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Reproductive toxicity and histopathological effects of heavy metal exposure in male Nile tilapia from the Brantas River, Indonesia

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Abstract. *Tuska HSA, Hariati AM, Sanusi A, Ciptadi G, Rumhayati B, Susanto H, Residiwati G, Aulanni'am A. 2025. Reproductive toxicity and histopathological effects of heavy metal exposure in male Nile tilapia from the Brantas River, Indonesia. Biodiversitas 26: 5169-5180.* Heavy metal pollution in freshwater ecosystems poses critical threats to fish health, particularly reproductive function. The Brantas River, one of Indonesia's most economically important watersheds, has experienced escalating contamination due to industrial, agricultural, and domestic waste discharges, leading to ecological concern. This study aimed to evaluate the bioaccumulation and toxicological effects of heavy metals on histopathological alterations (necrosis, inflammation, hyperemia, and hyperplasia) in gills and testes, and on sperm quality (viability, DNA integrity, and morphological abnormalities) of male Nile tilapia (*Oreochromis niloticus*) from the Brantas River Basin, Malang, East Java, Indonesia. Water and tissue samples were collected from five river sites (25 male fish; 5 fish per site). Heavy metal levels in water were analyzed using Atomic Absorption Spectrophotometry (AAS). Histopathological scores were evaluated semi-quantitatively. Sperm viability, DNA fragmentation, and morphology were assessed microscopically. Statistical analyses included One-Way ANOVA and Spearman correlation tests (IBM SPSS 23.0; $p<0.05$). Cadmium (Cd) and iron (Fe) were found at elevated concentrations, particularly at Kalisari (Cd = 0.0129 ppm; Fe = 7.690 ppm]), exceeding WHO (0.003 and 0.3 ppm) and USEPA (0.005 and 0.3 ppm) guidelines. Fish from this site exhibited the most severe histopathological alterations in both gills and testes: necrosis (1.8±0.24; 2.27±0.70), inflammation (2.13±0.27; 2.00±0.85), hyperemia (1.93±0.27; 2.60±0.63), and hyperplasia (2.07±0.27; 2.33±0.72), respectively. Sperm quality was significantly compromised: viability (60.27±1.17%), DNA fragmentation (59.4±0.87%), and morphological abnormalities (43±1.47%) ($p<0.05$). Strong positive correlations were found between Fe levels and testicular inflammation ($r = 0.909$), testicular hyperplasia ($r = 0.921$), and gill hyperplasia ($r = 0.913$) ($p<0.05$). Cd was negatively correlated with sperm plasma membrane integrity ($r = -0.927$) and DNA integrity ($r = -0.896$) ($p<0.05$). In conclusion, chronic exposure to Cd and Fe in the Brantas River is associated with marked tissue pathology and reduced reproductive performance in Nile tilapia. These findings underscore the urgent need for integrated river health monitoring and pollution control strategies to protect aquatic biodiversity and sustain ecosystem services in the Brantas River Basin.

Keywords: Brantas River, cadmium, iron, Nile tilapia, reproductive histopathology

INTRODUCTION

Heavy metal pollution in freshwater ecosystems poses serious risks to aquatic organisms and human health (Aziz et al. 2023; Piwowarska et al. 2024). Key contaminants—lead (Pb), cadmium (Cd), iron (Fe), and copper (Cu)—largely arise from industrial effluents, mining, agricultural runoff, and improper waste disposal (Afzaal et al. 2022; Melake et al. 2022; Sharma et al. 2024). These metals enter aquatic systems through surface runoff, atmospheric deposition, and direct discharge, compromising water quality and affecting biota (Haque et al. 2024; Saravanan et al. 2024). In fish, heavy metals cause molecular and physiological damage via oxidative stress, leading to excessive Reactive Oxygen Species (ROS), lipid peroxidation, DNA damage, and protein dysfunction (Bhat et al. 2024; Jarf et al. 2024). Mitochondrial dysfunction and immune suppression follow, along with altered gene expression and apoptosis (Kumar et al. 2023; Mani et al. 2023). Histopathological damage in gills, liver, and kidneys includes necrosis and inflammation (Paschoalini and Bazzoli 2021; Shahjahan et al. 2022), reducing fish survival and reproductive success and threatening biodiversity (El-Sharkawy et al. 2025; Oros 2025).

Bioaccumulation of metals in edible tissues increases chronic toxicity risks and human exposure via consumption (Ellwanger and Chies 2023; Ali et al. 2025), while enhancing trophic transfer and ecosystem disruption (Lipy et al. 2024; Singh and Sharma 2024). WHO/FAO limits for

Cd, Fe, Pb, and Cu in water and Tilapia muscle are 0.003/0.5, 0.3/100, 0.01/0.5, and 2/30 (ppm/mg·kg^{-1} wet weight), respectively (Saeed and Shaker 2008; Hamada et al. 2018; Mendoza et al. 2023). Heavy metals are potent reproductive toxicants in fish (Vicentini et al. 2021; Hou et al. 2024). Cd accumulates in gonads and liver, disrupts the hypothalamic-pituitary-gonadal axis, alters hormone expression, and reduces fertility (Su et al. 2023; Zhang et al. 2024). It impairs sperm motility, membrane integrity, and DNA stability (Acosta et al. 2016). Although Fe is essential, in excess it induces oxidative stress and reproductive dysfunction (Liu et al. 2022; Das et al. 2023; Rajalakshmi et al. 2023). Cd and Pb also disrupt epigenetic regulation, affecting DNA methylation and histone modifications in genes like SYCP3 and DAZL (Jenardhanan et al. 2016; Zhang et al. 2025), reducing sperm quality and testicular function (Mukherjee et al. 2022). Reproductive toxicity leads to population declines and ecosystem imbalance (Merçon et al. 2021). Evidence from Egypt and Pakistan shows Cd, Fe, and Cu bioaccumulation in tilapia correlates with testicular and ovarian degeneration, altered gene expression, and hormone suppression (Wu et al. 2024a, 2024b; Suleman et al. 2025). Similar findings occur in African catfish from Nigeria and Turkey (Opasola et al. 2019; Turan et al. 2020; Ibrahim and Sabiu 2022; Jim-Halliday et al. 2024).

In Indonesia, heavy metal contamination in rivers is a rising concern (Prayoga et al. 2022; Aristawidya et al. 2023). Our recent study in the Brantas River found high Cd and Fe levels associated with gonadal damage and poor oocyte quality in female tilapia, especially at Kalisari (Tuska et al. 2025). The Brantas supports high biodiversity and human livelihoods (Hasan et al. 2022), but pollution could destabilize its ecosystem and food web (Sigmund et al. 2023; Ziliotto et al. 2024). Although Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)) is an invasive alien species in Indonesia (Hasan and Tamam 2019), it is widely used as a sentinel species due to its ecological relevance, stress tolerance, and well-studied reproductive physiology (Bavia et al. 2022, 2024; Dos Santos Ferreira et al. 2024). This study aims to evaluate the effects of Pb, Cd, Fe, and Cu on the gills, testes, and sperm quality of male Nile tilapia from the Brantas River Basin, Malang, Indonesia. Our reproductive and histopathological approach provides baseline data to support pollution control, regulation, and sustainable freshwater ecosystem management.

MATERIALS AND METHODS

Research location

This study applied a field-based observational design adapted from Au et al. (2022) and Hossain et al. (2021), and was approved by the Institutional Animal Care and Use Committee at Universitas Brawijaya (NO: 201-KEP-UB-2024). Sampling was conducted at five sites along the Brantas River Basin in Malang, East Java, Indonesia, selected based on differing levels of anthropogenic impact. Sites included: i) Control-Sumber Brantas, Bumiaji, Batu (−7.754075, 112.526640), ii) Selorejo-Pandansari, Ngantang (−7.873486, 112.356285); iii) Kalisari-Mangliawan, Pakis (−7.950162, 112.664542); iv) Sengguruh-Sengguruh, Kepanjen (−8.18292, 112.549957), and v) Karangkates-Sumberpucung (−8.15639, 112.434519). The control site (Sumber Brantas) was chosen due to minimal upstream human activity, while the remaining sites reflected varying exposure from industrial, agricultural, and residential sources. Location coordinates were recorded using a handheld GPS device (Table 1).

Nile tilapia sampling

Nile tilapia sampling was conducted bi-monthly across five time points—February, April, June, August, and September 2024—capturing seasonal transitions between rainy-to-dry and dry-to-rainy periods. This timing aligned with the reproductive cycle of Nile tilapia, which typically spawns during warmer, late-dry seasons. The assumption of chronic exposure is supported by documented year-round pollution in the Brantas River from industrial and agricultural discharges (Fulazzaky 2009; Lusiana et al. 2023; Fitriani et al. 2024).

A total of 25 mature male Nile tilapia (5 from each site) was collected using hand nets (mesh size: 0.2 cm). Standard taxonomic keys were used for identifying wild-caught Nile tilapia based on morphological features, namely body shape, coloration, and fin structure as described in Nico et al. (2025). Only mature male tilapia (length: 15-20 cm; weight: 100-150 g) were selected for the study. Fish were wild-caught directly from river reaches (not from farms or exposure trials). The fishing permit has been obtained from local authorities. Fish were transported alive in oxygenated bags within insulated boxes to minimize stress. Upon arrival at the Faculty of Veterinary Medicine, Universitas Brawijaya, they were humanely euthanized using MS-222 (tricaine methane sulfonate, 150 mg/L) following ethical protocols. No laboratory exposure to heavy metals was applied.

Water and heavy metal analysis

Five liters of surface water were collected per site using acid-washed polyethylene containers, sealed with foil, and stored at 4°C after acidification with HNO_3 (pH<2). Heavy metal concentrations (Pb, Cd, Fe, Cu) were analysed using Atomic Absorption Spectrophotometry (Shimadzu AA 7800), following APHA (2017; Method 3120-B) and Aristawidya et al. (2023). Samples were digested with HNO_3 and $HClO_4$ at 95°C for 2 hours. Quality control included blanks, spiked recoveries, certified reference materials, and calibration curves ($R^2>0.999$). Limits of Detection (LOD) were 0.001 ppm (Cd), 0.002 ppm (Pb), 0.005 ppm (Cu), and 0.01 ppm (Fe); with LOQs of 0.003, 0.006, 0.015, and 0.03 ppm, respectively. Recovery ranged from 93-105%. Supporting parameters—BOD (mg/L), COD (mg/L), and DO (O_2/L)—were measured using APHA (2017) Method 5210 B, SNI 6989:2:2019, and APHA (2017) Method 4500-O G. Water was sampled once per site (n = 1) to reflect conditions during fish collection, not for inferential statistics.

Table 1. Sampling location coordinates were recorded using a handheld GPS device

Sampling location	Coordinates
Sumber Brantas (the river source/control site)	-7.754075, 112.526640
Selorejo	-7.873486, 112.356285
Kalisari	-7.950162, 112.664542
Sengguruh	-8.18292, 112.549957
Karangkates	-8.15639, 112.434519

Table 2. Histopathological scoring on gills and testes

Score	Severity	Level of changes			
		Inflammation	Necrosis	Hyperemia	Hyperplasia
0	Normal	0-1%	0-1%	0-1%	0-1%
1	Mild	2-15%	2-20%	2-20%	2-40%
2	Moderate	16-25%	21-30%	21-30%	41-60%
3	Severe	>25%	>30%	>30%	>60%

Histopathological assessment

Gills and testes were dissected, fixed in 10% formalin for 24 h, dehydrated through ethanol series, cleared in xylene, and embedded in paraffin at 60°C. Tissues were sectioned at 5 µm, mounted on albumin-coated slides, and stained with Hematoxylin and Eosin (H&E). Five non-overlapping fields per section were observed under 100× and 400× magnification. Lesions (inflammation, necrosis, hyperemia, hyperplasia) were semi-quantitatively scored from 0-3 (adapted from Bernet et al. 1999; Green et al. 2014; Table 2). Three blind observers evaluated all slides. The average score per fish was calculated from five fields and three observers. Photomicrographs were captured using a calibrated digital microscope, and lesion annotations were processed with ImageJ (Wayne Rasband).

Collection and examination of Nile tilapia sperm quality

Sperm quality was assessed based on three key parameters: DNA integrity, plasma membrane integrity, and morphological abnormalities, following the protocol adapted from Residiwati et al. (2020). Semen was collected non-lethally via abdominal stripping by gently applying pressure along the lateral abdomen, and samples were immediately transferred into sterile microtubes to prevent contamination and activation. DNA integrity was evaluated using 0.1% Trypan Blue staining. A 30 µL semen aliquot was smeared onto clean glass slides, air-dried, and fixed with a cold ethanol-acetone solution (1:1) at 4°C for 30 minutes. Following fixation, slides were hydrolyzed with 0.1 N HCl at 4°C for 5 minutes and restained with Trypan Blue. Two hundred spermatozoa per sample were examined under bright-field microscopy (Olympus® BX51, 1000× magnification), and DNA integrity was calculated as the percentage of sperm cells exhibiting intact chromatin (blue-stained heads) relative to the total counted.

Plasma membrane integrity was assessed using eosin-nigrosine staining. 10 µL of semen were mixed with 20 µL of eosin 1% for 30 seconds, followed by 20 µL of nigrosine 10% for another 30 seconds. The mixture was smeared, air-dried, and observed under a light microscope (1000×). Sperm cells were classified as either viable (unstained, intact membranes) or non-viable (pink-stained, damaged membranes). The percentage of viable spermatozoa was calculated accordingly. Sperm morphology was analysed to detect structural abnormalities such as deformed heads, coiled or bent tails, and other morphological defects associated with reduced fertility. 100 sperm cells were evaluated per sample, and the Percentage of Abnormal Spermatozoa (PAS) was calculated as:

$$\text{PAS} = \frac{\text{Number of abnormal sperm}}{\text{Totals counted}} 100\%$$

A PAS exceeding 20% is generally considered indicative of compromised reproductive potential. All assessments were conducted in triplicate for each individual fish, and evaluations were independently performed by three trained observers to ensure methodological consistency, inter-observer reliability, and reproducibility of results.

Data analysis

Statistical analysis was conducted using IBM SPSS v23.0. Normality was tested via the Shapiro-Wilk test ($p>0.05$). For normally distributed data, one-way ANOVA followed by LSD post-hoc tests ($p<0.05$) was used. Non-normally distributed data were analyzed using Kruskal-Wallis and Mann-Whitney U tests ($p<0.05$). Spearman's correlation ($p<0.05$) assessed the relationship between heavy metals and biological endpoints. Sample size: n = 5 male tilapia per site (total n = 25). Results are reported as mean±SD.

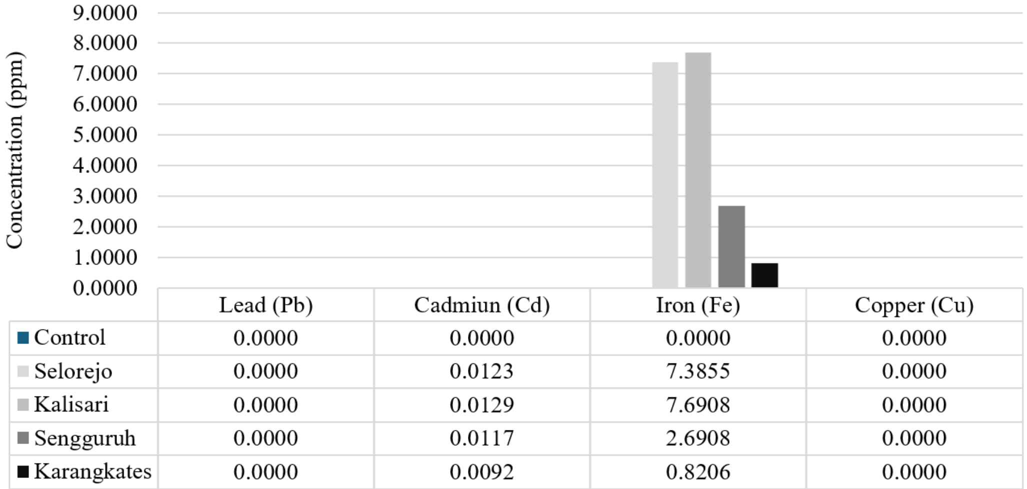
RESULTS AND DISCUSSION

Heavy metal concentrations in water

The levels of heavy metals in water samples, including Lead (Pb), Cadmium (Cd), Iron (Fe), and Copper (Cu), collected from five different sites along the Brantas River are shown in Figure 1. It revealed differences in the metal concentrations across the various sampling sites. Lead (Pb) and Copper (Cu) concentrations were either very low or undetectable at all sites, with recorded values of 0.0000 ppm. The highest Cadmium concentration was found at Kalisari (0.0129 ppm), followed by Selorejo (0.0123 ppm), Sengguruh (0.0117 ppm), and Karangkates (0.0092 ppm). In contrast, Iron concentrations were significantly higher at Kalisari (7.6908 ppm) and Selorejo (7.3855 ppm), while much lower levels were observed in Sengguruh (2.6908 ppm) and Karangkates (0.8206 ppm). The spatial variation in metal concentration indicates localized pollution hotspots (especially Kalisari and Selorejo), which may pose long-term threats to riverine biodiversity through reproductive and population-level impairments in aquatic species. The measurement of heavy metal concentrations in water was conducted once for each site without replication (n = 1 per site), as this study aimed to establish a spatial profile of contamination across the river rather than conduct temporal or replicate-based monitoring. Habitat characteristics for each sampling location (without replication; n = 1 per site), namely Biological Oxygen Demand (BOD; mg/L), Chemical Oxygen Demand (COD; mg/L), and Dissolved Oxygen (DO; O_2/L) are presented in Table 3.

Impact of heavy metal contamination on the gill of male Nile tilapia

Histopathological alterations were noted in the gills, including necrosis, inflammation, hyperemia, and hyperplasia (Figure 2). The results of the histopathological examination of the gills from male Nile tilapia are presented in Figure 3. Nile tilapia collected from Kalisari consistently exhibited the highest lesion scores across all assessed parameters. Necrosis was most pronounced in Kalisari (2.27±0.70), with significantly lower scores observed in Selorejo (0.73±0.70), Sengguruh (0.47), Karangkates (0.53±0.74), and the Control group (0.40±0.51; $p<0.05$). A similar pattern was observed for inflammation, where Kalisari (2.00±0.85) showed significantly higher values compared to Control (0.40±0.51) and Karangkates (0.60±0.63; $p<0.05$), while Selorejo (1.20±0.41) and Sengguruh (0.87±0.64) presented intermediate scores without significant differences. Hyperemia scores were also elevated in Kalisari (2.60±0.63; $p<0.05$), followed by Selorejo (1.40±0.51) and Karangkates (1.00±0.65), all of which were significantly greater than those recorded in Sengguruh (0.67±0.62) and Control (0.20±0.41; $p<0.05$). Hyperplasia followed a comparable trend, with the highest score in Kalisari (2.33±0.72), followed by Selorejo (1.40±0.51), whereas significantly lower scores were noted in Karangkates (0.60±0.63), Sengguruh (0.53±0.52), and the Control (0.20±0.41; $p<0.05$). The intensity of gill damage in Kalisari and Selorejo sites highlights chronic physiological stress that may impair respiratory efficiency and increase mortality in wild populations, which may potentially reduce species resilience and alter community composition.



	Lead (Pb)	Cadmiun (Cd)	Iron (Fe)	Copper (Cu)
■ Control	0.0000	0.0000	0.0000	0.0000
■ Selorejo	0.0000	0.0123	7.3855	0.0000
■ Kalisari	0.0000	0.0129	7.6908	0.0000
■ Sengguruh	0.0000	0.0117	2.6908	0.0000
■ Karangkates	0.0000	0.0092	0.8206	0.0000

Figure 1. Comparative concentrations (in ppm) of four heavy metals (Pb, Cd, Fe, Cu) detected at five sampling sites along the Brantas River, Malang District, Indonesia

Table 3. Comparison of BOD, COD, DO, and pH across sampling sites against standard thresholds for Nile tilapia health (Deswati et al. 2020) and WHO drinking-water quality guidelines (WHO 2022)

Standard range	**A: 0.48-4.64; B : ≤6**	**A: 9.6-25.9; B: ≤50**	**A: 5.5-7.7; B: >5**	**A: 7.7-8.7; B: 6.5-8.5**
Sampling location	**BOD (mg/L)**	**COD (mg/L)**	**DO (O_2/L)**	**pH**
Control	4.51	15.33	6.3	8
Selorejo	17.6	65.27	4.0	9.1
Kalisari	14.22	42.92	4.4	9.9
Sengguruh	8.09	27.02	4.9	13
Karangkates	8.09	27.02	4.9	7.9

Note: A: recommended water quality range for Nile tilapia health based on Deswati et al. (2020), B: Drinking water quality standards based on WHO guidelines (WHO 2022), BOD: Biochemical Oxygen Demand (mg/L), COD: Chemical Oxygen Demand (mg/L), DO: Dissolved Oxygen (O_2/L), pH: Potential of Hydrogen, Bolded values: Exceedance of the recommended standards, non-bolded values: Within acceptable limits. Water samples were analyzed once per site. Results are intended to provide a representative snapshot of water quality at each location

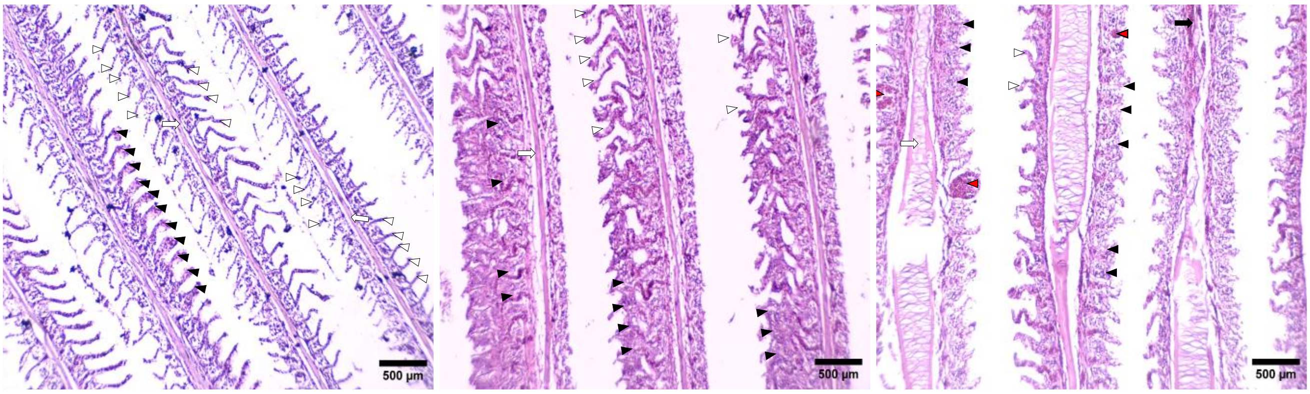
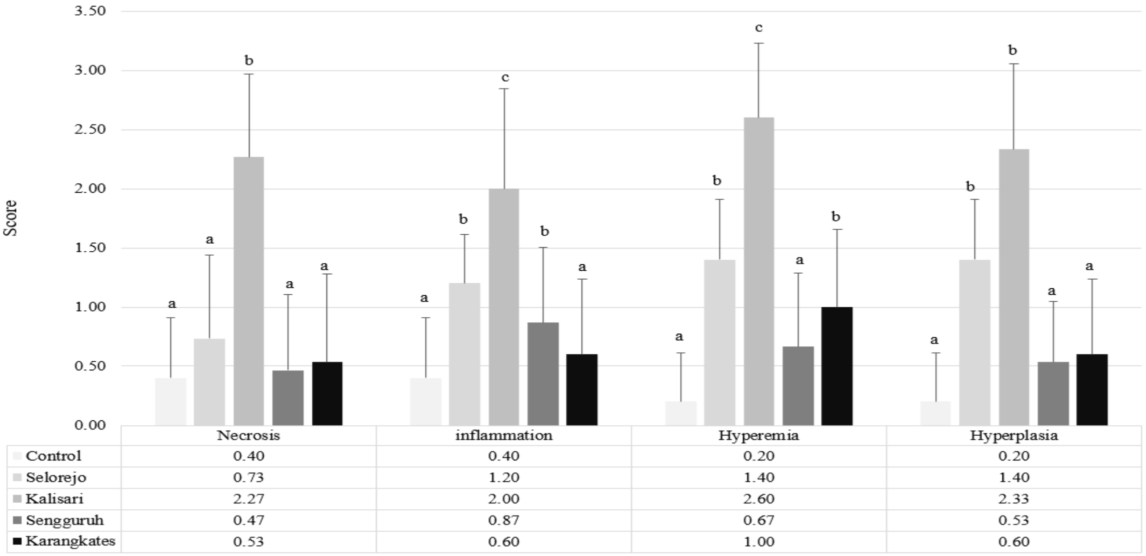


Figure 2. Histopathological alterations observed in gill of male Nile tilapia. White arrowhead: Normal secondary lamellae, Black arrowhead: Hypercellular secondary lamellae, White arrow: Normal primary lamellar, Black arrow: Inflammation primary lamellar, HE staining with 400× magnification (Personal Documentation 2024)



	Necrosis	inflammation	Hyperemia	Hyperplasia
▫ Control	0.40	0.40	0.20	0.20
▫ Selorejo	0.73	1.20	1.40	1.40
■ Kalisari	2.27	2.00	2.60	2.33
■ Sengguruh	0.47	0.87	0.67	0.53
■ Karangkates	0.53	0.60	1.00	0.60

Figure 3. Graphical representation of histopathological alterations observed in gill of male Nile tilapia; different letter subscripts indicate statistically significant differences between groups ($p<0.05$; CI 95%) for each observed parameter across the locations (total 25 male fish, 5 fish per site). Data is presented as mean±SD

Impact of heavy metal contamination on the testes of male Nile tilapia

The testes of male Nile tilapia exposed to heavy metals showed notable pathological changes, including inflammation in the periportal areas and hyperemia (Figure 4). The histopathological findings for the testes of male Nile tilapia are presented in Figure 5. Fish from Kalisari consistently exhibited the highest histopathological alteration scores across all parameters, with inflammation (2.13±0.27), necrosis (1.80±0.24), hyperemia (1.93±0.27), and hyperplasia (2.07±0.27) significantly elevated compared to all other locations ($p<0.05$). Selorejo showed moderate scores for inflammation (1.33±0.23), hyperemia (1.00±0.14), and hyperplasia (1.33±0.16), which were significantly lower than Kalisari but higher than Control (0.40±0.13, 0.20±0.11, and 0.33±0.13, respectively; $p<0.05$). Necrosis scores in Selorejo (0.47±0.17) did not differ significantly from those in Control (0.20±0.11). Sengguruh presented intermediate values for all parameters-ranging from 0.93 to 1.33 without significant differences from Karangkates in most cases. Karangkates, along with the Control, exhibited the lowest scores overall, particularly for necrosis (0.47±0.17 and 0.20±0.11, respectively), hyperemia (0.60±0.16 and 0.20±0.11), and hyperplasia (0.67±0.19 and 0.33±0.13; $p<0.05$), suggesting comparatively mild testicular alterations in these populations. Such damage to testicular structure indicates impaired spermatogenesis, which-if persistent across reproductive seasons-may reduce fertility rates and recruitment in local tilapia populations, with implications for natural stock stability and ecological balance.

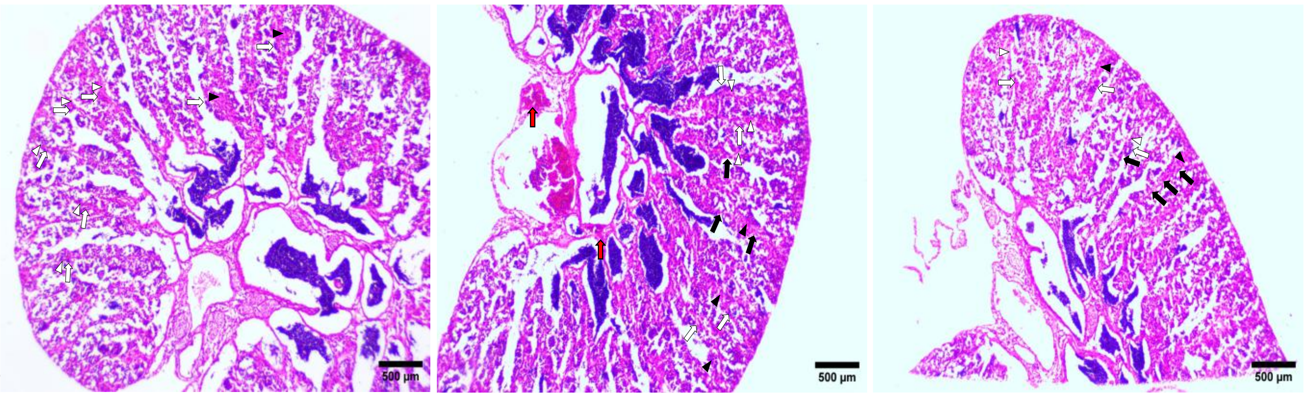
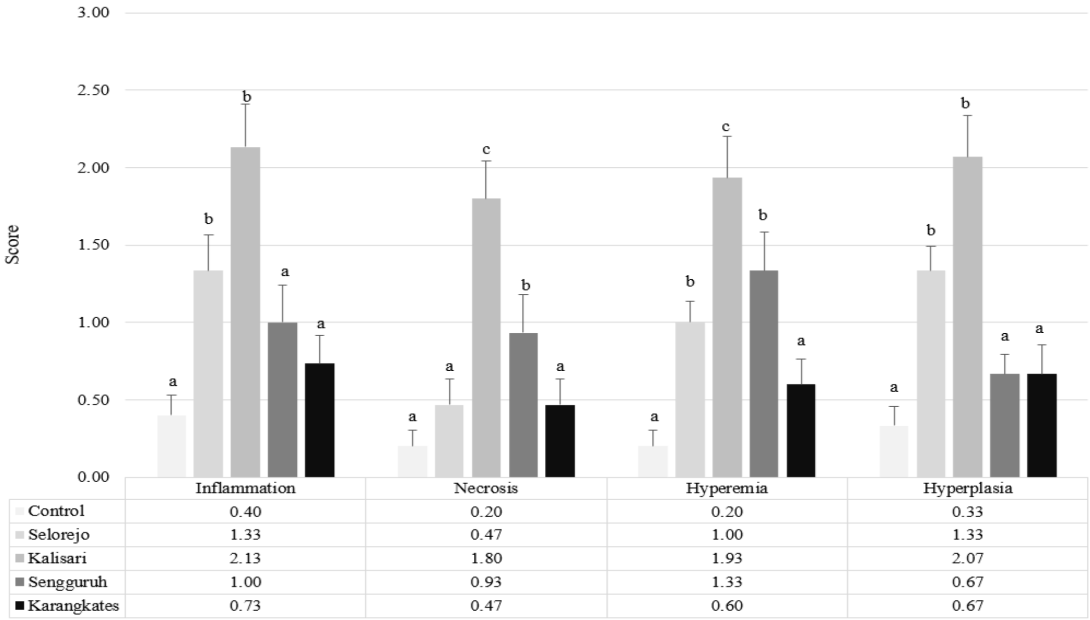


Figure 4. Histopathological alterations observed in test of male Nile tilapia. White-headed arrow: Normal interstitial tissue, White arrow: Normal epithelial spermatogonium, Black arrowhead: Hypercellular interstitial/inflammation, Black arrow: Necrotic epithelial spermatogonium, HE staining with 400× magnification (Personal Documentation 2024)



	Inflammation	Necrosis	Hyperemia	Hyperplasia
▫ Control	0.40	0.20	0.20	0.33
▫ Selorejo	1.33	0.47	1.00	1.33
■ Kalisari	2.13	1.80	1.93	2.07
■ Sengguruh	1.00	0.93	1.33	0.67
■ Karangkates	0.73	0.47	0.60	0.67

Figure 5. Graphical representation of histopathological alterations observed in test of male Nile tilapia. Different letter subscripts indicate statistically significant differences between groups ($p<0.05$; CI 95%) for each observed parameter across the locations (total 25 male fish, 5 fish per site). Data is presented as mean±SD

Impact of heavy metal contamination on gamete quality of male Nile tilapia

Graphical results of sperm quality examination are presented in Figure 6. Fish derived from Control exhibited the highest sperm quality in all parameters, with viability at 108.67±1.84%, DNA integrity at 114±2.48%, and morphological abnormalities at 13.27±1.20%. Fish from Selorejo demonstrated lower viability (68.07±1.75%) and DNA integrity (70.87±0.89%), as well as increased morphological abnormalities (18.33±0.61%), compared to Control (108.67±1.84%, 114±2.48%, 13.27±1.20%, respectively; $p<0.05$). Kalisari fish had even lower sperm viability (60.27±1.17%) and DNA integrity (59.4±0.87%), as well as higher morphological abnormalities (43±1.47%) compared to Control. Sengguruh showed the lowest sperm quality, with viability (53.87±1.02%) and DNA integrity (68.2±0.50%) significantly lower, and morphological abnormalities (39.47±1.19%) significantly higher than those from the Control ($p<0.05$). Karangkates exhibited slightly better sperm quality than Sengguruh, with DNA integrity (56.2%±1.54) and viability (59.8±0.81%) still significantly reduced, while morphological abnormalities (28.93±0.96%) were lower than those from Sengguruh but still higher than Control ($p<0.05$). These sublethal impairments in sperm structure and function suggest reduced male fertility potential, which, at the population level, may lead to declining spawning success and ultimately jeopardize reproductive sustainability in affected ecosystems.

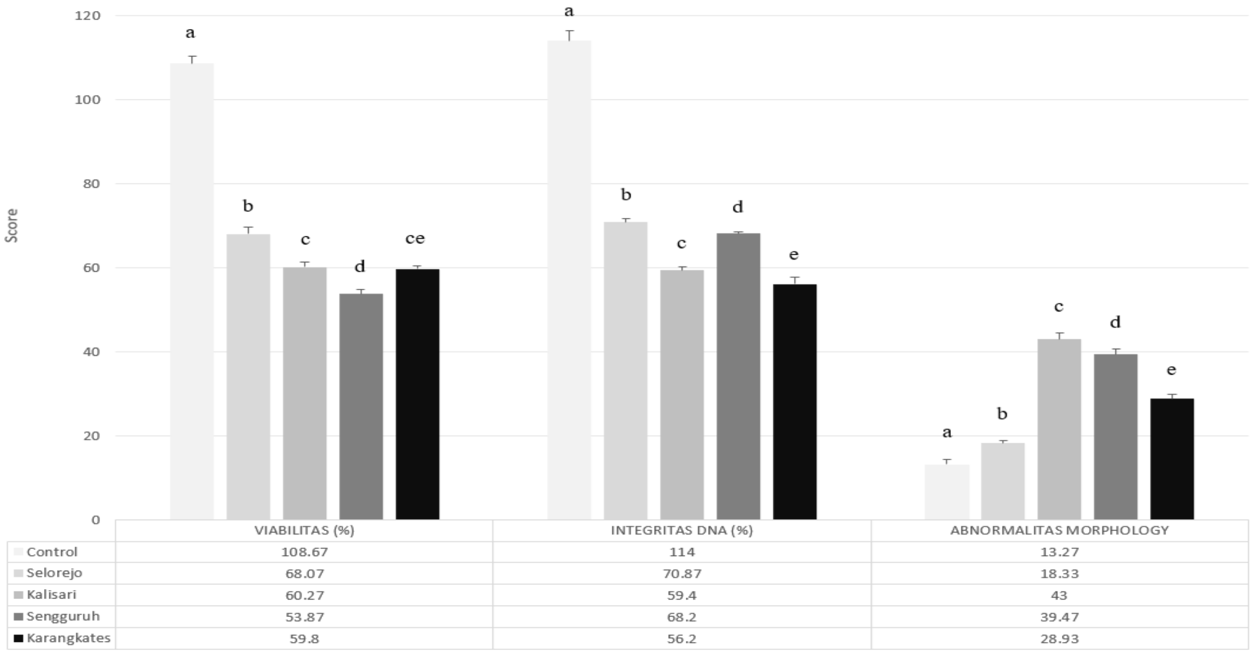
Correlation analysis of heavy metal concentrations across various parameters

The correlation analysis of cadmium (Cd) and iron (Fe) concentrations with various biological parameters is presented in Table 4. The correlation values between gill lesions (GN, GI, GHM, GHP), Testes Inflammation (TI), and sperm quality parameters (SPMI, SDI, SMA) were predominantly positive, ranging from 0.281 to 0.817 under cadmium (Cd) exposure. These results indicate a moderate to strong positive relationship among these variables. However, the correlations of SPMI, SDI, and SMA with gill lesions were generally weaker compared to the correlations observed among the gill lesions themselves. Negative correlations were also observed between Sperm Membrane Integrity (SPMI), Sperm DNA Integrity (SDI), and several tissue lesions, such as gill necrosis and testes hyperplasia, with correlation values of -0.927 and -0.896, respectively. Under iron (Fe) exposure, the correlation values were consistently higher, with most relationships exhibiting very strong positive correlations (e.g., SPMI with GHM: 0.844; SDI with GHP: 0.913), indicating a stronger impact of iron exposure on histopathological alterations and sperm quality. The parameters SPMI, SDI, and SMA showed significantly higher correlations with lesions such as testes inflammation (TI: 0.909) and testes hyperplasia (THP: 0.921). Several negative correlations were also found, particularly between sperm integrity parameters and testes lesions, with the most notable negative correlations being -0.447 for SPMI with TI and -0.476 for SDI with THM. These strong correlations reinforce the mechanistic link between environmental metal stressors and biological damage and suggest that continued exposure in key spawning habitats may progressively reduce reproductive output, threatening population viability and altering freshwater fish community dynamics.

Table 4. Correlation between cadmium, iron and various parameters in male Nile tilapia

CO	GN	GI	GHM	GHP	TI	TN	THM	THP	SPMI	SDI	SMA
Cd	0.281	0.709	0.703	0.673	0.758	0.632	0.817	0.697	-.927*	-.896*	0.688
Fe	-0.133	.905*	0.844	.913*	.909*	0.636	0.776	.921*	-0.447	-0.476	0.344

Note: CO: Correlation, Cd: Cadmium, Fe: Iron, GN: Gill Necrosis, GI: Gill Inflammation, GHM: Gill Hyperemia, GHP: Gill Hyperplasia, TI: Testes Inflammation, TN: Testes Necrosis, THM: Testes Hyperemia, THP: Testes Hyperplasia, SPMI: Sperm Plasma Membrane Integrity, SDI: Sperm DNA Integrity, SMA: Sperm Morphology Abnormality. *Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level, -: An increase in the row variable is followed by a decrease in the column variable



	VIABILITAS (%)	INTEGRITAS DNA (%)	ABNORMALITAS MORPHOLOGY
Control	108.67	114	13.27
Selorejo	68.07	70.87	18.33
Kalisari	60.27	59.4	43
Sengguruh	53.87	68.2	39.47
Karangkates	59.8	56.2	28.93

Figure 6. Graphical results of sperm quality examination (plasma membrane-, DNA integrity, and morphological abnormalities) in Nile tilapia. Different letter subscripts indicate statistically significant differences between groups (p<0.05; CI 95%) for each observed parameter across the locations (total 25 male fish, 5 fish per site). Data is presented as mean±SD

Discussion

This study underscores the significant ecological risks associated with heavy metal contamination -including lead (Pb), cadmium (Cd), iron (Fe), and copper (Cu)- in freshwater environments, with particular concern for the reproductive health and gamete quality of male Nile tilapia in the Brantas River Basin, Malang, East Java, Indonesia. Water quality assessments revealed substantial deviations from both ecological and public health standards, highlighting spatial heterogeneity in pollution pressures and their biological consequences. The control site provided optimal conditions, with BOD 4.51 mg/L, COD 15.33 mg/L, DO 6.3 mg/L, and pH 8.0, all within the recommended ranges for Nile tilapia health (BOD 0.48-4.64 mg/L, COD 9.6-25.9 mg/L, DO 5.5-7.7 mg/L, pH 7.7-8.7; Deswati et al. 2020) and compliant with WHO guidelines for drinking water (BOD ≤6 mg/L, COD ≤50 mg/L, DO >5 mg/L, pH 6.5-8.5; WHO 2022). In contrast, Selorejo exhibited critical pollution levels, with BOD 17.6 mg/L, COD 65.27 mg/L, DO 4.0 mg/L, and pH 9.1, indicating high organic loading, oxygen depletion, and alkalinity beyond safe thresholds. Kalisari showed similar concerns (BOD 14.22 mg/L, COD 42.92 mg/L, DO 4.4 mg/L, pH 9.9), consistent with anthropogenic inputs and eutrophication risk. The most extreme case was Sengguruh, where pH reached 13.0 -far exceeding ecological and human safety limits- accompanied by BOD 8.09 mg/L, COD 27.02 mg/L, and DO 4.9 mg/L, reflecting a caustic and biologically hostile environment. Karangkates showed intermediate risk, with BOD 8.09 mg/L, COD 27.02 mg/L, DO 4.9 mg/L, and acceptable pH (7.9). Further, Fe was the most prevalent heavy metal across sites, followed by Cd, while Pb and Cu were either very low or undetectable. At Kalisari, Fe concentrations (7.6908 ppm) greatly exceeded the WHO and USEPA threshold of 0.3 ppm, and Cd (0.0129 ppm) surpassed permissible WHO and USEPA limits of 0.003-0.005 ppm (Zhou et al. 2020). These anomalies -elevated organic matter, extreme alkalinity, and excessive Cd and Fe- align with observed histopathological lesions and impaired gamete quality, demonstrating a strong link between water quality degradation and reproductive toxicity.

These findings highlight the existence of localized pollution hotspots within the Brantas River Basin, underlining the urgent need for continuous monitoring and effective mitigation. The proximity of Kalisari to residential and industrial zones suggests anthropogenic activities and effluent discharges as primary contributors, consistent with similar pollution patterns documented across Indonesia (Prayoga et al. 2022). Parallel contamination profiles have been reported in major Southeast Asian rivers, including the Citarum (Indonesia), Chao Phraya (Thailand), Tonle Sap-Bassac (Cambodia), and Saigon (Vietnam), where heavy metals such as Al and Pb significantly contribute to cumulative ecological risks (Chanpiwat and Sthiannopkao 2013; Fadlillah et al. 2023). Likewise, African rivers such as the Ogun (Nigeria) and Molopo (South Africa) frequently exceed international safety guidelines for toxic metals in both water and sediments, primarily due to industrial effluents, urban runoff, and untreated sewage (Mohajane and Manjoro 2022; Khan et al. 2023). Persistent pollution pressures of this nature threaten aquatic biodiversity, as early-life-stage or reproductively active fish exposed to metals experience long-term population declines, species imbalance, and degradation of ecosystem services (Sharma et al. 2020; Madesh et al. 2024).

Histopathological evaluations unequivocally confirmed the adverse effects of heavy metal exposure on tilapia organs. Fish collected from Kalisari displayed pronounced gill and testicular lesions, including necrosis, hyperplasia, and inflammation, which are hallmarks of oxidative stress-mediated tissue injury caused by Reactive Oxygen Species (ROS) following chronic metal exposure. Both Cd and Fe are well documented to disrupt cellular redox balance, induce oxidative stress, lipid peroxidation, DNA damage, and apoptosis in fish organs (Hossain et al. 2021; Morshdy et al. 2021). In addition, they impair systemic physiology, including antioxidant enzyme suppression, acetylcholinesterase inhibition (neurotoxicity), and cytokine dysregulation, collectively reflecting biochemical and immunological stress (Morshdy et al. 2021; Reda et al. 2025). Biologically, gill damage compromises oxygen uptake and ionic regulation, while testicular lesions disrupt seminiferous epithelium integrity and viable spermatozoa development (Lahnsteiner et al. 2004; Hossain et al. 2021). These alterations were less pronounced at Karangkates and the control site, consistent with observed gradients in contamination levels.

Comparable histopathological and molecular disruptions have been observed in other freshwater systems, which exceed the WHO and FAO toxic threshold. Research in the Brantas River (Tuska et al. 2025), the Eastern Delta (Shaalan 2024), and Egypt's Middle Delta (Alm-Eldeen et al. 2018) confirmed correlations between elevated heavy metal levels and severe histopathological alterations. Such consistency across diverse ecosystems reinforces the value of Nile tilapia as a sentinel species for reproductive toxicity monitoring in tropical rivers. As a dominant species, reproductive impairment in tilapia threatens inter- and intraspecific interactions, destabilizes trophic dynamics, and weakens fish community resilience (Jere et al. 2021; Shuai and Ji 2022).

Sperm quality assessments corroborated the histopathological findings, demonstrating a clear decline in reproductive performance with increasing contamination. Control fish showed high viability and intact DNA integrity, while tilapia from Kalisari exhibited markedly reduced viability (60.27%), DNA integrity (59.4%), and elevated morphological abnormalities. Such gamete-level impairments, consistent with previous reports in *Danio rerio* (Acosta et al. 2016), *Cyprinus carpio* (Chyb et al. 2001a, b), and tilapia from Lake Manzala, Egypt (Elgaml et al. 2019), threaten recruitment, population abundance, and genetic diversity (Bobe 2015; Ohlberger et al. 2022).

The ecological ramifications of heavy metal-induced reproductive impairment in male Nile tilapia are profound, extending beyond individual pathology to population-level consequences and ecosystem stability. Chronic dysfunction of spermatogenesis reduces larval recruitment and effective population size, with risks of local extirpation, especially where tilapia act as keystone species (Hu et al. 2021). At the cellular level, exposure induces metallothionein and heat shock protein expression as stress responses, though often insufficient to prevent reproductive damage (Morshdy et al. 2021). Resultant population declines can disrupt trophic interactions, alter community structure, and destabilize food webs, thereby undermining ecosystem resilience. These ecological disturbances also carry socio-economic consequences, particularly for artisanal fisheries and rural communities dependent on tilapia for protein and income (Hu et al. 2021; Morshdy et al. 2021).

Mechanistically, heavy metals compromise sperm motility through multiple cellular pathways. Their high permeability into teleost sperm enables binding to flagellar proteins, disrupting beat symmetry and motility activation (Lahnsteiner et al. 1999, 2004). They also impair energy metabolism by inhibiting sulfhydryl-dependent enzymes crucial for axonemal integrity, while their affinity for macromolecules facilitates DNA binding and chromatin damage. Cd and Fe further exacerbate reproductive toxicity by promoting oxidative stress and ferroptosis. Cadmium triggers excessive autophagy and ferritinophagy-mediated iron overload in spermatogonia (Ali et al. 2023; Jia et al. 2024), while impairing Sertoli and Leydig cell function, reducing exosomal secretion, and suppressing steroidogenic enzymes (Gao et al 2015; Ali et al. 2023). Collectively, these effects reduce sperm motility, increase abnormal morphology, and damage testicular architecture, with Fe amplifying oxidative injury (Jia et al. 2024).

Correlation analyses reinforced these links, showing strong associations between histopathological lesions and reductions in sperm viability and DNA integrity. These results align with studies documenting Cd-induced disruption of hormonal cycles, spermatogenesis, and testicular necrosis (Kime 1998, 1999; Mousa and Mousa 1999). Additionally, Cd competes with zinc a critical element in spermatogenesis, motility, antioxidant defence, chromatin condensation, and zinc-dependent enzymatic phosphorylation-thereby compounding reproductive dysfunction (Kvist et al. 1987; Favier 1992; Reyes et al. 1993; Devi et al. 1997; Gu and Hecht 1997). Beyond these pathways, inhibition of aquaporins by metal ions disrupts osmotic regulation, leading to cellular swelling and further compromising sperm motility (Preston et al. 1993; Kuwahara et al. 1997; Dietrich et al. 2010).

Taken together, these findings indicate that chronic exposure to Cd and Fe in the Brantas River poses a severe risk to Nile tilapia reproduction and, consequently, to ecosystem integrity. Given the species' ecological and socio-economic roles, the decline of tilapia may trigger cascading effects in aquatic food webs and threaten local food security (Chakraborty 2021; Bera et al. 2022; Taslima et al. 2022; Oros 2025). From a biodiversity policy perspective, the Brantas River represents a vital freshwater corridor requiring urgent protection (Islamy et al. 2025a, 2025b). Few regional policies currently incorporate sublethal reproductive endpoints into biodiversity risk assessments. Our study reinforces Nile tilapia's utility as a bioindicator in tropical freshwater systems, where reproductive biomarkers can serve as early-warning signals of environmental stress and biodiversity erosion. To complement histopathological and gamete analyses, future studies should also measure heavy metal accumulation in edible tissues (e.g., muscle and liver) and model human health risks in relation to FAO/WHO intake limits. In response to these findings, robust mitigation strategies are urgently needed, including stricter regulation of industrial discharges, regular enforcement of water quality standards, and the application of bioremediation approaches using metal-accumulating plants and microorganisms (Fulke et al. 2024). Promoting sustainable aquaculture can further support recovery of impacted populations while safeguarding food safety. Protecting reproductive health in sentinel fish species is essential not only for preserving biodiversity and ecosystem resilience (Gárriz et al. 2019; Lionetto et al. 2023) but also for reducing risks to public health, as Nile tilapia represents a critical protein source for communities along the Brantas River. Effective conservation and pollution management will therefore require multisectoral collaboration, supported by community education and stakeholder engagement to ensure sustainable practices and long-term river health.

In conclusion, this study demonstrates that cadmium (Cd) and iron (Fe) are the predominant heavy metals exerting deleterious effects on the reproductive health of male Nile tilapia in the Brantas River Basin, as indicated by significant histopathological alterations in gill and testicular tissues, reduced sperm viability, impaired DNA integrity, and increased morphological abnormalities. These findings underscore high risk of reproductive dysfunction, potentially leading to reduced fecundity, population decline, and compromised aquatic biodiversity. Importantly, given that Nile tilapia is a widely consumed freshwater fish, the presence of heavy metals may also pose a serious threat to public health through bioaccumulation and trophic transfer within the food chain. However, this study is limited by the absence of tissue-level metal bioaccumulation and other environmental stressors, which may have contributed synergistically to the observed reproductive impairments. Future investigations should adopt a more integrative ecotoxicological framework, combining tissue metal quantification, multi-stressor assessment, and longitudinal monitoring of reproductive biomarkers to better elucidate the cumulative impact of environmental pollutants on fish reproductive health and inform sustainable environmental and public health management strategies.

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