plastic particles smaller than 5 mm, originate from both primary sources such as cosmetic microbeads and industrial abrasives, as well as secondary sources resulting from the degradation of larger plastic debris (Batel et al., 2016; Wagner et al., 2014). Studies indicate that while much research on microplastics has traditionally focused on marine environments, emerging evidence highlights rivers not only as receivers but also as significant conduits for microplastic transport from terrestrial sources to oceans, emphasizing the need to understand their impact on freshwater systems (McNeish et al., 2018; Zhao et al., 2022). In Indonesia, for instance, the Brantas River, a crucial freshwater system in East Java, is heavily impacted by industrial activities, urbanization, and agricultural expansion, all contributing to the influx of microplastic pollutants (de Oliveira et al., 2020).

Freshwater fish, particularly those with benthic and omnivorous-herbivorous feeding behaviors, are especially vulnerable to microplastic ingestion due to their interactions with sediments and detritus-rich substrates. The role of herbivorous fishes is ecologically significant; they contribute to controlling primary production and maintaining the aquatic trophic balance, thus serving as valuable bioindicators of environmental health (McIntyre et al., 2016; Serdiati et al., 2024). Research has documented various sublethal effects of microplastic ingestion in fish, which include reduced feeding efficiency, oxidative stress, inflammation, and histopathological damage, underscoring the multifaceted impacts of microplastics in aquatic ecosystems (ma et al., 2019; Rochman et al., 2013). Moreover, the interaction of microplastics with chemical pollutants – such as persistent organic pollutants and heavy metals – can lead to genotoxic effects, inducing DNA damage through both physical irritation and chemical routes (Islamy et al., 2017; Islamy et al., 2024; Kilawati and Islamy, 2019).

The micronucleus (MN) assay employed in peripheral erythrocytes is a recognized cytogenetic technique for evaluating genotoxicity in aquatic organisms. The presence of micronuclei and other nuclear abnormalities signals chromosomal breakage or disruption of the mitotic spindle, both indicators of genomic instability attributable to environmental stressors (Ali et al., 2019; Santos et al., 2014). This assay is especially favorable for freshwater fish species due to the ease of blood collection and the sensitivity of erythrocytes to genotoxic agents (Kumari, 2024). Despite growing concerns over freshwater microplastic pollution in Indonesia, studies exploring the cytogenotoxic implications among native wild fish populations, especially herbivorous species of high ecological and economic importance, remain scarce (Zhao et al., 2022). Therefore, this study aims to (1) quantify and characterize microplastics ingested by wild herbivorous fish from three segments of the Brantas River in Malang, East Java, and (2) assess potential genotoxic effects by analyzing the frequency of micronuclei and other nuclear abnormalities in peripheral erythrocytes. The findings are poised to yield critical insights for risk assessment and policy development related to plastic pollution and the conservation of freshwater biodiversity.

MATERIAIS AND METHODS

Study area and sampling sites

Fish samples were collected from three segments of the Brantas River in Malang, East Java, Indonesia, selected based on upstream-to-downstream gradients of anthropogenic activity (Table 1).

Fish collection and identification

A total of 180 wild herbivorous fish specimens (n = 60 per site) were collected using gill nets and cast nets during the dry season (August–September 2024). Six species were targeted based on their herbivorous feeding habits and local abundance: *Osteochilus hasselti*, *Puntius binotatus*, *Barbonymus balleroides*, *Neolissochilus soroides*, *Leptobarbus hoevenii*, and *Tor tambroides*. While the species composition varied slightly among sites depending on local habitat conditions and fish availability, all six species were represented in the overall sampling effort. Fish were transported alive to the laboratory in aerated containers and identified using standard taxonomic keys.

Microplastic extraction and analysis

Each fish was anesthetized with clove oil and dissected to remove the gastrointestinal tract. Contents were weighed and digested with 10% potassium hydroxide (KOH) at 60 °C for 48 hours. The digested material was vacuum-filtered through 0.45 μm cellulose nitrate membranes. Filters were dried and observed under a stereomicroscope (Olympus SZ61) at 40× magnification.

Microplastics were categorized based on morphology (fibers, fragments, films, beads) 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°51'45.0"S, 112°31'30.0"E	Urban transition zone with reduced riparian vegetation	Moderate; domestic runoff and minor industrial activity
C (Kepanjen)	8°07'37.8"S 112°33'28.1"E	Highly urbanized with limited natural riparian cover	High; domestic sewage, solid waste, and possible industrial discharge

size class (< 100 μm, 100–500 μm, > 500 μm). Suspected microplastic particles were confirmed using the hot needle test. Representative particles were selected for FTIR analysis (Bruker Tensor 27) to identify polymer types.

Micronucleus (MN) assay in erythrocytes

Peripheral blood samples were collected via caudal vein puncture using heparinized syringes. Blood smears were prepared immediately on clean glass slides, air-dried, and fixed in absolute methanol for 10 minutes. Slides were stained with 10% Giemsa for 15 minutes, rinsed, and dried.

For each fish, 2.000 mature erythrocytes were examined under a light microscope (Olympus CX23, Tokyo, Japan) at 1000× magnification using oil immersion. Micronuclei were identified as small, round or oval bodies in the cytoplasm, distinct from the main nucleus, and measuring less than one-third of the main nucleus. Other nuclear abnormalities (binucleated cells, blebbed, lobed, and notched nuclei) were also recorded following standardized criteria (Hernández-Cabanyero et al., 2023).

Histological analysis of gill lamellae

From each sampling station (Sites A, B, and C), three representative individuals per species (totaling 18 specimens per site) were selected for histological analysis. Fish were chosen based on similar external health conditions, and efforts were made to control for biological variability by selecting individuals of comparable size (± 10% in total length) and body weight range, thereby reducing the influence of ontogenetic differences on gill morphology. Although sex was not used as a primary selection criterion due to the difficulty of external sex identification in some species and life stages, individuals exhibiting signs of sexual maturity or abnormal development were excluded to maintain sample consistency. This approach ensured that histological variations could be attributed more reliably to environmental exposure rather than intrinsic physiological factors (Kilawati et al., 2025; Kilawati et al., 2024; Kilawati et al., 2024).

Gill tissues were immediately fixed in 10% neutral-buffered formalin (NBF) for 48 hours at room temperature. After fixation, samples were rinsed in distilled water and stored in 70% ethanol until further processing. Fixed gill tissues were dehydrated in a graded ethanol series (70%, 80%, 95%, 100%), cleared in xylene, and embedded in paraffin wax. Serial sections (5 μm thick) were cut using a rotary microtome and mounted onto poly-L-lysine-coated glass slides. Sections were deparaffinized and stained using hematoxylin and eosin (H&E) for general histological evaluation.

Histological slides were examined under a light microscope (Olympus CX23, Tokyo, Japan) at magnifications of 100× and 400×. Lesions in gill lamellae were evaluated following established criteria (Mitchell et al., 2023), focusing on the following key alterations:

- Lifting of epithelial layer,
- Hyperplasia of epithelial cells,
- Fusion of secondary lamellae,
- Hypertrophy of epithelial or chloride cells,
- Edema or congestion in blood vessels,
- Lamellar necrosis or degeneration.

Lesions were scored using a modified gill histopathological index (GHI) system, which allows for semi-quantitative assessment of gill damage severity and has been widely validated in ecotoxicological studies involving freshwater fish (Król et al., 2020). This method provides a reliable means of comparing histopathological alterations across spatial gradients of pollution exposure.

Recent literature underscores the critical importance of gill histopathology in monitoring aquatic health in polluted environments. For instance, (Mitchell et al., 2023) highlighted that lifting or separation of gill epithelium is frequently observed in various studies of irritants and toxins, which aligns with the findings in this study. Similarly, (Król et al., 2020) discussed the significance of integrating histopathology and morphology in assessing gill health, emphasizing the need to recognize how these alterations relate to broader ecological impacts. Meanwhile, (Wu et al., 2012) provided insights into specific histological changes observed in fish gills in response to environmental stressors, reinforcing the relevance of using such assessments in ecotoxicological research. 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

Data were analyzed using SPSS v25.0. Microplastic abundance and MN frequency were expressed as mean ± standard deviation. Differences among sites were evaluated using one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$). Pearson's correlation was used to analyze the relationship between microplastic load and MN frequency.

RESULTS AND DISCUSSION

Microplastic abundance and micronucleus frequency

Microplastic abundance and micronucleus frequency is shown in Table 3.

Prevalence and distribution of microplastics

The detection of microplastics in all examined herbivorous fish species across the Brantas River underscores the pervasive nature of plastic contamination within this freshwater ecosystem. A clear spatial gradient was evident, with fish from downstream Site C (Kepanjen) exhibiting significantly higher microplastic loads ($p < 0.001$) compared to upstream Site A (Batu), consistent with escalating anthropogenic pressures such as domestic wastewater discharge, industrial effluents, and agricultural runoff. This downstream intensification mirrors patterns observed in other Southeast Asian River systems where inadequate waste infrastructure exacerbates plastic pollution (Gad et al., 2023; Limbago et al., 2020; Permatasari et al., 2023).

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)

Across all sites, fiber-type microplastics – predominantly originating from synthetic textiles – were the most frequently encountered, reflecting the prevalence of untreated wastewater effluents (Grigorakis and Drouillard, 2018; Zhao et al., 2022). The detection of microbeads, particularly concentrated in fish from Site C, highlights additional contamination sources such as personal care products and industrial abrasives, pointing to systemic gaps in regulatory enforcement and wastewater treatment.

Importantly, the species-specific patterns of microplastic burden further reveal how ecological traits modulate exposure and accumulation risk. *Tor tambroides*, *Leptobarbus hoevenii*, and *Neolissochilus soroides*, which exhibited the highest microplastic concentrations (up to 11.0 ± 2.4 items/individual), are benthic-detritivorous feeders that consume organic matter, algae, and sediments – habitats known to retain microplastic particles. Their foraging behaviors and benthic habitat preferences inherently increase their likelihood of ingesting sediment-associated plastics, a phenomenon documented in other demersal freshwater fish (McMullen et al., 2024; Roch et al., 2020). In contrast, more pelagic or column-feeding species such as *Barbonymus* and *Puntius* spp. demonstrated lower, though still concerning, levels of microplastic ingestion.

These patterns support the premise that trophic ecology – specifically feeding modality and substrate interaction – is a critical determinant of microplastic exposure in freshwater fish. The observed trend whereby demersal, bottom-feeding species (Islamy and Hasan, 2020; Isroni et al., 2023) accumulate higher microplastic loads reinforces prior findings that sediment-bound particles represent a major vector for plastic entry into aquatic food webs (McIlwraith et al., 2021). Moreover, the correlation between larger body mass and elevated plastic burden, as seen in *Tor tambroides*, may indicate potential for

Table 3. Microplastic abundance and micronucleus frequency in peripheral erythrocytes of wild herbivorous fish from Brantas River, Malang

Fish species	Sampling site	Sample size (n)	Microplastic abundance (items/individual)	Microplastic type detected	Micronucleus frequency (‰)	Other nuclear abnormalities (%)
Osteochilus sp.	Site A (Batu)	10	2.1 ± 0.7	Fibers (blue, black), fragments	1.8 ± 0.3	5.4 ± 1.1
Puntius sp.	Site A (Batu)	10	2.4 ± 0.5	Fragments, films	2.1 ± 0.4	6.0 ± 1.2
Barbonymus sp.	Site A (Batu)	10	1.9 ± 0.6	Fibers (black), fragments	1.7 ± 0.2	4.8 ± 0.9
Neolissochilus sp.	Site A (Batu)	10	2.6 ± 0.8	Fragments, microbeads	2.3 ± 0.5	5.9 ± 1.0
Osteochilus sp.	Site B (Malang City)	10	5.7 ± 1.2	Fibers (red, black), films	3.6 ± 0.6	8.7 ± 1.3
Puntius sp.	Site B (Malang City)	10	6.2 ± 1.0	Fragments, fibers	3.9 ± 0.8	9.1 ± 1.0
Barbonymus sp.	Site B (Malang City)	10	5.0 ± 1.1	Films, fibers (white, blue)	3.4 ± 0.5	8.2 ± 1.4
Neolissochilus sp.	Site B (Malang City)	10	6.7 ± 1.3	Microbeads, fragments	4.2 ± 0.9	9.8 ± 1.1
Osteochilus sp.	Site C (Kepanjen)	10	8.4 ± 1.5	Fragments (transparent), microbeads	5.2 ± 0.9	12.3 ± 1.5
Puntius sp.	Site C (Kepanjen)	10	9.1 ± 1.8	Microbeads, fibers (green, black)	5.6 ± 0.7	13.0 ± 1.7
Barbonymus sp.	Site C (Kepanjen)	10	7.6 ± 1.6	Fibers (black), films	4.9 ± 0.8	11.2 ± 1.3
Neolissochilus sp.	Site C (Kepanjen)	10	10.2 ± 2.0	Microbeads, fragments, fibers	6.1 ± 1.0	14.5 ± 1.6
Leptobarbus sp.	Site C (Kepanjen)	10	9.8 ± 2.1	Fibers (blue, red), microbeads, films	5.9 ± 0.9	14.1 ± 1.5
Tor tambroides	Site C (Kepanjen)	10	11.0 ± 2.4	Microbeads, fibers, fragments (colorless)	6.5 ± 1.2	15.0 ± 1.8

Note: all values are mean ± standard deviation; micronucleus frequency and other abnormalities are indicators of genotoxic stress; common microplastic types: fibers (from textiles), fragments (from larger plastic debris), films (packaging), microbeads (from cosmetics); microplastic abundance: average number of microplastic particles found per individual fish; micronucleus frequency (‰): number of micronuclei observed per 1000 erythrocytes; other nuclear abnormalities: includes lobed, notched, or binucleated cells.

bioaccumulation over time, especially in long-lived species with broader foraging ranges.

Collectively, the integration of spatial contamination gradients and species-specific ecological roles provides a nuanced understanding of microplastic distribution and impact in freshwater environments. These findings not only emphasize the need for site-specific pollution control measures but also highlight the importance of selecting appropriate bioindicator species – particularly benthic herbivores – for effective biomonitoring and ecological risk assessment in microplastic-contaminated river basins.

Micronucleus assay and genotoxic impacts

The application of the micronucleus assay in this study revealed a statistically significant increase in MN frequency in fish collected from downstream sites (Station C – Kepanjen), which also exhibited the highest levels of microplastic accumulation. This clear spatial trend suggests a correlation between microplastic exposure and chromosomal damage, reinforcing the hypothesis that microplastics contribute to genotoxicity in aquatic organisms. The MN assay, a widely accepted and sensitive biomarker of genotoxic stress, is particularly suitable for field-based biomonitoring due to its ability to detect early and sublethal chromosomal alterations (Hernández-Cabanyero et al., 2023).

The elevated MN frequency observed in this study (up to 6.5‰ in *Tor tambroides* at Site C) is comparable to values reported in heavily polluted freshwater systems such as the Citarum River in West Java (7.1‰) (Ilmi et al., 2023) and the Yamuna River in India (6.9‰) (Sharma et al., 2021), both of which are recognized for chronic chemical contamination. However, unlike those rivers, where industrial solvents and heavy metals are the primary genotoxicants, the Brantas River's increasing genotoxic profile correlates more closely with plastic-related pollutants, especially fibers and microbeads originating from household, textile, and agro-industrial sources (Figure 1).

Microplastics can induce genotoxic effects through several distinct yet potentially synergistic mechanisms (Figure 2). Firstly, physical stress and inflammation may result from the ingestion of sharp-edged or rigid microplastic fragments, which mechanically irritate the gastrointestinal lining. This irritation can lead to chronic inflammation and stimulate the overproduction of reactive oxygen species (ROS), triggering oxidative stress, lipid peroxidation, and eventually causing DNA damage in somatic cells. (Jeyavani et al., 2023) demonstrated this mechanism in zebrafish, where exposure to polyethylene microplastics significantly increased ROS levels and induced DNA strand breaks, especially at higher concentrations. Secondly, microplastics act as vectors for hydrophobic contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), bisphenol A (BPA), and various heavy metals. These substances, adsorbed onto the plastic surface, can be desorbed in the gut and enter systemic circulation, where they may interact directly with genetic material, leading to mutagenic effects or disruption of DNA repair processes. Supporting this, (Ventura et al., 2017) found that Nile tilapia exposed to cadmium-laden microplastics exhibited significantly higher micronucleus frequencies compared to those exposed to microplastics alone, underscoring the role of combined toxicity. Lastly, endocrine disruption and interference with DNA replication represent additional pathways. Plastic additives such as phthalates and alkylphenols are known endocrine disruptors that can alter hormonal regulation of cell proliferation and DNA synthesis. These disruptions may lead to altered gene expression in pathways controlling the cell cycle, apoptosis, and DNA repair, thereby increasing the likelihood of nuclear anomalies and chromosomal instability. Collectively, these mechanisms highlight the complex and multifactorial genotoxic potential of microplastic exposure in aquatic organisms.

The study of micronucleus (MN) frequencies in fish from upstream sites (e.g., Site A – Batu) provides insight into the genotoxic effects of environmental stressors. In these areas, MN frequencies were relatively low, approximating 1.7–2.3‰. This finding can be interpreted as reflecting background genotoxic levels, which are likely due to minimal anthropogenic exposure combined with natural environmental stressors such as UV radiation and atmospheric deposition of particulates (Kontaş and Bostancı, 2020).

Environmental influences are demonstrated as significant factors in determining MN frequencies in aquatic organisms. For instance, research indicates that inherent environmental conditions like UV exposure contribute to baseline levels of genotoxicity (Chen et al., 2022) and corroborate the correlation between natural factors and MN frequency variations (Liang et al., 2022). The substantial difference in MN frequencies—over threefold – between upstream and downstream sites underscores that genotoxicity is influenced primarily by environmental conditions rather than being randomly distributed. Such a conclusion aligns with findings noting how anthropogenic stressors significantly amplify genotoxic

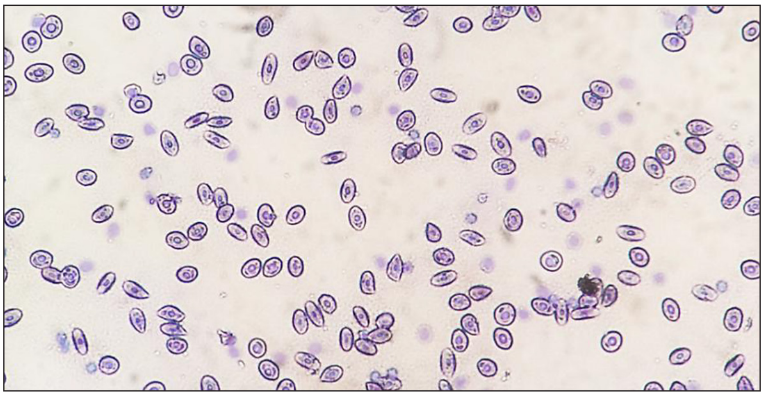

Figure 1. The micronucleus assay revealed a significant increase in MN frequency in fish collected

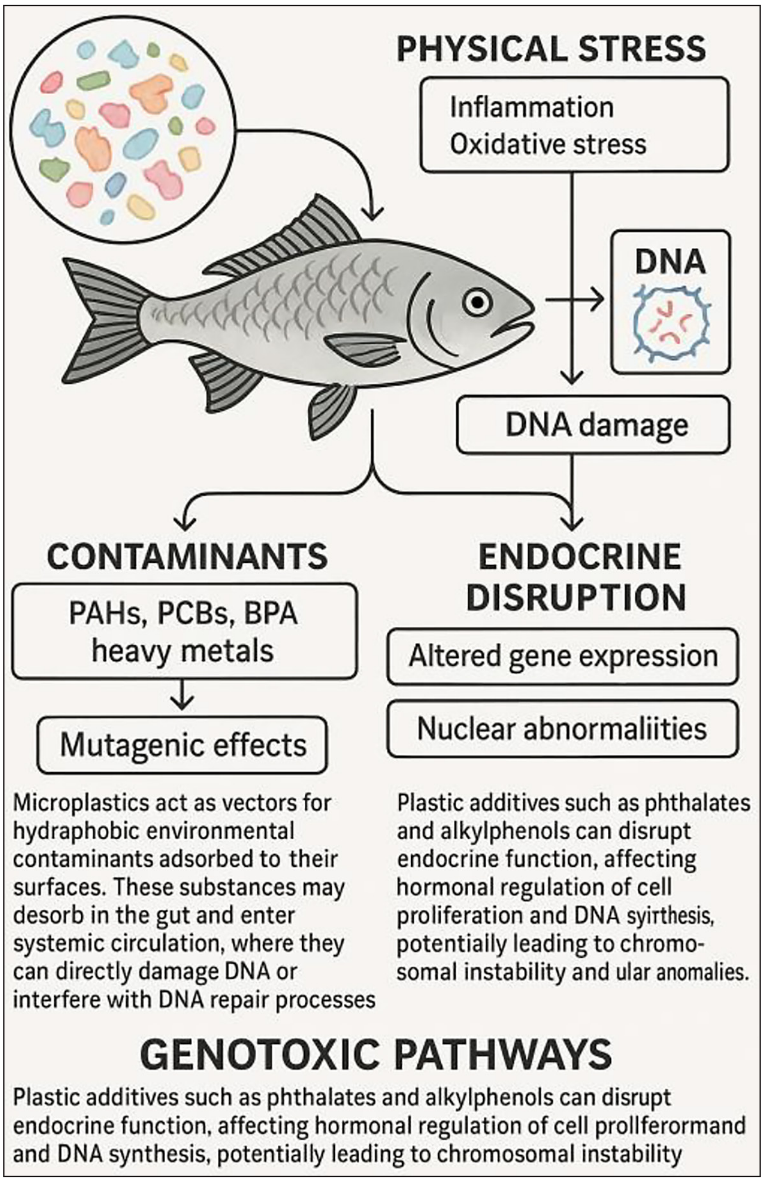

Figure 2. Illustration of genotoxic pathway

responses within aquatic ecosystems due to increasing pollution loads (Wu et al., 2024).

The association between elevated MN frequencies and oxidative stress caused by exposure to pollutants, particularly heavy metals and microplastics, has been well-documented across various fish species. Studies reveal that exposure to metals such as cadmium and mercury can significantly enhance DNA damage and precipitate MN formation, supporting the notion that the observed variations in genotoxicity are intricately linked to environmental stressors, affirming those upstream locations – less impacted by human activities – maintain lower genotoxic levels (Thakur et al., 2020; Zou et al., 2022). Downstream areas, conversely, are often significantly impacted by anthropogenic sources, resulting in elevated genotoxic risks (da Silva et al., 2020).

Moreover, additional nuclear abnormalities like binucleated cells and notched nuclei were noted at higher frequencies in fish collected from downstream locations. These anomalies provide crucial auxiliary evidence of chronic stress responses that could be elicited by pollutants. Research indicates that such nuclear alterations may signify sustained stress responses rather than isolated acute exposures (Germanov et al., 2019). Studies within temperate ecosystems, such as along the Thames River and the Danube, report moderate increases in MN frequencies attributed to pharmaceutical residues and urban effluents, suggesting that tropical freshwater systems, particularly in developing regions characterized by inadequate waste management practices, face heightened vulnerability to genotoxic pollution (Barboza et al., 2018).

Furthermore, this study highlights the ecological importance of monitoring herbivorous species for genotoxic impacts. Unlike predatory fish that bioaccumulate contaminants through trophic magnification, herbivorous fish are exposed directly through sediment and periphyton ingestion. Their frequent contact with benthic substrates makes them effective early warning indicators of sediment-associated genotoxins, including microplastics and their chemical payloads. The implications of these findings are multifaceted. At the population level, sustained DNA damage can reduce reproductive fitness, increase developmental deformities, and compromise immunity – factors that ultimately threaten population viability. At the ecosystem level, reductions in herbivore populations can disrupt nutrient cycling and algal control, leading to cascading ecological effects. Given these observations, continued research integrating MN assays with molecular biomarkers (e.g., DNA strand break assays, oxidative stress enzymes) and histopathological endpoints is warranted. Such approaches will deepen mechanistic understanding and enhance the predictive value of genotoxicity as a tool for ecological risk assessment in microplastic-contaminated environments.

Histopathological alterations in gill lamellae

Histological analysis of gill lamellae showed increasing degrees of structural damage from upstream to downstream stations. At Station A, lamellae appeared mostly intact with minor hyperplasia or epithelial lifting (mean Gill Histopathological Index [GHI] = 3.1 ± 0.9). At Station B, moderate fusion of secondary lamellae, hyperplasia, and localized necrosis were observed (GHI = 6.8 ± 1.7). At Station C, severe degeneration, extensive hyperplasia, epithelial hypertrophy, and lamellar fusion dominated the histological profile (GHI = 11.2 ± 2.4) (Figure 3).

Figure 1 illustrates the histological structure of gill tissues from wild herbivorous fish collected from three distinct sites (A, B, and C) along the Brantas River, representing a presumed gradient of microplastic contamination and associated environmental stress. At Site A, the gill filaments display relatively normal histoarchitecture, characterized by well-arranged secondary lamellae and minimal signs of epithelial lifting or fusion. The absence of significant pathological alterations suggests that fish at this site are experiencing minimal environmental stress, likely correlating with lower levels of microplastic exposure and genotoxic agents. This finding aligns with prior studies that emphasize the importance of spatial heterogeneity in microplastic contamination across various ecosystems, underscoring the need for localized assessments of freshwater pollution sources (Ahmad et al., 2020; H. Zhao et al., 2022).

The examination of gill tissue from Site B reveals moderate pathological changes, including partial lamellar fusion, mild epithelial hyperplasia, and clubbing of secondary lamellae. These alterations are suggestive of sub-chronic exposure to irritants, particularly microplastics and their associated chemical additives, such as phthalates and bisphenol A (BPA) (Barboza et al., 2018). Both phthalates and BPA have been documented to induce oxidative stress and inflammatory responses in aquatic organisms, thereby pointing to the potential risks associated with chemical contaminants in microplastics (Barboza et al., 2018; McNeish et al., 2018). The observed histological abnormalities support the hypothesis that microplastics, even at moderate concentrations, can elicit inflammatory and proliferative responses in fish gill tissues, thereby compromising respiratory efficiency and ion regulation (McNeish et al., 2018; Rojas et al., 2023).

At Site C, the most severe histological damage is characterized by widespread lamellar fusion, epithelial lifting, necrosis, and cellular degeneration, which indicate chronic and high-intensity exposure to environmental stressors (Rojas et al., 2023). This severe gill pathology is likely exacerbated by microplastics and their adsorbed pollutants, such as PAHs and heavy metals (McNeish et al., 2018). The extensive disruption of gill architecture is consistent with findings that demonstrate microplastic particles can induce physical abrasion, oxidative stress, and cellular apoptosis in gill epithelial cells (McNeish et al., 2018). These pathologies are further complicated by the potential genotoxicity associated with microplastic ingestion, which is supported by results from comet assays and micronucleus tests.

The gradation of histopathological severity from Site A to Site C correlates closely with the increasing microplastic load and genotoxicity markers reported in this study (Rojas et al.,

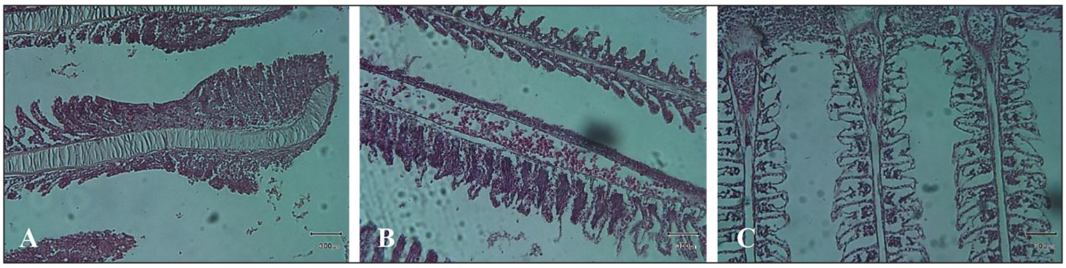

Figure 3. Histological alteration of wild herbivorous fish during the observation. A: Site A; B: Site B; C: Site C

2023). This pattern offers compelling evidence for the utility of gill histopathology as a reliable biomarker for environmental monitoring in freshwater ecosystems impacted by anthropogenic pollution (Malla-Pradhan et al., 2022). Moreover, the findings illustrate that microplastic contamination is not solely a marine issue; it significantly impacts freshwater biota, including fish species essential for maintaining ecological balance in river systems. Continued investigation of the combined histological, genotoxic, and accumulation data underscores the urgent need for regulatory interventions and the formulation of comprehensive microplastic mitigation strategies in riverine ecosystems (Ribeiro et al., 2017; Weber et al., 2021). As microplastic pollution emerges as a critical environmental challenge, ongoing research into its effects on freshwater organisms is imperative for developing impactful conservation strategies that address ecological and public health concerns.

Integrated interpretation and environmental implications

The combined evidence from microplastic quantification, micronucleus frequency, and gill histopathology strongly indicates a gradient of environmental degradation along the Brantas River. The consistent pattern – lowest effects in the upstream site and highest in the downstream site – demonstrates the cumulative impact of urban and industrial discharges (Dent et al., 2023; Samudra et al., 2024). Importantly, this study highlights the vulnerability of wild herbivorous fishes to pollution, despite their non-predatory trophic level. Their benthic and detritivorous feeding modes may increase exposure to sediment-associated microplastics and contaminants (Buwono et al., 2021a). As these fish play a vital ecological role in maintaining algal balance and nutrient cycling, their physiological impairments could disrupt riverine ecosystem functioning (Buwono et al., 2021a). Physiological impairments – such as gill tissue damage and genotoxic stress – can reduce herbivorous fishes' feeding efficiency, reproductive success, and survival rates, potentially leading to localized population declines. These declines may trigger cascading ecological effects, particularly in riverine ecosystems where herbivorous species play a pivotal role in controlling algal biomass and facilitating nutrient cycling. A reduction in grazing pressure can lead to excessive algal growth (algal blooms), which in turn may cause hypoxic conditions and disrupt aquatic food webs. Over time, this trophic imbalance can result in reduced species diversity, the loss of sensitive taxa, and the dominance of pollution-tolerant organisms, thereby compromising ecosystem stability and resilience.

From a broader perspective, the findings call for urgent mitigation strategies, including improved wastewater management, microplastic filtration technologies, and public awareness campaigns on plastic usage and disposal (Buwono et al., 2021b; El-Sappah et al., 2022). The complexity of microplastic pollution necessitates a multi-faceted approach to address both ecological impacts and public health concerns surrounding the consumption of contaminated fish. Studies have shown that chronic exposure to microplastics can lead to adverse health outcomes, including reproductive toxicity and bioaccumulation of harmful substances in edible tissues, which is essential to further investigate (Limbago et al., 2020; Xu et al., 2020).

Furthermore, as microplastics have been recognized as an emerging pollutant across various freshwater environments, ongoing research is pivotal to understand the dynamics of their distribution and the potential risks they pose to both aquatic life and human health (Hossain et al., 2024). Monitoring programs should integrate the measurement of microplastics and their effects on biota as biological indicators of the ecosystem's health and pollutant exposure levels. This can aid in formulating effective environmental policies and public health strategies aimed at mitigating the impacts of plastic pollution in freshwater systems, especially in densely populated areas like the Brantas River basin (Ahmad et al., 2020). The findings underscore the urgency of implementing comprehensive management protocols to tackle the pervasive issue of microplastic pollution, thereby enhancing ecosystem resilience and safeguarding public health in freshwater environments.

Ecological and public health implications

The findings of this study have significant ecological and public health implications. The consistent detection of microplastics, increased genotoxic biomarkers, and histopathological alterations in wild herbivorous fish from the Brantas River suggest chronic exposure to environmental pollutants that may not only threaten local aquatic biodiversity but also disrupt ecosystem functioning (Uy and Johnson, 2021; Cousin et al., 2020). Herbivorous fish play an essential role in maintaining trophic balance, controlling algal biomass, and facilitating nutrient cycling in riverine systems (Susetyo et al., 2023). Physiological impairments – such as gill tissue damage and genotoxic stress – can reduce their feeding efficiency, reproductive success, and survival rates, potentially leading to population declines. Such declines could trigger cascading effects across the aquatic food web, affecting both higher trophic level species and ecosystem resilience (Bucci et al., 2020; Cousin et al., 2020).

From a public health perspective, these fish species are often consumed by local communities. The ingestion of microplastics by fish raises concerns about human exposure to both plastic particles and associated toxicants such as persistent organic pollutants (POPs) and heavy metals that can adsorb onto microplastic surfaces (Gouin, 2020). Although the degree of human health risk via trophic transfer remains under investigation, chronic exposure to these contaminants has been linked to endocrine disruption, immunotoxicity, and even carcinogenicity in humans (Bucci et al., 2020). Moreover, the presence of genotoxic effects in fish erythrocytes serves as a biological warning signal of deteriorating water quality. Monitoring such biomarkers in sentinel species should be integrated into environmental surveillance programs, especially in rivers that support fisheries and provide water resources to dense populations, such as the Brantas River (Cousin et al., 2020; Wu and Seebacher, 2020).

The results underscore the urgent need for multi-sectoral actions – ranging from stricter wastewater regulations, and improved plastic waste management, to public education—to mitigate microplastic pollution and safeguard both aquatic ecosystem health and human well-being. Environmental policies must also emphasize the need for comprehensive studies that evaluate the biotransfer and impacts of microplastics on the entire food web, ensuring the integration of ecological health metrics into public health frameworks.

Comparative context and future directions

When contextualized with similar studies across Southeast Asia and other regions experiencing rapid urbanization and industrial expansion, the present findings align with a growing body of evidence linking microplastic contamination to genotoxic and histopathological stress in freshwater fish. Comparable levels of micronuclei frequencies and gill tissue alterations have been documented in polluted rivers such as the Citarum in West Java and the Ganges in India – regions facing similar socio-environmental challenges including plastic waste mismanagement, domestic effluents, and agricultural runoff (Labcom et al., 2024; Siddiqa et al., 2025). However, few studies to date have simultaneously assessed microplastic burden, genotoxic biomarkers, and histological gill integrity in wild herbivorous fish, which this study aims to address.

This study bridges the gap in the existing literature by providing an integrative biomonitoring framework that utilizes the ecological role of herbivores that directly interact with sediments, periphyton, and detritus – compartments known to accumulate microplastics. Given the alarming biological responses observed even in upstream segments (Station A), it is essential for future research to prioritize longitudinal monitoring that captures seasonal variations and identifies plastic polymer types along with any associated adsorbed contaminants (Labcom et al., 2024). Additionally, thorough multi-tissue toxicological assessments (e.g., of liver, intestine, and gonads) are required to better understand organ-specific impacts resulting from microplastic exposure (Zhang et al., 2023). The inclusion of molecular markers such as oxidative stress enzymes, apoptotic genes, or inflammatory pathways can further elucidate the mechanistic underpinnings of microplastic toxicity (Baras and Lagardère, 1995).

Moreover, integrating socio-economic dimensions – such as the dietary dependence of local populations on these fish – can help connect environmental exposure to public health risk assessments. Recognizing the public health implications of these findings is crucial, given the consumption of contaminated fish and the potential risks associated with microplastics and adsorbed toxicants (Santillo et al., 2017). Collaborative efforts across academic, governmental, and community sectors are essential for translating scientific findings into actionable policies. River basins like the Brantas should be regarded not only as critical environmental sentinels but also as high-priority intervention sites for addressing plastic pollution challenges (Muñoz et al., 2022). The methodological framework developed in this study can serve as a model for future biomonitoring efforts in other freshwater systems threatened by plastic pollution and its associated toxicants.

Following the outcomes of this study, future research should broaden the biomonitoring framework to better understand the ecological impacts of microplastic pollution in freshwater ecosystems. Incorporating a wider range of sentinel organisms, such as aquatic snails (Islamy and Hasan, 2020; Isroni et al., 2023), can provide valuable insights into microplastic accumulation at the benthic level due to their sedentary nature and close association with sediments. Expanding bioindicator species to include both native and non-native freshwater fish from Indonesian river (Fadjar et al., 2019; Hasan et al., 2020; Islamy, Valen, et al., 2025; Valen et al., 2019) systems will allow researchers to assess species-specific responses to microplastic exposure and trophic-level bioaccumulation. Additionally, freshwater periphytic diatoms (Masithah and Islamy, 2023) and aquatic macrophytes should be integrated into future studies, as these primary producers can serve as early indicators of microplastic-associated stress and are critical to the stability and productivity of aquatic food webs.

To complement biomonitoring, future investigations should also explore biological treatment strategies aimed at enhancing organismal resilience to microplastic exposure. One promising approach is the use of natural immunostimulants (Armando et al., 2021; Islamy et al., 2024a) and detoxifying agents derived from medicinal plants and marine algae. Candidate botanicals such as seaweed extracts (Islamy et al., 2024; Islamy et al., 2024; Islamy et al., 2025; Kilawati et al., 2025), Ipomoea pes-caprae (Islamy et al., 2024b), Alligator weed (*Alternanthera philoxeroides*) (Serdiati et al., 2024), and Neem (*Azadirachta indica*) leaves (Gupta et al., 2017; Islamy et al., 2024a) are known to contain bioactive compounds with antioxidant, anti-inflammatory, and cellular protective properties. These herbal-based interventions may help mitigate oxidative stress and tissue damage associated with chronic microplastic ingestion, particularly in fish. Evaluating the efficacy of these treatments under experimental and field conditions could lead to novel, sustainable approaches for protecting freshwater biota in polluted environments, while supporting conservation and aquaculture resilience in microplastic-affected regions.

CONCLUSIONS

This study demonstrates that wild herbivorous fishes from the Brantas River, Malang, are experiencing significant ecological stress due to microplastic contamination, as evidenced by the increasing abundance of microplastics, genotoxic damage, and histopathological alterations observed from upstream to downstream sites. The findings of this study have significant ecological and public health implications. Among the species studied, *Tor tambroides*, *Neolissochilus soroides*, and *Leptobarbus hoevenii* exhibited the highest levels of microplastic accumulation, elevated micronucleus frequencies, and severe gill histopathological alterations, indicating that these benthic-detritivorous herbivores are biologically the most affected. Their feeding behavior and habitat preference make them particularly vulnerable to sediment-bound contaminants. Physiological impairments – such as genotoxic stress and respiratory dysfunction – could reduce their reproductive output and survival rates, leading to population declines. These declines may result in cascading ecological effects, such as uncontrolled algal growth, nutrient imbalance, and reduced aquatic biodiversity, ultimately threatening the integrity and resilience of the Brantas River ecosystem. Monitoring these key species as sentinel bioindicators is therefore critical for effective environmental assessment and conservation planning. The predominance of synthetic fibers, elevated frequencies of micronuclei in peripheral erythrocytes, and progressive damage to gill lamellae (such as epithelial lifting, hyperplasia, and lamellar fusion) reflect not only the intensity of pollution but also its biological impact on aquatic organisms. These findings underscore the role of herbivorous fish as sensitive bioindicators of freshwater pollution, particularly because of their ecological position and feeding behavior that increase their exposure to contaminated sediments and water. The health implications extend beyond aquatic life, posing potential risks to humans who consume these fish regularly. Therefore, we recommend implementing regular biomonitoring programs using integrated biomarkers, improving waste management systems to reduce plastic input, expanding toxicological studies across multiple tissues and species, and promoting public awareness and policy reforms targeting plastic reduction at the source. Ultimately, the Brantas River exemplifies the urgent need for coordinated ecological and public health responses to mitigate microplastic pollution and preserve the integrity of freshwater ecosystems.

Acknowledgment

The authors express their sincere gratitude to Universitas Brawijaya, Universitas Airlangga, Universiti Sultan Zainal Abidin, Universiti Malaya, and Universitas Bangka Belitung for their invaluable support and provision of research facilities throughout this study. Special thanks are also extended to the Integrated Research Laboratory Brawijaya University for technical assistance and infrastructure support during the experimental phases.

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