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Bioaccumulation of Nickel and Lead in Fish Tissues and Associated Histological Impacts in Head Balloki, Kasur

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Abstract

Fish serve as a vital food source and, are characterized by streamlined bodies, fins, and gill-based respiration. Heavy metals are essential in trace amounts for metabolic processes but become toxic at abnormal concentrations. This study evaluates the bioaccumulation of nickel and lead in various fish tissues and the associated histological impacts caused by contamination in Head Balloki, Kasur. Samples of three fish species—*Catla catla*, *Wallago attu*, and *Labeo rohita*—were collected with the assistance of experienced fishermen. Heavy metal concentrations in organs (liver, gills, intestine, kidney, muscle, skin) and freshwater were determined using Atomic Absorption Spectrometry. Results were compared against the World Health Organization's permissible limit of 0.5 mg/kg. The concentrations of both nickel and lead exceeded WHO standards, with the highest levels observed in *Wallago attu*, followed by *Catla catla* and *Labeo rohita*. Nickel accumulation was highest in the kidneys of *Wallago attu* (66 mg/kg) and lowest in the muscles of *Labeo rohita* (4.75 mg/kg). Lead followed a similar pattern, with the highest levels found in the muscles of *Wallago attu* (56 mg/kg). The elevated heavy metal levels likely result from agricultural, industrial, and human activities around Head Balloki, which negatively impact fish health and pose risks to human consumers. The bioaccumulation of lead and nickel in fish tissues poses significant risks to human health. When people consume fish contaminated with these heavy metals, they can be exposed to toxic levels that may lead to neurological, developmental, and cardiovascular problems. Lead exposure is particularly harmful to children, affecting cognitive development and behavior, while nickel can cause allergic reactions, kidney damage, and an increased risk of certain cancers. Long-term consumption of contaminated fish can lead to chronic toxicity, underscoring the need for stringent environmental monitoring and management practices to ensure food safety.

Keywords: Bioaccumulation, heavy metals, *Catla catla*, *Labeo rohita*, *Wallago attu*, Head Balloki

Introduction

Water is one of the most abundant natural resources and a fundamental necessity for life. It plays a critical role in various biological processes, including metabolism, energy production, and cellular development (Can and Austin, 2024). As aquatic vertebrates, fish are entirely dependent on water for respiration, nutrition, waste excretion, reproduction, and osmoregulation. They inhabit a wide range of aquatic ecosystems, from freshwater to marine environments, and constitute a vital component of the global food supply due to their high nutritional value, particularly their rich protein content (Geist and Hawkins 2016).

Despite their ecological and economic importance, fish are increasingly vulnerable to environmental contamination, particularly from heavy metals (Can et al. 2012). Heavy metals, including nickel (Ni) and lead (Pb), are naturally occurring elements required in trace amounts for metabolic processes but become toxic at elevated concentrations. These metals enter aquatic ecosystems through industrial and agricultural runoff, domestic sewage, and other anthropogenic sources (Tulasi et al., 1992). Nickel,is an essential trace element for some biological processes, becomes toxic at raised concentrations. Research has demonstrated that chronic exposure to nickel can lead to bioaccumulation in fish tissues, particularly in the liver, kidney, and gills. These organs are critical for detoxification and respiration, making them primary targets for metal-induced tissue damage. Nickel exposure often results in histopathological changes, such as necrosis, swelling, and deterioration of hepatocytes in the liver (Pane et al., 2004). Fish gills exposed by nickel causes epithelia damage, hyperplasia, and lamellar fusion, that causes prejudice oxygen exchange and osmoregulatory functions (Athikesavan e al., 2006; Jasim et al., 2022).

Lead is a non-essential metal known for its high toxicity even at low concentrations. In aquatic environments, lead primarily accumulates in the gills, liver, kidney, and brain of fish (Lee et al., 2019). The liver, being a major detoxification organ, shows noticeable variations including cellular vacuolization, pyknotic nuclei, and sinusoidal dilation upon lead exposure (Khidr et al., 2012). Kidney tissues also exhibit glomerular degeneration and tubular necrosis, which impair excretory functions (Javed & Usmani, 2011). In addition to morphological changes, lead induces hematological and biochemical abnormalities. It alters hemoglobin levels, red blood cell counts, and plasma enzyme activities, disrupting normal physiological homeostasis (Kim et al., 2025). In gills, lead exposure causes structural damage, such as lamellar disorganization and increased mucous cell proliferation, which compromises respiration (Curcio et al., 2022). Oxidative stress is another major outcome of lead toxicity. Several studies have linked lead exposure to elevated reactive oxygen species (ROS) production and weakened antioxidant defenses in fish tissues (Lopes et al., 2016; Lee et al., 2019). These oxidative disparities subsidize to cellular damage and apoptosis, affecting the overall health and survival of fish.

Once absorbed by fish through the gills, diet, or surrounding water, heavy metals bioaccumulate in various organs, resulting in physiological disturbances and histopathological alterations (Can et al. 2012; Talib Jawad S et al., 2022). These include lamellar fusion, hypertrophy, and hyperplasia in the gills, as well as degeneration and necrosis of liver cells. The persistent accumulation of heavy metals in fish tissues poses considerable health risks to human consumers, highlighting its significance as a global public health concern. Therefore, detecting and monitoring heavy metals is crucial not only for environmental protection but also for ecological perspective such as food web alterations (De Giorgi et al. 2024; Gencer, 2025).

This study investigates the bioaccumulation of nickel and lead in three fish species (*Catla catla, Wallago attu, and Labeo rohita*) collected from the Balloki Headworks, a critical water management structure on the Ravi River in Pakistan. The site plays a pivotal role in regulating irrigation and water supply for extensive agricultural lands. Its proximity to industrial and urban areas makes it a key location for assessing water quality and pollutant loads, as industrial effluents and agricultural runoff contribute to the introduction of contaminants, including heavy metals, into the aquatic ecosystem. Understanding these impacts is essential for sustainable water resource management and the protection of downstream ecosystems and communities. The primary objective of this study is to evaluate heavy metal concentrations in various fish tissues and assess their compliance with World Health Organization (WHO) standards. The findings underscore the effects of environmental contamination on fish health and the associated risks to human consumers.

Materials and Methods

Study Design

This study was designed to assess heavy metal contamination in water samples and fish tissues collected in October and November from upper stream of Headworks of Balloki, Lahore, Punjab. Gps location of. 31°21'56.9"N+74°02'57.9"E

Sample Collection

Water Samples

Water samples were collected from multiple sites upper stream of Headworks of Balloki, Lahore, Punjab. Gps location of. 31°21'56.9"N+74°02'57.9"E. Each 1000 mL sample was stored in HDPE bottles and transported to the Kinnaird College Laboratory under refrigerated conditions (-20°C). Samples were digested using aqua regia (HCl 37%: HNO3 67%) to analyze heavy metal content. Mineralization was performed with a microwave digester, (Wise Therm FM-15DAIHAN Scientific. CO. LTD made in Korea) and the levels of heavy metals (lead, and nickel) were determined using Atomic Absorption Spectrophotometer USA(Perkin Elmer AA700) (Radulescu et al., 2014).

Fish Samples

Fish specimens were collected using Malian nets and other traditional fishing gear. During the afternoon, the water temperature ranged between 25–26 °C. The captured fish were immediately transferred to cooled ice containers and transported to the laboratory at Kinnaird College. Three different species were examined: *Labeo rohita*, *Wallago attu*, and *Catla catla*. A total of nine individuals from each species were selected. From each specimen, four organs—kidney, liver, intestine, and gill—were dissected. Each organ was sectioned in triplicate for the purposes of heavy metal detection and histological analysis. The fish species were identified based on morphological characteristics (Ayanda et al., 2019). Each specimen was washed with Milli-Q water, descaled, and dried with blotting paper for further analysis (Quader et al., 2022).

Morphological Analysis

The physical parameters of the fish, including weight and total length, were measured using a weighing scale and measuring tape (Table 1). Environmental parameters such as water temperature and pH were also recorded morphological characteristics of each fish species were documented as per previous study (Younis et al., 2021).

Table 1. Morphological Characteristics of Fish Species

Specimen Name and Area	Skin Color	Weight (g)	Total Length (cm)
Labeo rohita (Rahu)A	Greyish brown from dorsal side and silvery sheen on flanks and whitish on belly	249	20
Labeo rohita (Rahu)B	Greyish brown from dorsal side and silvery sheen on flanks and whitish on belly	342	26
Labeo rohita (Rahu) C	Greyish brown from dorsal side and silvery sheen on flanks and whitish on belly	315	24.5
Wallago attu (Malli) A Bulloki	Greyish from dorsal and pale white from belly side	331	24
Wallago attu (Malli) B Bulloki	Greyish from dorsal and pale white from belly side	479	31.2
Wallago attu (Malli) C Bulloki	Greyish from dorsal and pale white from belly side	324	24
*Catla catla (*Thalla) A Bulloki	Silvery-gray body with a darker shade on the dorsal side and lighter whitish belly scales give shiny appearance.	178	19
*Catla catla (*Thalla*)* B	Silvery-gray body with a darker shade on the dorsal side and lighter whitish belly scales give shiny appearance.	188	20
Catla catla (Thalla) C	Silvery-gray body with a darker shade on the dorsal side and lighter whitish belly scales give shiny appearance.	205	21

Procedures

Fish Dissection

Fishes were dissected using standard dissection tools, and their organs were separated and stored in labeled containers.

Tissue Digestion

Fish tissues were subjected to acid digestion using nitric and per chloric acid in a 1:1 ratio as per standard protocol for tissue digestion for heavy metal study. After overnight digestion, additional acid was added, and the mixture was heated at 200–250°C until a clear solution was obtained. The samples were diluted to a final volume of 10 mL with distilled water. Lead and Nickel concentrations were measured using Atomic Absorption Spectrophotometry USA (Perkin Elmer AA700) at graphite furnace mode at wavelength of 232nm for nickel and 283.3nm for lead. Reference material had LOD 15µg/kg from scallop tissue and QA an Qccertified value was 2.4ppm while observed value read by instrument was 2.38ppm (Quader et al., 2022).

(BAFs values are identical to those represented in the graph, as the concentrations of nickel and lead in the water samples were 1 mg/L (equivalent to 1000 µg/L). Therefore, the values observed in the tissue samples correspond directly to the concentrations in the water.).

Organ Digestion

Selected fish organs were ashed in a muffle furnace at ~450°C. The ash was digested in nitric and sulfuric acids, filtered, and analyzed for nickel and lead using Atomic Absorption Spectrometer USA(Perkin Elmer AA700) as per above mentioned method (Sarkar et al., 2022).

Histological Analysis

Sample Preparation

Fish tissues (liver and gills) were fixed in a solution of 60% ethanol, 30% formalin, and 10% acetic acid for 3–4 hours at room temperature. Tissues were dehydrated in graded ethanol solutions,

cleared in xylene, and embedded in paraffin wax. Sections (5–10 µm) were cut using a microtome and stained with hematoxylin and eosin for microscopic analysis (Jabeen et al., 2013).

Steps in Histological Processing

Tissue samples were fixed in formaldehyde for 6–24 hours. Samples were treated with graded ethanol (70%–100%) to remove water. Xylene was used as a clearing agent. Specimens were embedded in paraffin wax at 60°C. Thin sections were cut, mounted on slides, and stained. Stained sections were examined under a light microscope (Khan et al., 2022)

Data Analysis

Data were expressed as mean ± standard error (SE). One-way ANOVA was conducted to compare lead concentrations among different fish species across each organ. Tukey's HSD post hoc test was applied for pairwise comparisons. A p-value less than 0.05 was considered statistically significant. Graphs were annotated with asterisks denoting significance: $p < 0.05$ (), $p < 0.01$ (), $p < 0.001$ (). Statistical analysis was performed using GraphPad Prism v9.

The concentration of heavy metals in water and fish tissues was compared with WHO standards for permissible limits. Data were analyzed and visualized using GraphPad Prism software and represented as graphs and charts (Alesci et al., 2022).

Results

Concentration of Nickel in Fish Organs

The analysis of Nickel concentrations in various organs of *Labeo rohita*, *Wallago attu*, and *Catla catla* collected from the Headworks of Balloki revealed levels exceeding the WHO permissible limit of 0.5 mg/kg.

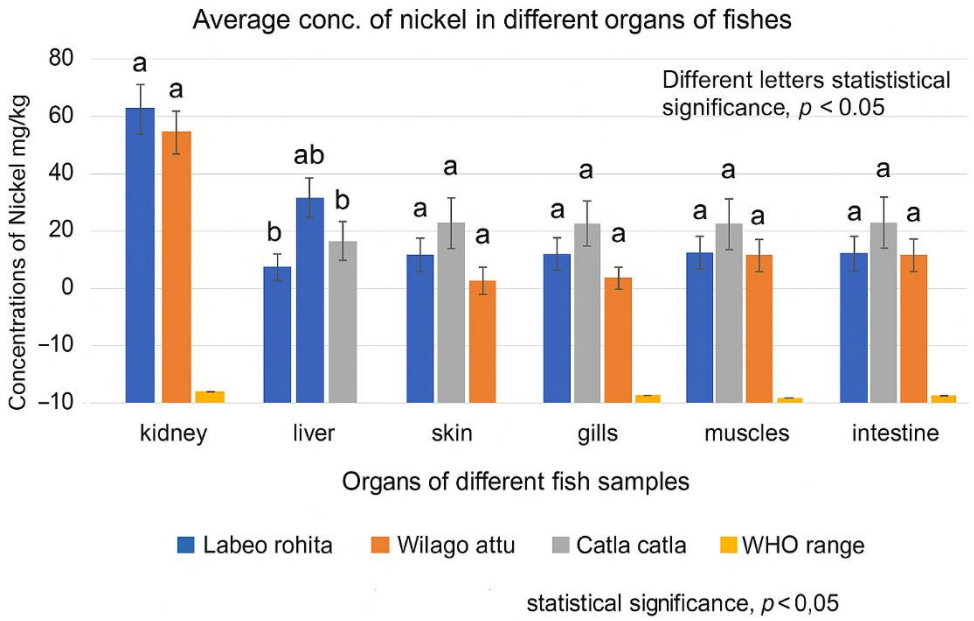


Figure 1. Concentration of Nickel in different organs of *Labeo rohita, Wallago attu* and *Catla*

The highest nickel concentration in the kidney was recorded in *Wallago attu* (Sample 2) at 84 mg/kg, while concentrations in *Labeo rohita* ranged from 44 to 72 mg/kg. In the liver, *Wallago attu* (Sample 1) exhibited the highest concentration at 54 mg/kg, with *Labeo rohita* showing values between 29 and 38 mg/kg. Similarly, in the skin, *Wallago attu* (Sample 1) had the highest concentration at 41 mg/kg, significantly surpassing *Labeo rohita* (7–9.75 mg/kg). Nickel concentrations were elevated in the gills across all species, with *Wallago attu* (Sample 3) reaching 31 mg/kg and *Labeo rohita* displaying values between 12 and 31 mg/kg. In muscle tissue, concentrations varied from 3 to 33 mg/kg, with the highest level observed in *Wallago attu* (Sample 3). Finally, in the intestine, *Catla catla* (Sample 1) exhibited the highest nickel concentration at 32 mg/kg, while *Labeo rohita* ranged from 6 to 7 mg/kg.

Concentration of Lead in Fish Organs

Lead concentrations in the fish samples exceeded the WHO permissible limit of 2 mg/kg across all examined organs. In the kidney, the highest concentration was recorded in *Wallago attu* at 56 mg/kg, while *Labeo rohita* exhibited levels ranging from 11.7 to 14.3 mg/kg. The liver showed a maximum lead concentration of 64 mg/kg in *Wallago attu* (Sample 3), whereas *Labeo rohita* and *Catla catla* displayed concentrations between 35.1 and 47 mg/kg. In the skin, *Wallago attu* had the highest concentration at 35 mg/kg, surpassing *Labeo rohita*, which ranged from 9 to 13 mg/kg. The gills exhibited the highest lead accumulation in *Catla catla* at 71 mg/kg, significantly exceeding the levels found in *Labeo rohita* and *Wallago attu*. Muscle tissue showed the highest lead concentration in *Wallago attu* at 61 mg/kg, with *Labeo rohita* ranging from 7 to 15 mg/kg. Finally, in the intestine, the peak concentration of lead was observed in *Catla catla* (Sample 3) at 57 mg/kg (Figure 2) .

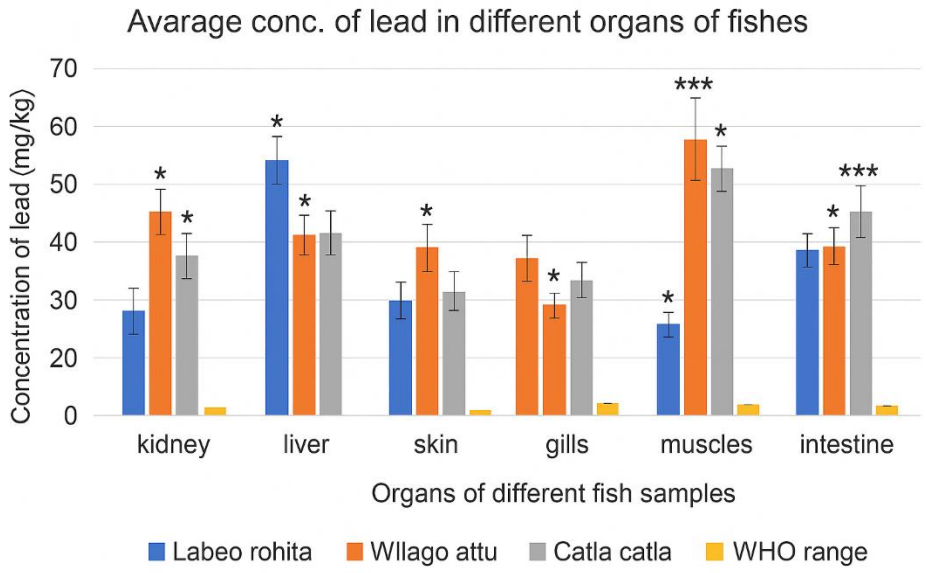


Figure 2. Average concentrations of Lead in different organs of *Labeo rohita, Wallago attu and Catla catla*

Concentration of Heavy Metals in Water Samples

The concentration of heavy metals in water samples from the Headworks of Balloki also exceeded WHO permissible limits:

Nickel: The concentration was 1.002 ppm, exceeded the WHO permissible limit of 0.005 ppm.

Lead: The concentration of 1.035 ppm was above the WHO permissible limit of 0.015 ppm.

Mean value of Nickel and Lead in different organs of *Labeo Rohita, Wallago attu* and *Catla*

The concentrations of nickel and lead in various organs of *Labeo rohita*, *Wallago attu*, and *Catla catla* exhibit significant variations. For nickel, the kidney showed the highest concentration in *Wallago attu* (66 µg/g), followed by *Labeo rohita* (55.89 µg/g), and the lowest in *Catla catla* (30.36 µg/g). In the liver, *Catla catla* had the highest nickel level (36.33 µg/g), followed by *Labeo rohita* (32.77 µg/g) and *Wallago attu* (27.66 µg/g). Nickel concentrations in the skin were highest in *Catla catla* (33 µg/g), slightly higher than *Wallago attu* (31 µg/g), and much lower in *Labeo rohita* (8.59 µg/g). For gills, *Catla catla* again showed the highest concentration (34.33 µg/g), followed by *Wallago attu* (31 µg/g) and *Labeo rohita* (24.11 µg/g). In muscles, *Wallago attu* had the highest nickel concentration (30.33 µg/g), with *Catla catla* (24.33 µg/g) and *Labeo rohita* (4.75 µg/g) trailing. Lastly, the intestines had the highest nickel concentration in *Wallago attu* (30.66 µg/g), followed by *Catla catla* (29.66 µg/g) and *Labeo rohita* (6.50 µg/g).

For lead concentrations, the kidney of *Wallago attu* contained the highest levels (39.66 µg/g), followed by *Catla catla* (31.8 µg/g) and *Labeo rohita* (13.10 µg/g). In the liver, *Labeo rohita* exhibited the highest lead concentration (45.99 µg/g), with *Wallago attu* (39 µg/g) and *Catla catla* (30 µg/g) having lower levels. The skin of *Wallago attu* showed the highest lead content (31.77 µg/g), followed by *Catla catla* (11.66 µg/g) and *Labeo rohita* (11.5 µg/g). For gills, *Labeo rohita* had the highest lead levels (21.66 µg/g), followed by *Wallago attu* (21 µg/g) and *Catla catla* (18.66 µg/g). In muscles, *Wallago attu* had the highest lead concentration (56 µg/g), followed by *Catla catla* (48.66 µg/g) and *Labeo rohita* (11.09 µg/g). Lastly, the intestines of *Catla catla* exhibited the highest lead levels (44 µg/g), followed by *Wallago attu* (33.33 µg/g) and *Labeo rohita* (29.55 µg/g). Overall, the distribution of nickel and lead among the species highlights distinct organ-specific accumulation patterns, with *Wallago attu* and *Catla catla* generally showing higher concentrations than *Labeo rohita* in most organs (Habib et al., 2023).

Both Nickel and Lead concentrations were significantly elevated in fish tissues and water samples, indicating bioaccumulation and potential ecological and health risks. Among the species studied, *Wallago attu* showed the highest accumulation of heavy metals across most organs, with *Catla catla* also displaying notable concentrations, particularly in gills and intestines. These findings underscore the need for stringent monitoring and regulation of heavy metal contamination in aquatic ecosystems.

Histological Findings

The histological analyses provided additional insights into the effects of heavy metals on fish organs:

Gills:The gills, being highly exposed to the surrounding environment, displayed varied responses among species. *Catla catla* exhibited muscle degeneration and blood vessel congestion, likely due to Lead toxicity, corroborating earlier findings (El-Naggar et al., 2009). *Wallago attu* showed relatively mild degeneration, and *Labeo rohita* maintained a largely normal architecture, possibly reflecting its mid-water feeding behavior, which reduces direct exposure to contaminants.

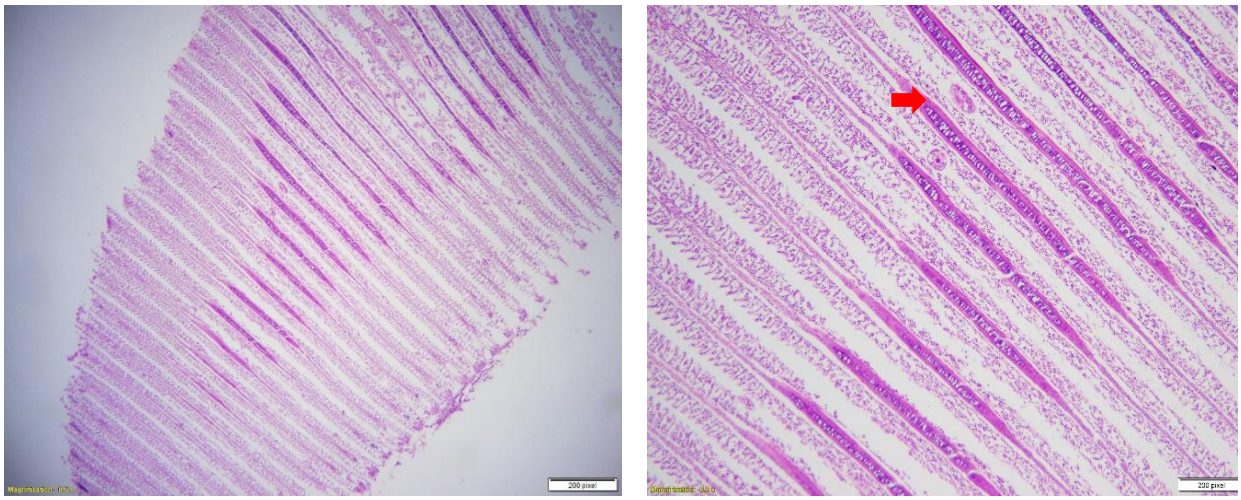
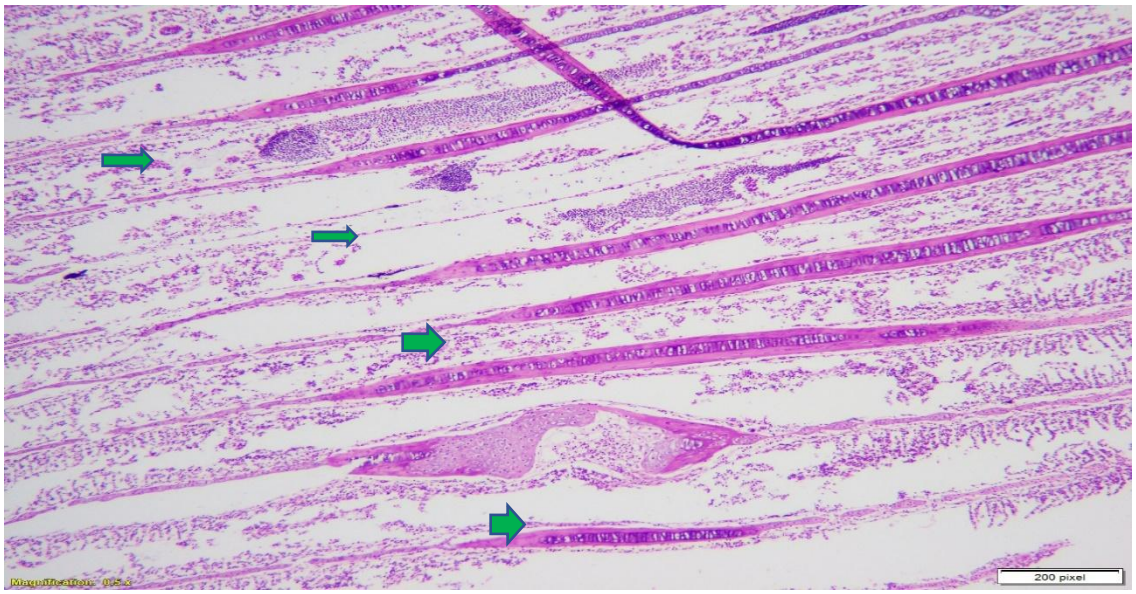


Figure 1.The photomicrograph of fish gill tissue shows relatively well-preserved lamellar architecture, but with notable pathological changes indicative of mild chronic gill disease. The secondary lamellae appear thickened and slightly fused in several regions, suggesting epithelial hyperplasia. Some lamellar capillaries appear congested (arrow) and disruption of normal respiratory surface. 4x and 10x

(Staining with Hematoxylin and Eosin)



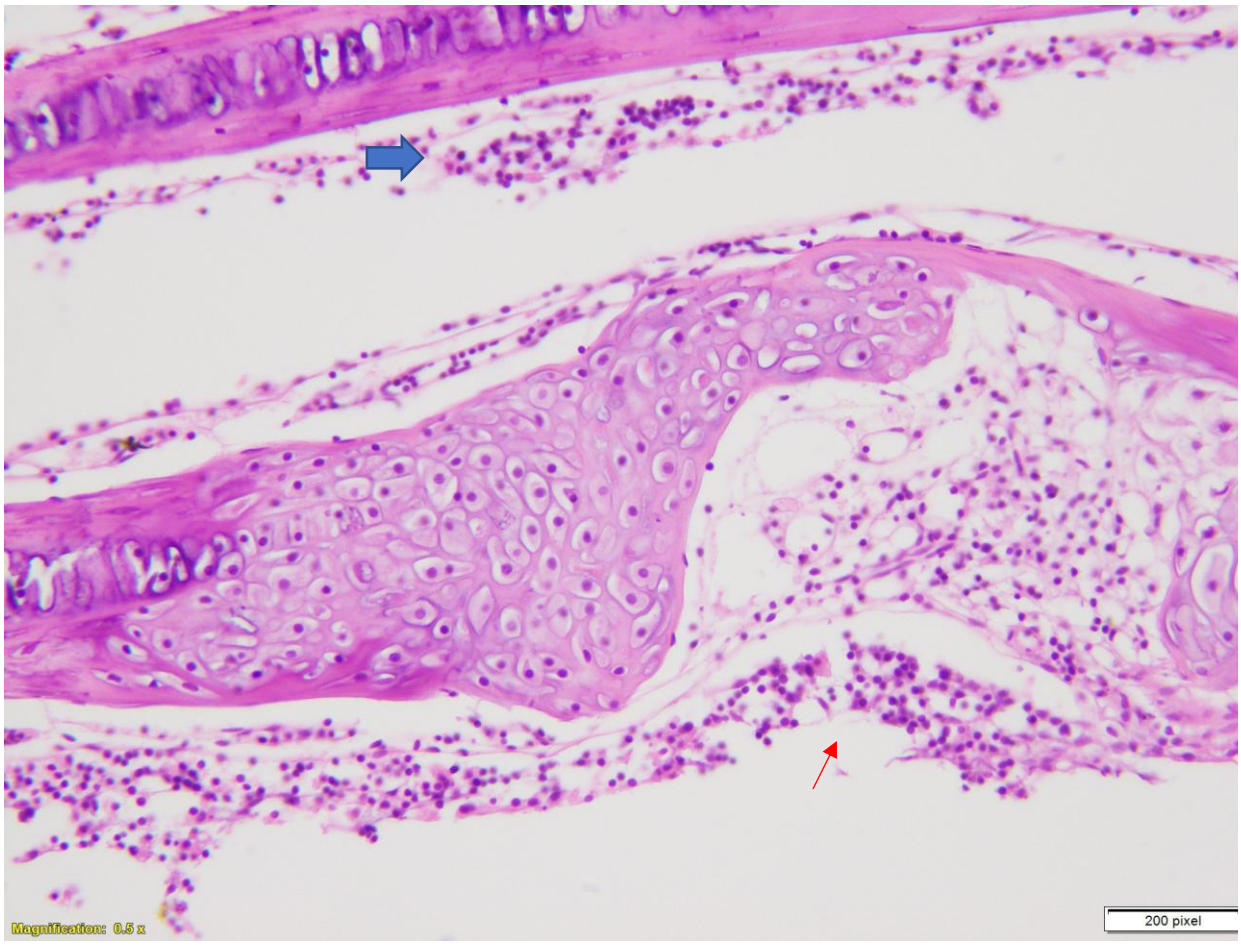
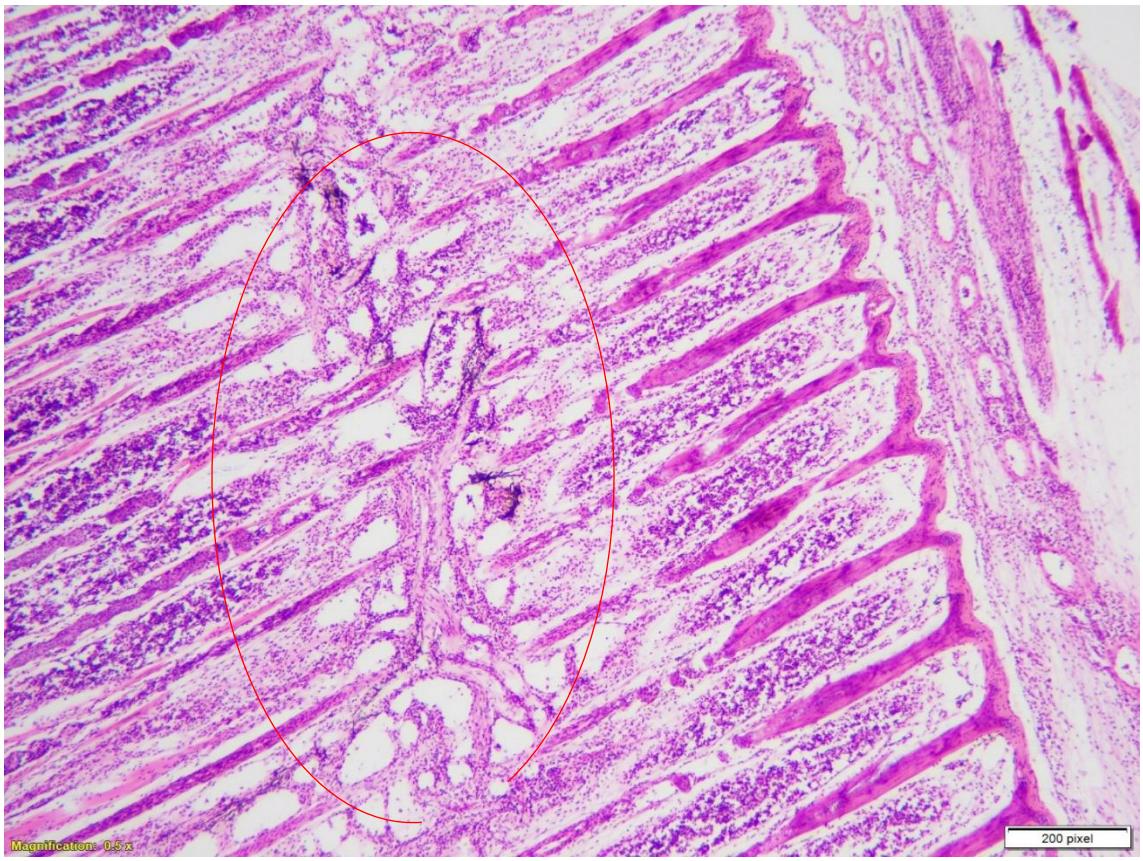
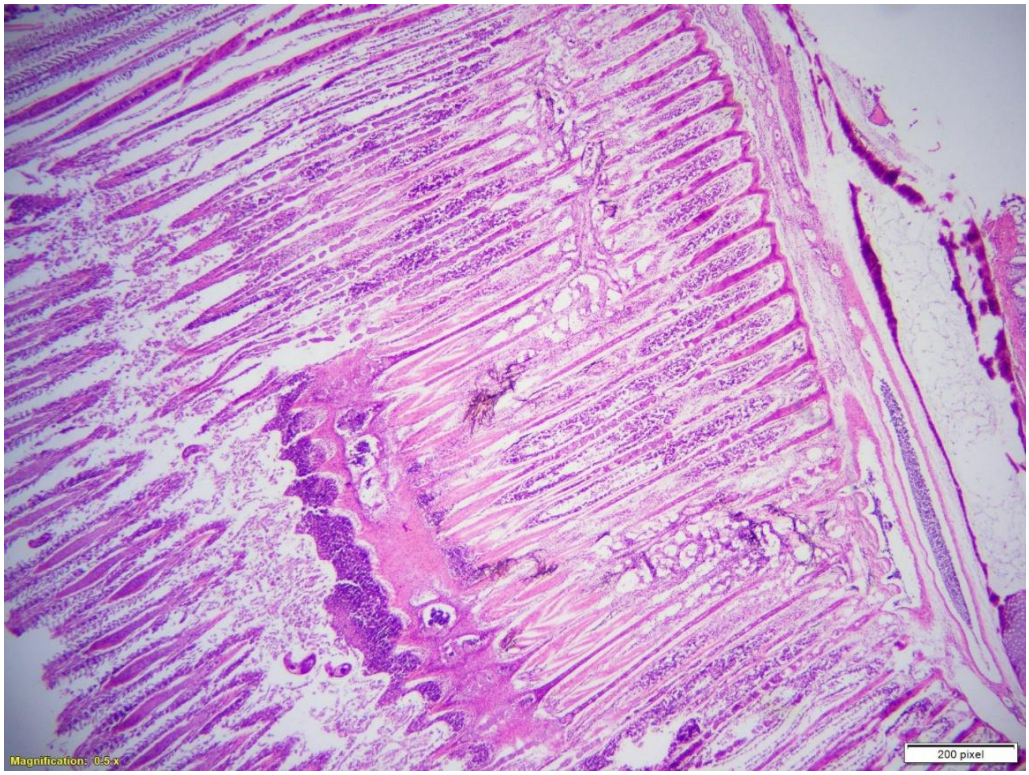


Figure 2 Photomicrograph of the fish gill demonstrates well-defined gill lamellae radiating from the primary gill filaments preserving the normal branchial architecture. However, multiple pathological features are present indicating chronic gill inflammation and mild hyperplasia. Several lamellae exhibit epithelial lifting and diffuse cellular infiltrates (arrowhead) predominantly composed of mononuclear inflammatory cells are scattered across the interlamellar spaces and filament core, indicative of a chronic inflammatory response. There is focal secondary lamellar fusion (arrows). 4x,10x and 40. (staining with Hematoxylin and Eosin)


Magnification: 0.5 x
200 pixel


Magnification: 0.5 x
200 pixel

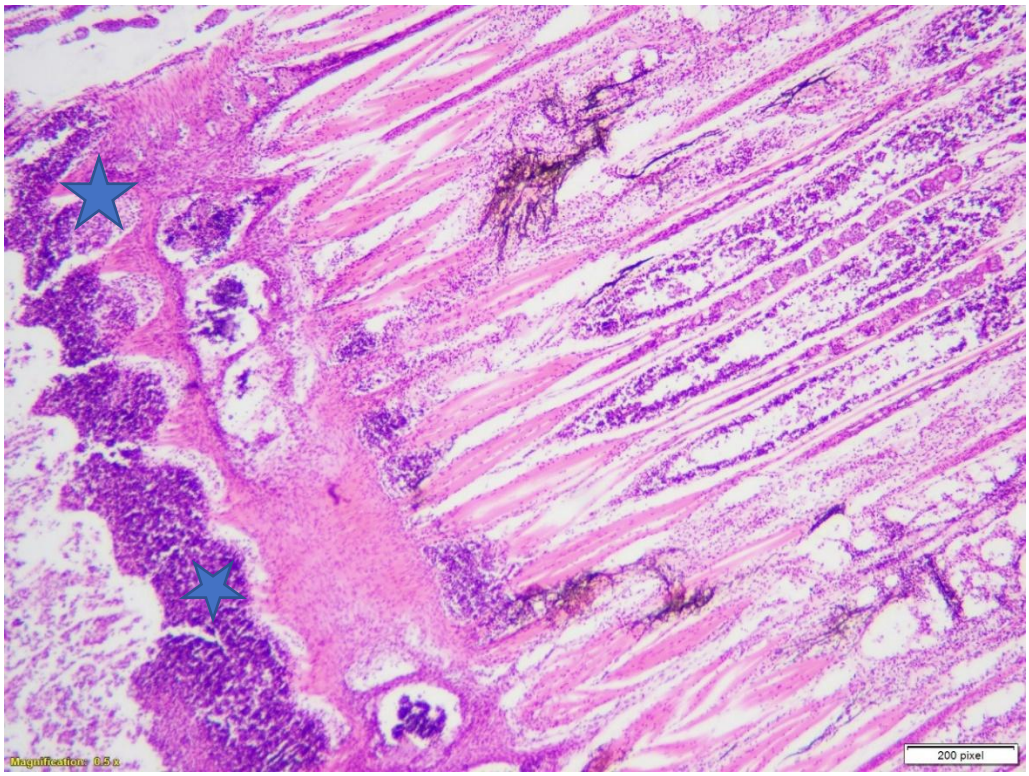
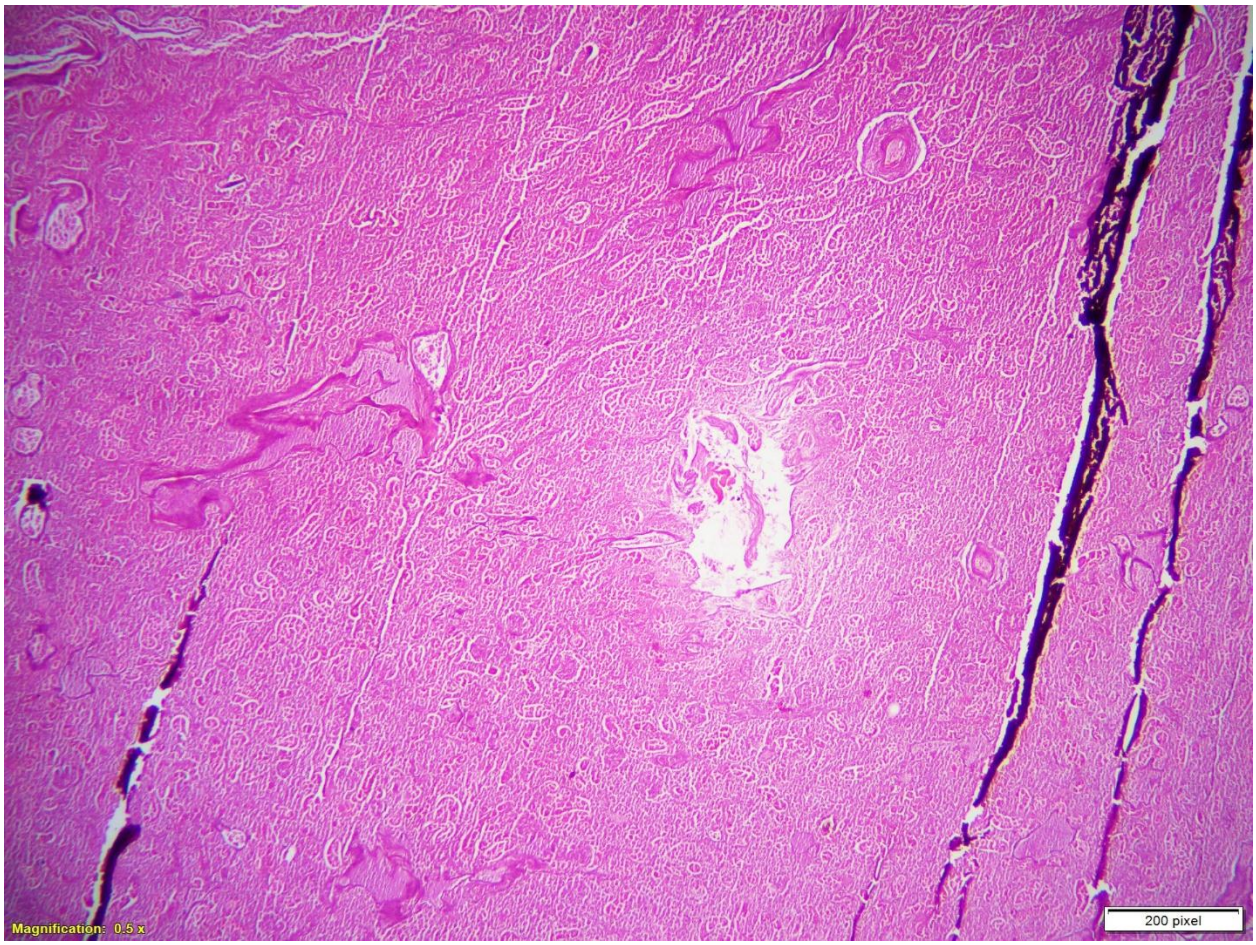
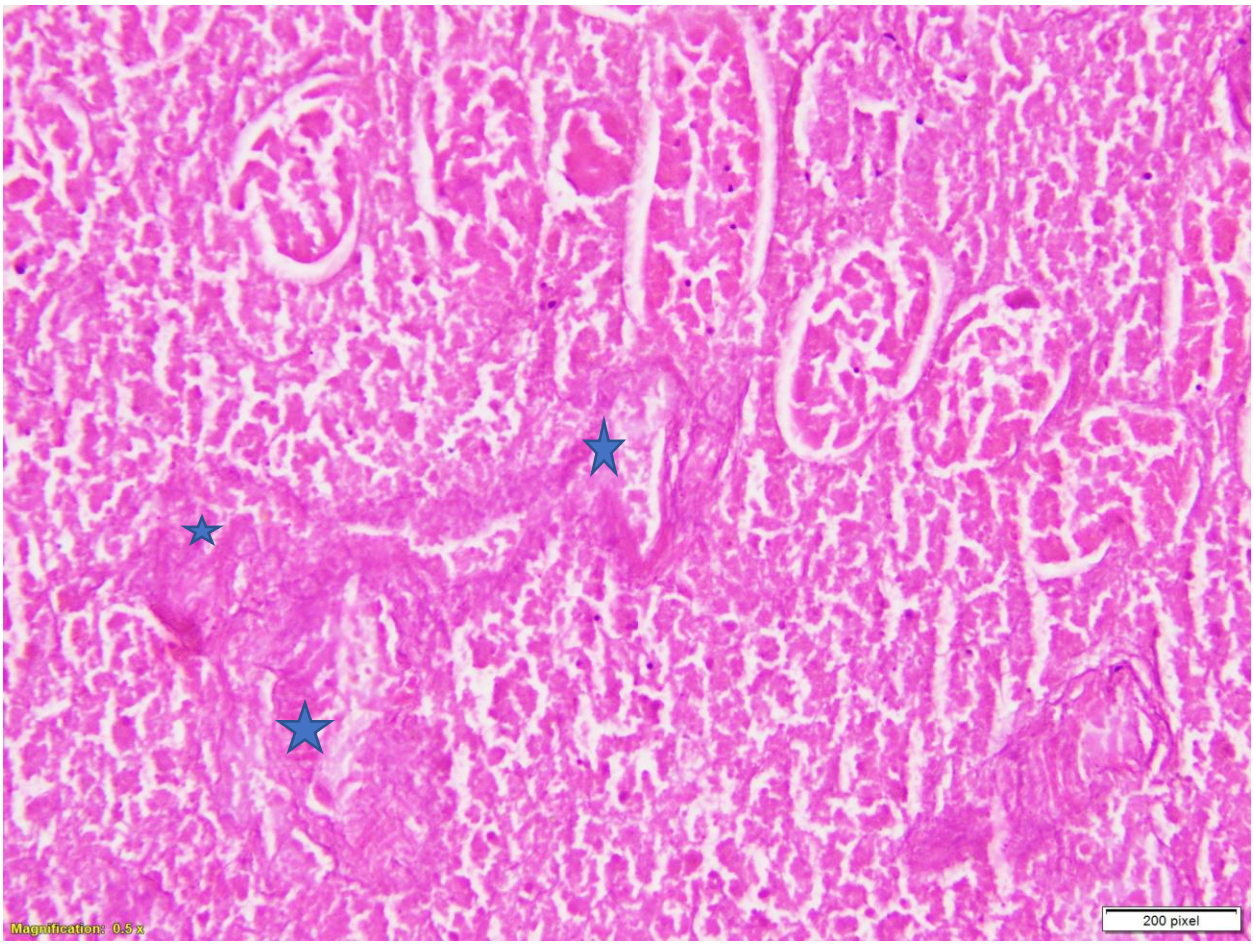


Figure 3. The photomicrograph of fish gill tissue demonstrates marked pathological alterations consistent with severe chronic bronchitis and lamellar remodeling. The primary and secondary lamellae show significant epithelial hyperplasia leading to fusion of adjacent lamellae (circle) and loss of distinct respiratory structures. There is prominent inflammatory cell infiltration (arrow) in the gill arch and filament core, particularly lymphocytes and mononuclear cells, suggesting chronic inflammation. Multiple areas exhibit lamellar clubbing, edema, and mucous cell proliferation (star). The architectural distortion compromises the gill's respiratory function. 4x, 10x and 40x

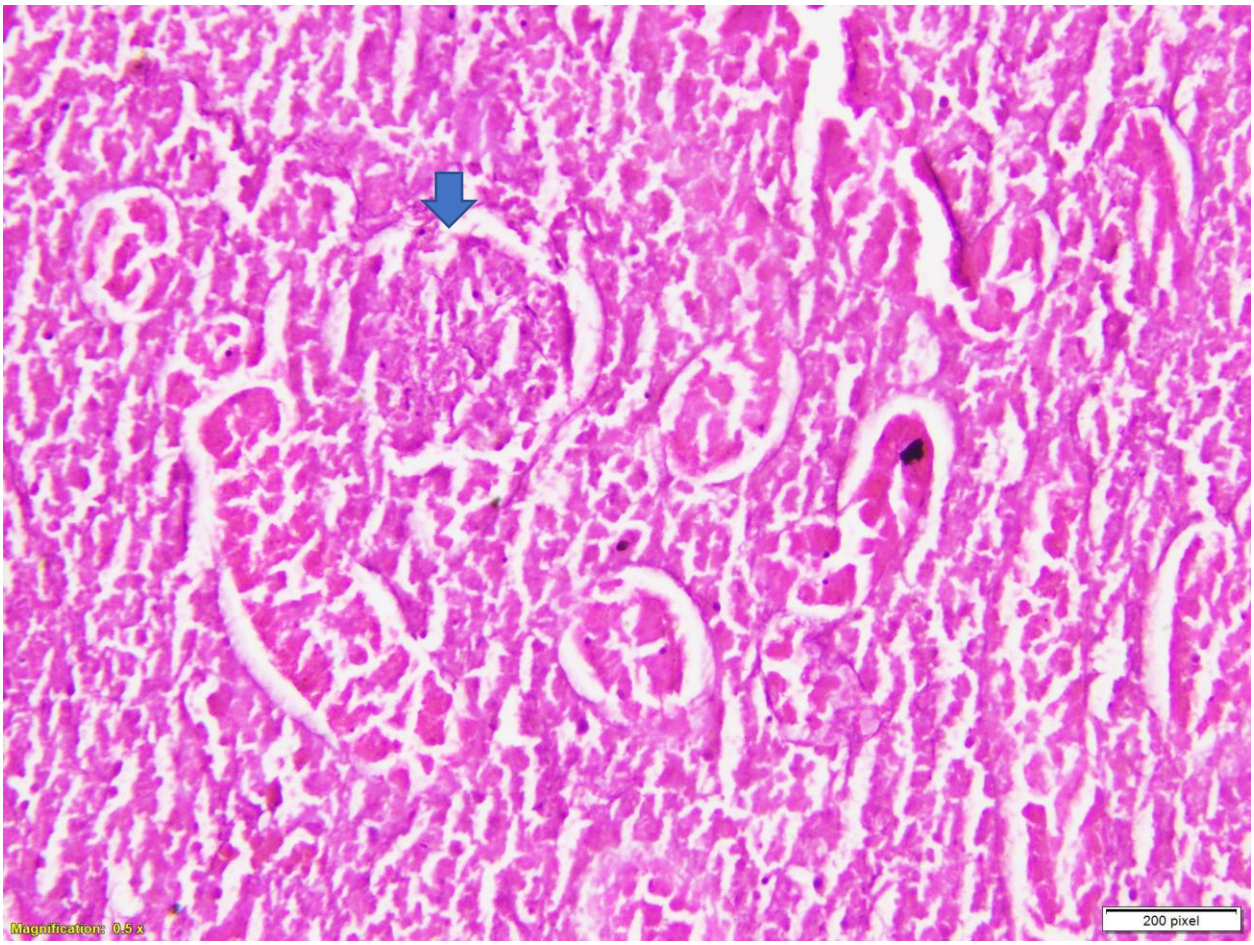
Kidney:The kidneys of all species exhibited morphological aberrations to varying degrees. *Wallago attu* showed the most severe changes, including necrosis, vacuolization, and hemosiderin deposits, indicative of heavy metal-induced oxidative stress and nephrotoxicity (Mario D.G. et al., 2022). *Labeo rohita* exhibited mild degeneration, reflecting its comparatively lower metal burden.



299 4(a)

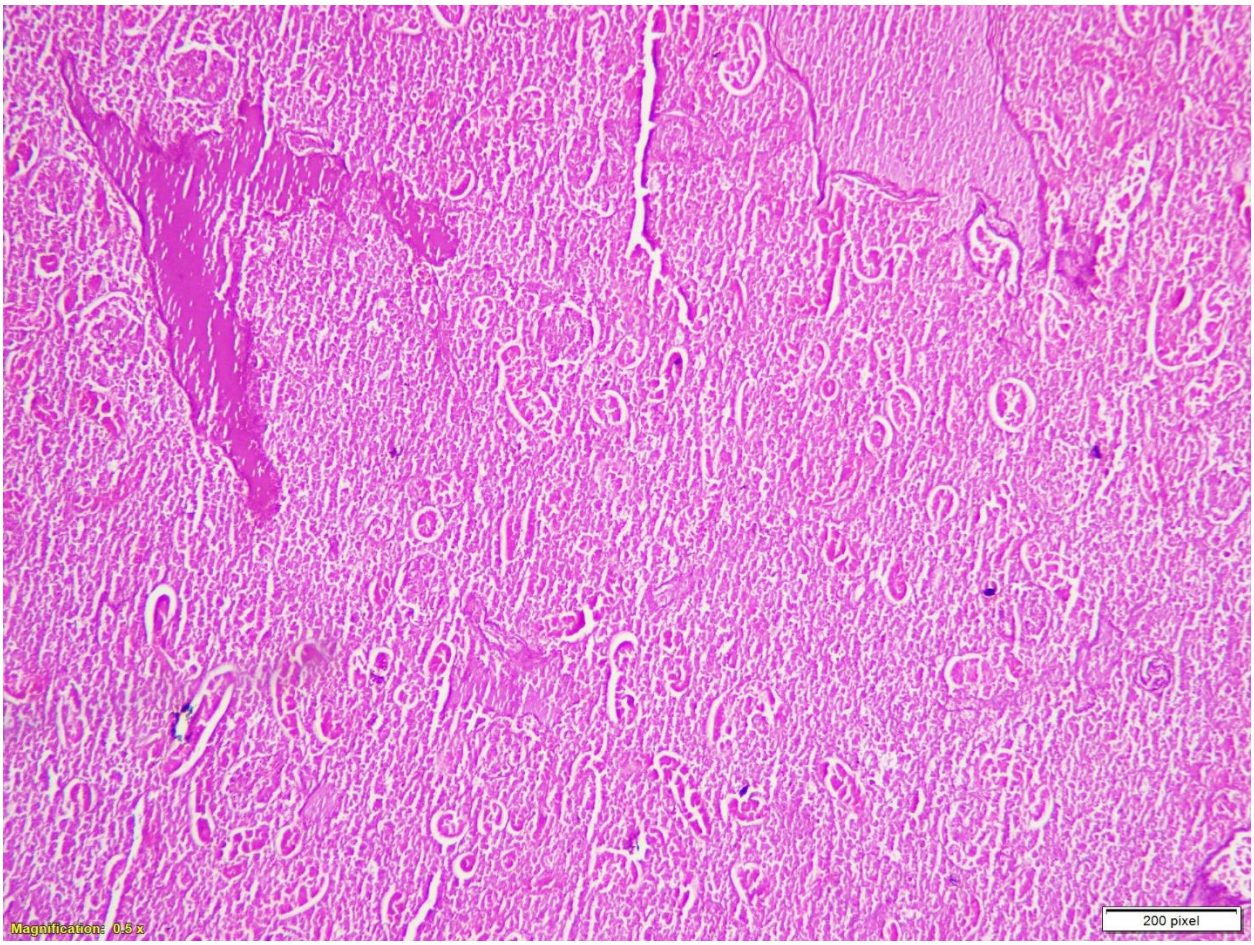


4(b)

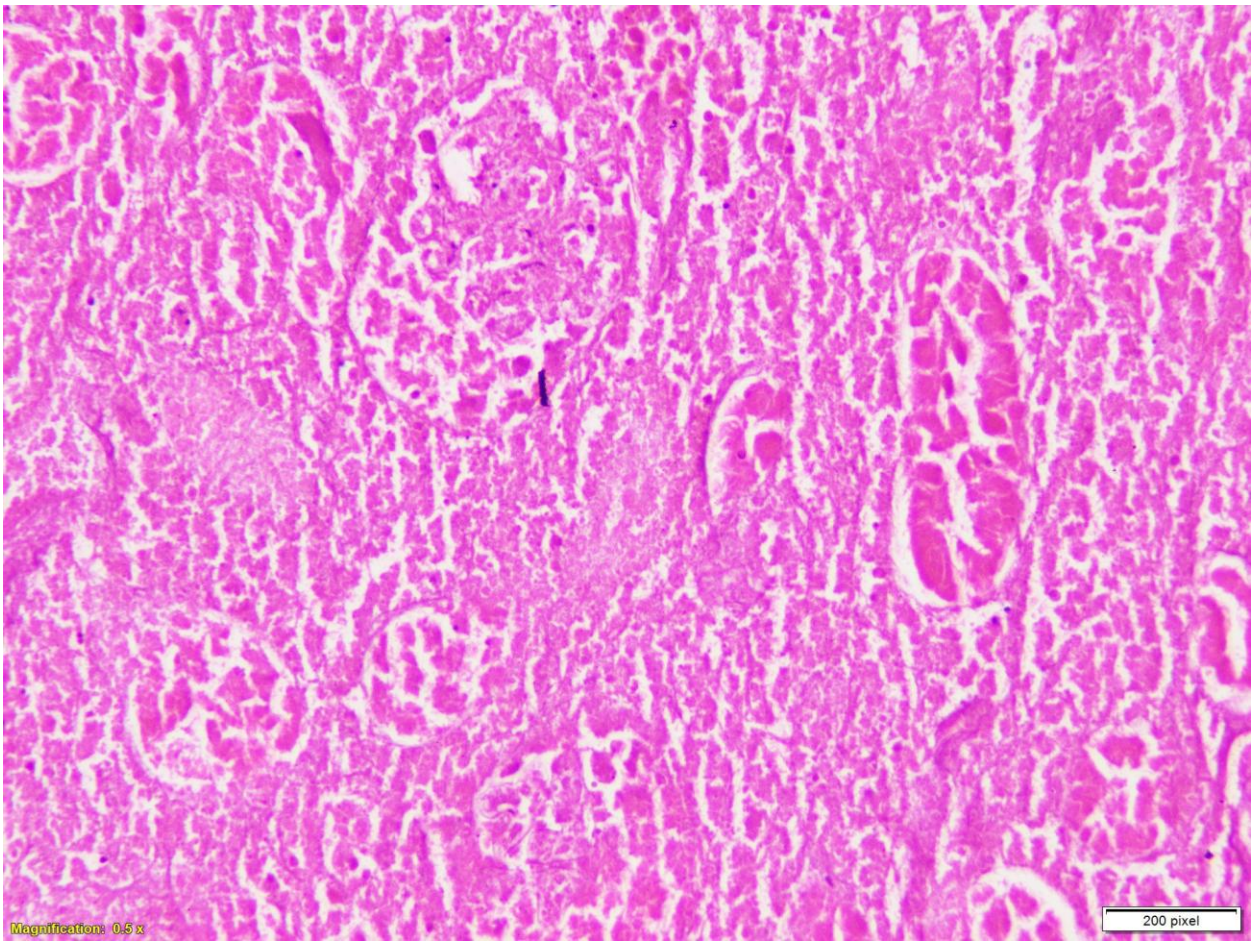


4 (c)

Figure 4(a,b,c) Photomicrograph of fish kidney displays renal parenchyma characterized by densely packed renal tubules and glomeruli, consistent with normal teleost renal architecture. However, histopathological alterations are evident. The renal tubules exhibit dilation and mild degenerative changes in epithelial lining cells (star), such as cytoplasmic granularity and mild vacuolization, suggestive of early tubular injury. Several glomeruli appear shrunken , with widened Bowman's spaces, indicating possible glomerular degeneration (arrow). 4X, 10X (staining with Hematoxylin and Eosin)



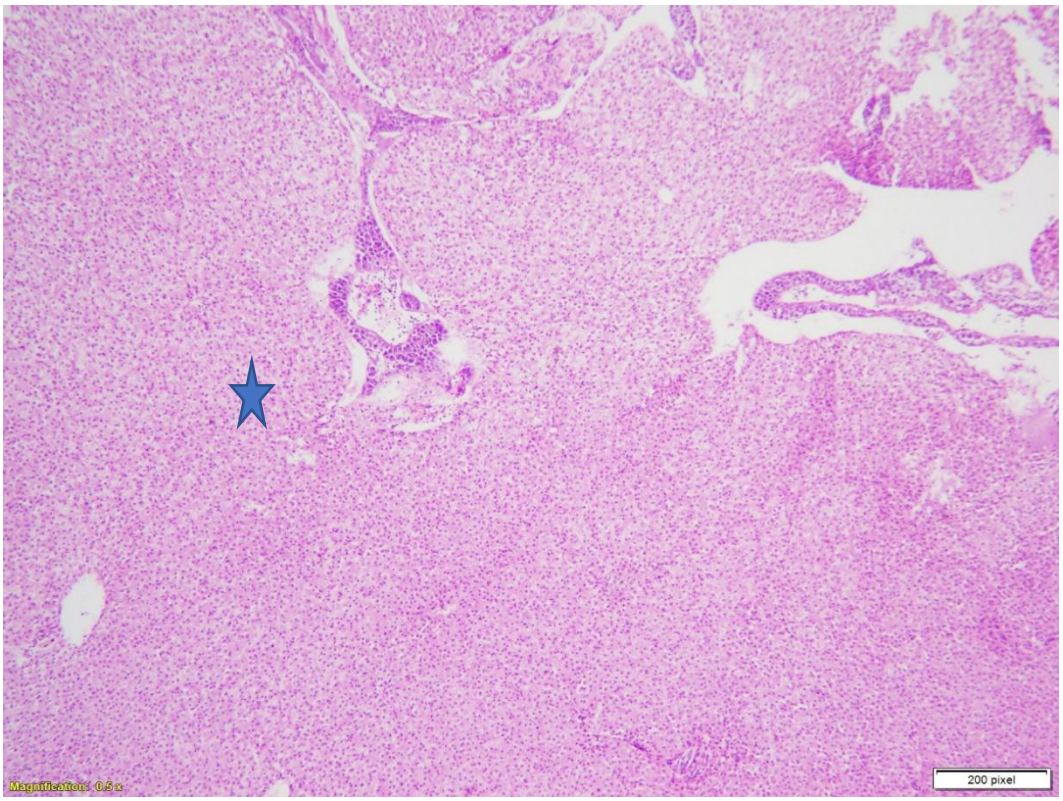
5(a)



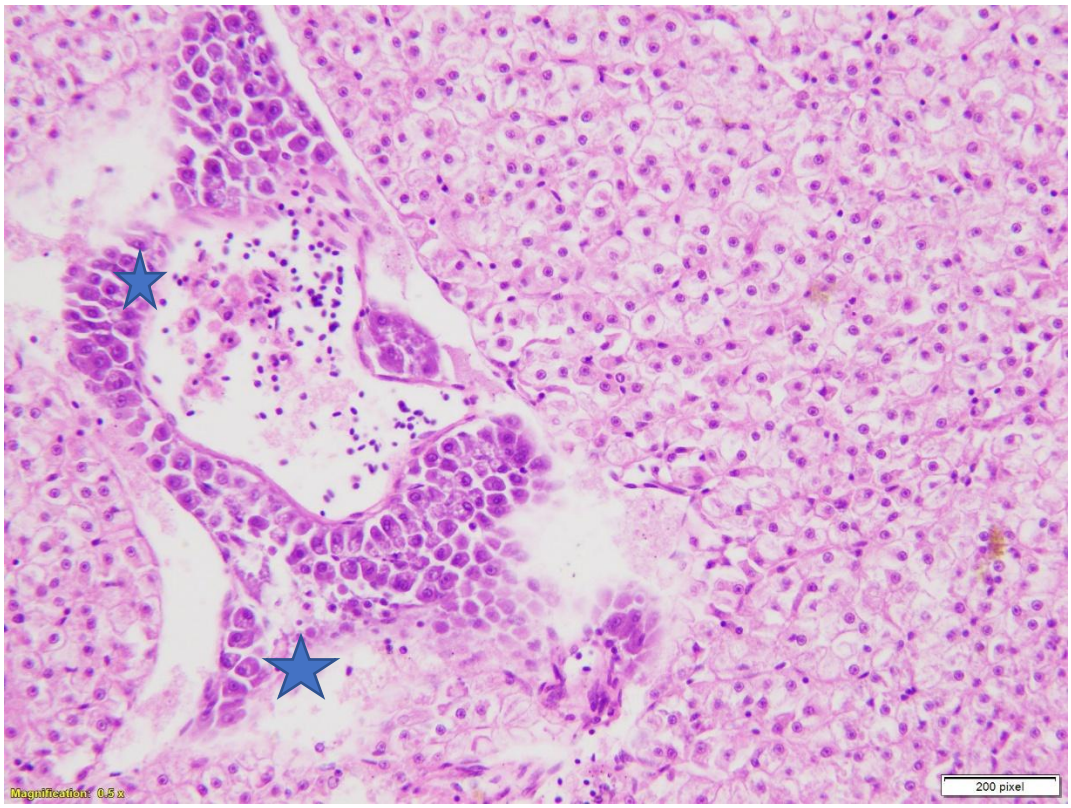
5(b)

Figure 5 (a,b) Photomicrograph of fish kidney displays renal parenchyma characterized by densely packed renal tubules and glomeruli. Several glomeruli appear degenerative and densely packed. There is marked area of tubular degeneration in renal paranchyma suggesting of renal tubule debris or necrotic material. 4X and 10X

Liver:Histological variations in the liver were most pronounced in *Catla catla* and *Wallago attu*, with findings of necrosis, vacuolization, and hemosiderin deposition. These changes are consistent with impaired detoxification and iron overload resulting from heavy metal exposure (Mehboob et al., 2019). In contrast, *Labeo rohita* displayed normal hepatic architecture, reaffirming its greater resistance to metal toxicity.



6(a)



6(b)

Figure 6 (a,b). Photomicrograph of the fish liver reveals marked histopathological changes indicative of biliary hyperplasia, hepatocellular degeneration, and inflammation. Prominently, there is ductular proliferation suggestive of biliary hyperplasia, characterized by multiple enlarged and irregularly shaped bile ducts lined with hyperplastic cuboidal epithelium accompanied by a periductular inflammatory infiltrate consisting primarily of mononuclear cells (star). The surrounding hepatocytes exhibit cytoplasmic vacuolation, implying fatty change or early hydropic degeneration, and many display mild nuclear pleomorphism. There is a focal collection of inflammatory cells within the lumen of one duct, suggesting a component of cholangitis.4x,10x and 40x (staining with Hematoxylin and Eosin)

Discussion

This study evaluates the bioaccumulation of heavy metals (Nickel and Lead) in three fish species—*Labeo rohita*, *Wallago attu*, and *Catla catla*—and assesses their histological impacts on various organs. The histological findings presented in Figures 1 through 3 offer a progressive illustration of gill damage in fish resulting from chronic exposure to lead (Pb) and nickel (Ni)**.** These heavy metals are known aquatic pollutants that induce dose-dependent histopathological, inflammatory, and structural remodeling in gill tissue**,** severely compromising the respiratory and osmoregulatory functions of affected fish. Figure 1 illustrates mild chronic gill disease, evidenced by epithelial hyperplasia and capillary congestion. These changes likely reflect early compensatory responses to metal irritation and inflammation. Figure 2 shows diffuse mononuclear inflammatory infiltration, epithelial lifting, and focal fusion, consistent with chronic gill inflammation. These changes impair gas exchange and indicate the onset of tissue remodeling**.** Figure 3 depicts severe pathological remodeling, including complete fusion of lamellae, clubbing, edema, and intense inflammatory cell accumulation. These alterations denote advanced lamellar collapse, leading to loss of respiratory architecture.Such histopathological alterations are well-documented in recent toxicological literature. According to Alsulami et al. (2024), lead and nickel exposure in *Oreochromis niloticus* results in epithelial hyperplasia**,** congestion, fusion, and chronic leukocytic infiltration—closely mirroring the structural deterioration observed here (Alsulami et al., 2024). Similarly, Athanasius and Richard (2025) noted that sub-chronic exposure to heavy metals like Ni and Pb in *Coptodon zillii* caused significant lamellar thickening, vascular congestion, and interlamellar edema, concluding that these metals disrupt cellular osmoregulatory functions and epithelial integrity (Athanasius & Richard, 2025).

The photomicrographs from Figures 4 and 5 highlight progressive renal damage in fish exposed to lead (Pb) and nickel (Ni)**,** two pervasive aquatic contaminants. The renal system, particularly the tubules and glomeruli, plays a critical role in osmoregulation and detoxification, making it a primary target for heavy metal-induced toxicity.Figure 4 shows early-stage nephrotoxicity, including dilated tubules**,** epithelial cell vacuolization, and cytoplasmic granularity, indicating the onset of tubular cell degeneration**.** The shrunken glomeruli with widened Bowman's space point toward incipient glomerular atrophy**.**Figure 5 reflects more advanced renal damage, with dense glomerular degeneration and areas filled with tubular debris or necrotic material, suggesting

chronic renal tubular necrosis.These findings are consistent with established literature on heavy metal nephrotoxicity in teleosts. Lead and nickel interfere with renal tubular reabsorption**,** induce oxidative stress, and disrupt glomerular filtration, leading to tubular degeneration**,** necrosis, and glomerular atrophy.A recent study by Alsulami et al. (2024) reported that *Oreochromis niloticus* exposed to Pb and Ni exhibited tubular vacuolation, epithelial sloughing, and glomerular shrinkage, mirroring the damage observed in the current micrographs Alsulami et al., 2024. Another study by Athanasius & Richard (2025) emphasized that even sub-chronic exposure to heavy metals leads to extensive nephron damage, evident through renal necrosis and degenerative glomeruli. The renal pathology observed highlights the fish kidney's sensitivity to lead and nickel toxicity, where early cellular changes rapidly progress to necrosis, severely impairing waste excretion, electrolyte balance, and osmoregulation**.**

The histological findings from Figure 6 demonstrate significant hepatic injury in fish exposed to lead (Pb) and nickel (Ni)**,** marked by biliary hyperplasia, hepatocellular degeneration, and inflammatory infiltration. These lesions are hallmark indicators of heavy metal-induced chronic liver toxicity in teleost fish, reflecting both direct cytotoxicity and immune-mediated damage. Biliary hyperplasia: Excessive ductular proliferation, lined by hyperplastic cuboidal epithelium, is a classic response to toxic insult and cholestasis. Hepatocellular degeneration: Manifested as cytoplasmic vacuolation (suggesting fatty change or early hydropic degeneration) and nuclear pleomorphism, indicative of metabolic and oxidative stress. Periductular inflammation**:** Infiltration by mononuclear inflammatory cells, along with focal cholangitis, denotes a chronic inflammatory state likely secondary to metal-induced hepatic insults. These observations are consistent with established research. Zheng et al. (2014**)** demonstrated that nickel exposure activates oxidative stress pathways (JNK signaling) leading to hepatocyte apoptosis in *Carassius auratus* . Likewise, Fatima et al. (2015) reported histopathological damage in the liver of fish exposed to lead and nickel, including fatty degeneration, nuclear alteration, and chronic inflammation, aligning with the current micrographic features. Furthermore, Hussain et al. (2025**)** showed that Ni exposure in *Hypophthalmichthys molitrix* led to marked hepatocellular necrosis and reduced enzymatic function, reinforcing the evidence that both metals disrupt liver function at both the cellular and systemic levels. In summary, the liver's histological alterations provide compelling evidence of heavy metal hepatotoxicity, where both Pb and Ni disrupt biliary architecture, induce metabolic dysfunction, and provoke chronic inflammation, collectively impairing hepatic performance in exposed fish.

Implications and Environmental Concerns

The observed histopathological changes and bioaccumulation patterns underscore the impact of industrial and agricultural pollution on aquatic ecosystems. The contamination of water at the Balloki Headworks, as a result of untreated industrial waste, poses significant risks to aquatic life and human health. Consuming fish with high concentrations of heavy metals, particularly in muscle tissue, can lead to bioaccumulation in humans, disrupting physiological functions and causing severe health issues (Talib Jawad S et al., 2022).

Recommendations

To mitigate these effects, regular monitoring of heavy metal concentrations in water and aquatic organisms is imperative. Implementing stringent waste treatment protocols before discharging industrial and agricultural effluents into water bodies is crucial. Public awareness campaigns are

also needed to highlight the dangers of heavy metal contamination and promote sustainable practices. Besides:

-Waste Management and Treatment Systems: Efficient treatment systems should be used to ensure that wastes from industrial facilities and agricultural areas are passed through before reaching water resources. The efficiency of existing wastewater treatment facilities should be increased and their heavy metal removal capacity should be strengthened.

-Environmental Policies and Regulations: In order to prevent heavy metal pollution, existing environmental regulations should be tightened, and inspections on waste discharges from industrial facilities should be increased. Stricter legal regulations should be introduced, especially for sectors that cause water pollution.

-Fishing and Aquatic Product Safety: Heavy metal levels in water and fish tissues in fishing areas should be analyzed regularly, and hunting should be restricted in areas exceeding the specified threshold values. Safe consumption levels should be determined and public awareness should be raised in order to protect human health.

-Community Awareness Programs: Local people, fishermen and farmers in the region should be made aware of the effects of heavy metal pollution and the protection of water resources. Education programs should be organized to increase awareness of reducing pollution.

-Alternative Agriculture and Industrial Practices: Chemical fertilizer and pesticide use should be reduced, and organic farming practices should be encouraged. In addition, environmentally friendly production processes should be adopted in industrial facilities.

-More Comprehensive Research: Future studies should investigate not only heavy metal pollution but also the effects of other pollutants (e.g. pesticides, microplastics) on fish health and human consumption. Biomarker studies should be conducted to understand long-term effects.

Competing interests

All the authors have no competing interests of any type.

Authors' contributions

All authors contributed to the conception and design of the study. IA prepare the initial draft of the Manuscript. EC provided mythological guidance on experimental procedure. Data collection and analysis were conducted under the supervision of IA and EC. FA, SA and HR contributed the revision and improvement of manuscript particularly in area of data interpretation. IA and EC rewrite the results and methodical refinement. EC and IA Reviewed and approved the final version of manuscript.

Conflict of Interest Statement

The authors declare no conflict of interests.

Data Availability Statement:

The data supporting this study's findings are available on request from the corresponding author.

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Ethics State Ethics Statement: All experimental procedures were approved by the institutional ethical committee and animal welfare committee of Kinnaird College for Women Lahore in compliance with local legislation.

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