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Effect of Mercury Contaminated Diet on Hematology and Histology of Liver, Kidney and Gills in Nile Tilapia (Oreochromis Niloticus)

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Abstract

The present study was designed to assess the impact of mercury contaminated diets on hematological parameters and histopathological alterations in vital organs of Nile tilapia. Nile tilapia is a widely cultivated freshwater species known for its rapid growth and high adaptability; however, its exposure to toxic heavy metals, especially mercury, can lead to significant physiological and structural damage. A total of 300 healthy fingerlings were randomly assigned to four treatment groups: T0 (control), T1 (0.5 mg/kg methylmercury), T2 (5 mg/kg), and T3 (10 mg/kg), with three replicates each. The feeding trial was conducted over a period of 105 days under controlled laboratory conditions. Water quality parameters were regularly monitored to assess the environmental impact of mercury exposure. In T3, significant increases were observed in ammonia, nitrite, phosphorus and iron indicating a deterioration in water quality and increased metabolic stress on the fish. Hematological parameters showed pronounced dose-dependent alterations. Hemoglobin levels declined significantly along with reductions in RBC count, mean corpuscular volume and mean corpuscular hemoglobin concentration. White blood cell counts were suppressed showing immune suppression induced by mercury toxicity. it is concluded that Mercury severely affected hematological and physiological organ system. Its contamination resulted in significant alterations in hematological parameters, including anemia and immune dysfunction, alongside severe histopathological damage in key organs such as the liver, kidneys, and gills. it is suggested to implement and enforce strict environmental policies to limit industrial and agricultural mercury discharge into water bodies.

Keywords; Nile tilapia, heavy metals, methylmercury, water quality, immune suppression, anemia

1 Introduction

Aquatic ecosystems are highly exposed to heavy metal contamination from industrialization, mining, agricultural runoff, and urban effluents (Saravanan et al., 2024). Unlike organic pollutants, heavy metals are persistent, non-biodegradable, and accumulate in sediments, water, and aquatic organisms. This persistence promotes bioaccumulation and biomagnification through food chains, posing risks to both fish and human health (Jeong et al., 2023; El-Sharkawy et al., 2025). Fish, due to their ecological and commercial significance, are sensitive bioindicators of environmental pollution (Ray & Vashishth, 2024). Chronic exposure to metals such as mercury, cadmium, and lead can impair physiology, immunity, reproduction, and survival, making heavy metal contamination a major concern for aquaculture, biodiversity, and food security (Singh & Sharma, 2024).

Mercury (Hg) is a naturally occurring heavy metal present in elemental, inorganic, and organic forms and is found in air, soil, and aquatic environments. When introduced into water bodies via industrial discharges, mining effluents, or atmospheric deposition, mercury undergoes transformations, including microbial methylation, which substantially increases its toxicity (Kumar et al., 2023; Aleku et al., 2024). Unlike many pollutants, mercury persists in ecosystems and bioaccumulates in fish tissues, particularly in the liver, kidney, and gills (Oros, 2025). It also biomagnifies through aquatic food webs, reaching high concentrations in predatory species, posing risks to fish health and human consumers through contaminated fish (Córdoba-Tovar et al., 2022; Wu et al., 2024). In aquaculture, feed represents a major route of mercury exposure, making the evaluation of diet-borne contaminants highly relevant for both fish health and food safety.

Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultured freshwater species globally, valued for its rapid growth, adaptability, and high market demand. It provides an essential source of animal protein in developing countries and contributes significantly to food security and rural livelihoods (Kwikiriza et al., 2025). Tilapia is frequently used as a model in ecotoxicological studies due to its well-characterized physiology, ease of handling, and sensitivity to environmental stressors, including heavy metals (Emam et al., 2025). Its omnivorous diet and ecological versatility render it particularly susceptible to pollutants in aquatic systems, making it an ideal species to study the effects of dietary mercury exposure on fish health (Topic Popovic et al., 2023).

Mercury exposure disrupts multiple physiological systems in fish, with hematological parameters and organ histology serving as sensitive indicators of toxicity (Yulianto et al., 2023). Hematological indices, including red blood cell count, hemoglobin concentration, hematocrit, and leukocyte profiles, are altered under mercury stress, reflecting anemia, impaired oxygen transport, and immune dysfunction (Ahmed et al., 2022; Witeska et al., 2023). Mercury also accumulates in metabolically active tissues such as the liver, kidney, and gills, causing pronounced histopathological damage. Typical lesions include hepatocyte vacuolation and necrosis in the liver, tubular degeneration and glomerular alterations in the kidney, and lamellar fusion, epithelial lifting, and hyperplasia in the gills, impairing respiratory efficiency. These effects compromise fish survival and performance and highlight the risk of mercury contamination to aquaculture productivity and food safety (Shahid et al., 2020; Mishra & Behera, 2023).

Although mercury toxicity has been widely studied in waterborne exposure, the specific effects of mercury contaminated diets on both hematology and histopathology in tilapia remain insufficiently explored. Studying both systemic (hematological) and tissue-level (histopathological) responses simultaneously provides a comprehensive assessment of mercury toxicity. Therefore, this study was designed with the aim to evaluate the effects of mercury-contaminated diets on hematological parameters and histopathological changes in the liver, kidney, and gills of Nile tilapia.

2 Materials and Methods

2.1 Ethical Approval

All experimental procedures involving fish were conducted in accordance with the guidelines for the care and use of laboratory animals and were approved by the Ethical

Review Committee of Shaheed Benazir Bhutto University of Veterinary and Animal Sciences (SBBUVAS), Sakrand with approval no. SBBUVAS/ORIC-ACERC/01/2025.

2.2 Experimental Animals and Acclimatization

A total of 300 Nile tilapia (Oreochromis niloticus) fingerlings were procured from the Fisheries Unit at SBBUVAS. Fish were selected based on health and uniform size, with an average body length of 2.0–3.5 cm and weight between 2.02 ± 3.79 g. Prior to the trial, fingerlings were acclimatized for 15 days in laboratory aquaria under controlled environmental conditions. Water temperature, dissolved oxygen, and pH were maintained within optimal ranges during this period.

2.3 Experimental Design

The trial was conducted using a completely randomized design with four dietary treatments as

- Control (T0) = without treatment (methyl mercury)
- Treatment 1 (T1) = 0.5 mg\kg feed
- Treatment 2 (T2) = 5 mg\kg feed
- Treatment 3 (T3) = 10 mg\kg feed

A total of 12 glass aquaria (18 × 12 × 12 inches; capacity ~42.5 L) were used, each stocked with 20 fingerlings, resulting in equal stocking density across treatments. Aeration and filtration were maintained with dual filters and airstones in each aquarium.

2.4 Preparation and Feeding of Diet

The basal diet was prepared using fish meal (45%), rice bran (49%), cottonseed cake (3%), vitamin mineral premix (1%), salt (1%), and binder (1%), totaling 1,000 g per batch (Table 3.3). Mercury exposure was introduced by supplementing diets with methyl mercury (CH_3Hg^+) at different concentrations according to treatment groups. Diets were pelletized, dried, and stored under dry conditions until use.

After acclimatization, experimental fish were fed their respective diets for 16 weeks. Feeding was carried out twice daily to apparent satiation. Leftover feed was avoided to maintain water quality, and feeding times were kept consistent throughout the experiment.

2.5 Water Quality Monitoring

Physicochemical parameters were monitored regularly. pH was measured using a digital pH meter (Hanna); ammonia using an ammonia ion selective electrode; nitrite and phosphorus by DR 1900 spectrophotometer (Hach); nitrate by NitraVer 5 kit (Hach); hardness by reagent test kits (Hach); total dissolved solids (TDS) by TDS-3 meter (HM Digital); iron by a LaMotte colorimeter; and salinity using HI98200 multiparameter (Hanna). Trace metals were analyzed by ICP-OES (Thermo Fisher iCAP 7000, lead), atomic absorption spectrometry (PerkinElmer PinAAcle, cadmium), and Mercury Analyzer (Thermo Fisher, mercury).

2.6 Samples Collection

Fish sampling was carried out at day 30 and subsequently at 15-day intervals up to 16 weeks. From each aquarium, three fish were randomly collected for hematological analysis, while four fish were euthanized at the end of the experiment for histological examination.

Blood was collected aseptically from the gills and caudal vein using sterile syringes and transferred into EDTA-coated tubes. Fish were restrained on a sampling board to minimize stress. Following collection, puncture sites were compressed with sterile gauze. Fish were monitored in aerated aquaria until recovery. Hematological indices included red blood cell count, hemoglobin concentration, hematocrit, and leukocyte profile.

For histopathology, tissues from the liver, kidney, and gills were excised post-euthanasia. Samples were fixed in 10% buffered formalin, dehydrated in graded ethanol series (70–100%), cleared in xylene, and embedded in paraffin wax. Sections (4–6 µm) were cut with a microtome, mounted on glass slides, and stained with hematoxylin and eosin (H&E). Slides were examined microscopically for histopathological lesions including hepatocyte vacuolation, necrosis, tubular degeneration, glomerular changes, and gill lamellae alterations.

Statistical Analysis

Data were tabulated and analyzed using Statistics 8.1. One-way analysis of variance (ANOVA) was employed to determine treatment effects, with $p < 0.05$ considered statistically significant.

3 Results

3.1 Physico-chemical parameters of water

Exposure of Nile tilapia to increasing dietary concentrations of methyl mercury (CH_3Hg^+) produced dose-dependent alterations in water quality parameters (Table 4.1). In the control group (T0), all values remained within normal ranges. The pH, hardness, total dissolved solids (TDS), and salinity showed no significant variation across treatments ($p > 0.90$). In contrast, significant increases were recorded in ammonia, nitrite, phosphorus, iron, and mercury concentrations with increasing dietary exposure. Ammonia rose from 0.01 mg/L in T0 to 0.12 mg/L in T3 ($p = 0.004$), nitrite increased from 0.02 to 0.10 mg/L ($p = 0.025$), phosphorus from 0.3 to 0.8 mg/L ($p = 0.001$), and iron from 0.2 to 0.6 mg/L ($p = 0.014$). Mercury concentration reached 0.03 mg/L in T3 (p

= 0.021). Lead and cadmium showed minor rises but remained statistically insignificant (p > 0.05).

Table 1: *Physico-chemical parameters of water in different treatment groups (Mean ± Standard error)*

Parameter	T0	T1	T2	T3	p-value
pH	7.5 ± 0.1	7.5 ± 0.1	7.5 ± 0.1	7.5 ± 0.1	0.98
Ammonia (mg/L)	0.01 ± 0.002	0.03 ± 0.003	0.08 ± 0.005	0.12 ± 0.006	0.004
Nitrite (mg/L)	0.02 ± 0.004	0.03 ± 0.005	0.07 ± 0.006	0.1 ± 0.007	0.025
Nitrate (mg/L)	10 ± 1.5	10.5 ± 1.5	11 ± 1.5	11.5 ± 1.5	0.34
Phosphorus (mg/L)	0.3 ± 0.05	0.4 ± 0.05	0.6 ± 0.05	0.8 ± 0.05	0.001
Hardness (mg/L)	150 ± 15	150 ± 15	150 ± 15	150 ± 15	0.99
TDS (mg/L)	1000 ± 100	1000 ± 100	1000 ± 100	1000 ± 100	0.97
Iron (mg/L)	0.2 ± 0.02	0.3 ± 0.02	0.5 ± 0.03	0.6 ± 0.03	0.014
Salinity (ppt)	2 ± 0.1	2 ± 0.1	2 ± 0.1	2 ± 0.1	0.99
Lead (mg/L)	0 ± 0	0.01 ± 0.001	0.02 ± 0.002	0.02 ± 0.002	0.18
Cadmium (mg/L)	0 ± 0	0.01 ± 0.001	0.02 ± 0.002	0.02 ± 0.002	0.21
Mercury (mg/L)	0 ± 0	0.01 ± 0.002	0.02 ± 0.003	0.03 ± 0.004	0.021

3.2 Clinical and macroscopic observations

No clinical abnormalities were noted in the control group (T0). Fish in mercury-exposed groups (T1–T3) exhibited dose-dependent behavioral disturbances including respiratory distress, surface swimming, rapid opercular movements, ataxia, and eventual mortality after prolonged exposure (Figure 1).

Fig. 1 Clinical signs and gross observations under different treatment groups.

3.3 Histopathological Alterations

3.3.1 Liver

The liver of control fish (T0) exhibited normal histoarchitecture with intact hepatocytes, central vein, and sinusoids. Mild lesions appeared in T1, including cytoplasmic vacuolation and occasional nuclear pyknosis. Moderate lesions in T2 comprised vacuolation, nuclear degeneration, bile duct hyperplasia, and hemosiderosis. Severe degeneration, necrosis, and inflammatory infiltration were recorded in T3, where hepatic cords were completely disorganized (Figure 2)

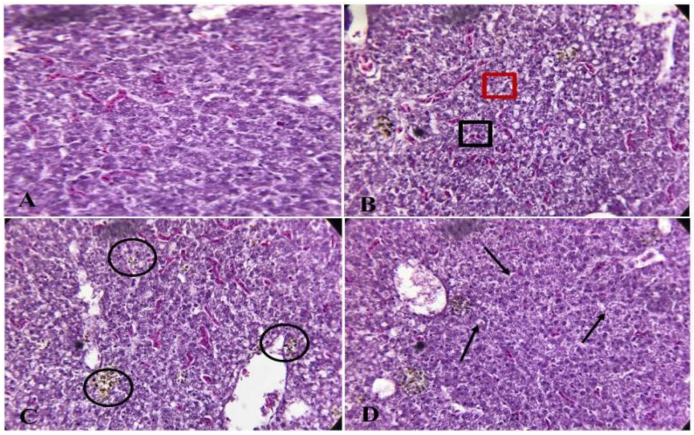

Fig. 2 Histopathological changes in liver

3.3.2 Kidney

Kidneys from T0 showed normal glomeruli, renal tubules, and hematopoietic tissues. In T1, mild congestion, hyperemia, and vacuolization of renal tubule cells were observed. Fish in T2 displayed marked tubular necrosis, pycnotic nuclei, and glomerular dilation. In T3, severe cortical damage, glomerular atrophy, capsular space dilation, and interstitial edema were evident (Figure 3).

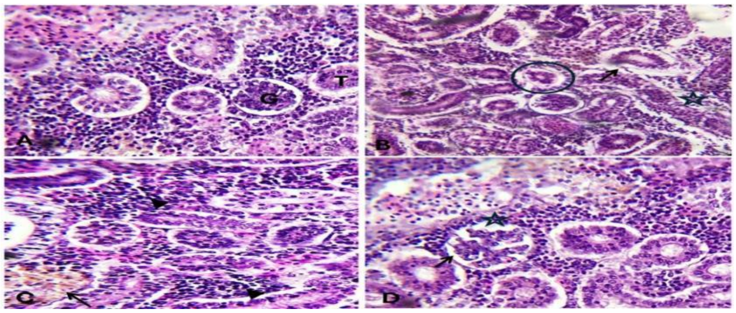

Fig. 3 Histopathological changes in kidney.

3.3.3 Gills

Gill architecture in T0 was normal, with well-structured primary and secondary lamellae. T1 exhibited epithelial damage, filament deformation, and lamellar thickening. In T2, vacuolation, blood pooling, and necrosis were evident. T3 showed extensive lamellar fusion, epithelial lifting, and complete necrosis of filaments, indicating severe respiratory impairment (Figure 4).

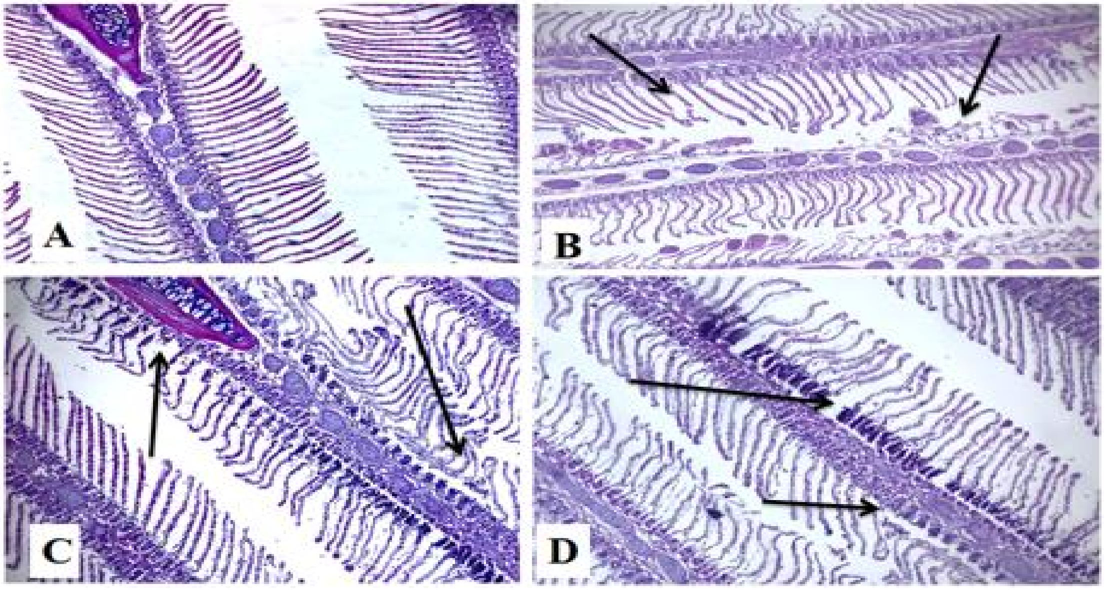

Fig. 4 Histopathological changes in gills

3.4 Hematological parameters

Hematological parameters across different time points (T0–T3) were noted over the study period. Red blood cells count decreased significantly from T0 to T3 at all measured intervals, with the control group (T0) consistently showing the highest values and T3 the lowest (p value= 0.0002). A similar significant declining pattern was observed for hemoglobin concentration, indicating a reduction in oxygen-carrying capacity as treatments progressed (p value=0.0003). White blood cell (WBC) counts followed a decreasing trend across T0 to T3, showing a gradual decline in leukocyte levels, which may reflect immunological or physiological changes over time (p=0.0004).

Mean corpuscular volume showed a slight decrease from T0 to T3, indicating a reduction in average RBC size across the groups(p=0.0012), while mean platelet volume also declined steadily, pointing to changes in platelet morphology and possibly production(p=0.0021). Mean corpuscular hemoglobin concentration (MCHC) remained relatively stable in T0 but showed slight decline in T1–T3 groups (p=0.0001), whereas mean corpuscular hemoglobin (MCH) values showed minor fluctuations with an overall decreasing trend (p=0.0012), particularly in the T3 group. These results show the effect of different treatment induced changes in hematological values, with the control groups maintaining high values, while the experimental or treatment groups (T1–T3) having gradual reductions due to potential dose- or time-dependent effect of the treatments.

Table 4.2: *Hematological parameters of Nile tilapia exposed to mercury-contaminated diets*

Parameter	T0 (Control)	T1 (0.5 mg/kg)	T2 (5 mg/kg)	T3 (10 mg/kg)	p-value
Hemoglobin (g/dL)	4.26 ± 0.12	2.40 ± 0.10	1.80 ± 0.08	1.45 ± 0.06	0.0003
RBC Count ($\times 10^6/mm^3$)	1.112 ± 0.05	0.920 ± 0.04	0.759 ± 0.03	0.589 ± 0.02	0.0002
WBC Count ($\times 10^3/mm^3$)	7.33 ± 0.03	6.02 ± 0.02	4.80 ± 0.01	4.01 ± 0.01	0.0004
Mean Platelet Volume ($\times 10^5/\mu L$)	213,000 ± 764	203,167 ± 1,014	195,167 ± 1,302	165,167 ± 1,302	0.0021
Mean corpuscular volume (fL)	128.57 ± 2.1	117.13 ± 1.9	102.10 ± 2.2	95.60 ± 1.8	0.0006
Mean corpuscular hemoglobin (pg)	36.80 ± 0.50	36.40 ± 0.45	34.10 ± 0.40	32.30 ± 0.35	0.0012
Mean corpuscular hemoglobin concentration (g/dL)	33.87 ± 0.41	31.80 ± 0.36	30.80 ± 0.34	30.00 ± 0.32	0.0001

4 Discussion

The results of this study show the effect of methyl mercury on various water parameters, consistent with many research that have explored the toxicological effects of mercury in aquatic environments. Mercury-contaminated diets induced a non-significant reduction in water pH. Although statistically unremarkable (p = 0.98), the observed decline reflects a trend toward acidification, consistent with previous studies that associate heavy metal exposure with lowered water pH due to acidic metabolic by-products and metal hydrolysis reactions (de Almeida et al., 2019; Jiménez-Oyola et al., 2023). Acidic water enhances the solubility and reactivity of heavy metals, thereby increasing their bioavailability and toxicity to aquatic life. According to Kibria et al. (2016), pH below 6.5 can increase mercury uptake in fish by up to 35%, leading to heightened oxidative stress and enzymatic disruption. Mercury significantly altered nitrogen metabolism. Elevated ammonia suggests inhibition of nitrifying bacteria and disrupted gill function. These findings echo Duan et al. (2024), who documented that mercury compromises ammonia excretion pathways in fish by damaging branchial ion transporters.

Hematological changes serve as sensitive biomarkers of physiological stress in fish exposed to environmental contaminants. Mercury caused significant hematological dysregulation in a dose-dependent manner. Mercury likely inhibits heme synthesis and induces hemolysis via oxidative stress. Ahmadivand et al. (2020), reported similar declines in various teleosts. Ciji et al., 2021) found that exposure to just 0.05 mg/L of methylmercury in zebrafish caused 40% hemoglobin depletion within 30 days. RBCs values significantly dropped across treatment groups. This reduction may result from suppressed erythropoiesis or direct lysis due to lipid peroxidation in RBC membranes (Calbet et al., 2006). In tilapia exposed to mercury in Brazil, found up to 50% RBC reduction, indicating impaired hematopoietic function. Vianna et al. (2019), reported increased leukocytosis in response to tissue inflammation and microbial invasion in mercury-exposed fish. WBC elevation may indicate the onset of systemic inflammation and adaptive immune stimulation. MCV and MCHC values reduced significantly that indicate microcytic hypochromic anemia. MPV values also declined, while RDW also dropped. Meenakshi et al. (2023) added that chronic mercury stress leads to thrombocytopenia, possibly explaining platelet volume reduction.

The liver, being the central organ for detoxification and metabolism, is particularly vulnerable to mercury toxicity. In this study, histopathological examination revealed progressive liver damage in a dose-dependent manner. Fish from the control group had normal hepatic architecture, while those in T1 showed mild vacuolar degeneration. In T2, hepatocytes showed swollen cytoplasm, bile duct proliferation, and congestion of blood sinusoids. The most severe changes were recorded in T3, where complete lobular disorganization, necrosis, and infiltration of inflammatory cells were observed. These lesions indicate cellular damage due to oxidative stress and disrupted metabolic function. Similar liver alterations were reported by de Oliveira et al. (2002), who found increased lipid peroxidation and glutathione depletion in mercury-exposed tilapia. The appearance of melano-macrophage centers and hyperplasia is a defensive response of hepatic tissues attempting to sequester and detoxify mercury. This pattern reflects the findings of Singh et al. (2024), who also associated hepatocyte vacuolization and necrosis with mercury-induced mitochondrial damage and impaired protein synthesis. Mercury had a pronounced nephrotoxic effect, evidenced by extensive kidney damage in treated groups. Control fish showed intact renal tubules and glomeruli, but in T1, mild tubular dilation was seen. T2 samples displayed cellular degeneration, necrosis of tubular epithelium, and narrowed glomerular spaces. The worst damage appeared in

T3, where tubules were necrotic, and glomeruli had collapsed. Protein casts in renal tubules further indicated compromised filtration and reabsorption. This damage likely results from mercury's binding with thiol groups in renal enzymes, disrupting cellular respiration and triggering apoptosis. Similar histological findings were reported by Agyemang et al. (2020) in tilapia from mercury-polluted lakes. Moreover, de Almeida et al. (2019) noted that chronic mercury exposure in fish led to interstitial fibrosis and infiltration of mononuclear cells in kidney tissues. These cumulative changes impair the osmoregulatory and excretory capacities of fish, increasing their vulnerability to other environmental stressors.

The gills, as the primary site for respiration and ion exchange, are highly sensitive to waterborne pollutants. In the current study, fish from T1 showed slight epithelial lifting and increased mucus secretion. T2 samples revealed lamellar fusion, cellular hypertrophy, and mild necrosis, while T3 tissues displayed extensive epithelial hyperplasia, clubbing of secondary lamellae, and hemorrhaging. These lesions disrupt normal gas exchange and compromise ionic regulation. Reda et al. (2025) observed similar patterns, noting that mercury exposure leads to structural collapse of gill lamellae and thickening of the basal membrane. The observed changes are a result of defense mechanisms such as increased mucus production and cellular regeneration in response to mercury irritation. Maharajan et al. (2016) suggested that such gill remodeling under chronic mercury exposure compromises aerobic metabolism and contributes to behavioral abnormalities.

5 Conclusion

Mercury contamination resulted in significant alterations in hematological parameters, including anemia and immune dysfunction, alongside severe histopathological damage in key organs such as the liver, kidneys, and gills. These findings show the critical issue of mercury bioaccumulation in aquatic ecosystems and its implications for food safety and public health. As Nile tilapia is an important species in global aquaculture, the risks associated with mercury exposure necessitate immediate environmental and regulatory interventions.

Conflict of Interest

Authors declare no conflict of interest.

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