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RESEARCH ARTICLE
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Assessment of Antioxidant Defense, Histological Modifications and Metal Bioaccumulation in Tilapia Fish Brain and Muscle Exposed to Aqueous Nickel and Chromium Co-Exposure

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ABSTRACT

Heavy metal contamination in aquatic ecosystems poses significant threats to aquatic organisms affecting their physiological and biochemical functions. This study evaluates the impact of aqueous nickel (Ni) and chromium (Cr) co-exposure on oxidative stress, antioxidant defense mechanisms, histopathological alterations, and metal bioaccumulation in brain and muscle tissues of *Tilapia* fish. Fish were divided into four groups: Group A (Control), Group B (Ni exposure), Group C (Cr exposure), and Group D (Ni-Cr co-exposure), and exposed to metals for a specific duration. Oxidative stress biomarkers, including thiobarbituric acid reactive substances (TBARS) and reactive oxygen species (ROS), were measured along with antioxidant enzyme contents such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and reduced glutathione (GSH). The results demonstrated a significant increase in TBARS and ROS levels, indicating oxidative stress, while antioxidant enzyme activities (SOD, CAT, POD, and GSH) declined significantly, suggesting impaired defense mechanisms. Histopathological examination revealed structural alterations in both brain (neuronal degeneration, necrosis of neurons, microgliosis, and vacuolization) and muscle (necrosis of myocytes, inflammation, and disorders in the arrangement of muscle fibers) in fish of the Ni-Cr co-exposure group. Metal bioaccumulation analysis showed a higher concentration of Ni and Cr in the brain compared to muscle tissue with the highest accumulation occurring in the combined metal exposure group. These findings highlight the toxic effects of Ni and Cr co-exposure in aquatic organisms, emphasizing the need for stricter pollution control measures to safeguard aquatic ecosystems.

Keywords: Nickel, Chromium, Tilapia fish, Oxidative stress, Antioxidant enzymes, GSH, Histopathology, Metal bioaccumulation.

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INTRODUCTION

Aquatic species are at risk of extinction due to metal pollution and global climate change. Moreover, fish and their products are deemed unsafe for human consumption and their exports have declined due to both inorganic and natural pollutants (Kumar et al., 2023). Due to expansions in industries and technology, the concentration of heavy

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metals in drinking water has surpassed the recommended thresholds set by global regulatory bodies. Contaminated drinking water remains the primary source of human exposure to heavy metals. The harmful effects of heavy metal contaminants including arsenic, lead, nickel, cadmium and mercury have increasingly drawn the attention of researchers. The general mechanism of heavy metal toxicity involves the generation of reactive oxygen species, leading to oxidative damage and subsequent detrimental health outcomes. Consequently, heavy metal-contaminated water significantly contributes to elevated morbidity and mortality rates worldwide (Fu and Xi, 2020).

Chromium, a prevalent and widespread metal pollutant in the environment (Bakshi and Panigrahi, 2018) enters aquatic ecosystems through industrial effluents from sectors such as textiles, tanneries, mining, electroplating, dyeing, printing, photographic processing, pharmaceuticals, stainless steel production, and rubber manufacturing (Bakshi and Panigrahi, 2018). The accumulation of chromium (Cr) in various body organs poses significant risks to human health (Chakraborty et al., 2022). Cr adversely affects bronchial epithelial cells, potentially through the dysregulation of apoptosis-related proteins, cytoskeletal proteins, and proteins associated with energy metabolism (Xia et al., 2022). Studies on the impact of Cr on fetal development during pregnancy suggest its toxic effects on fetal growth (Peng et al., 2018). Recognized as a carcinogen, Cr has been implicated in the onset of lung cancer (Baszuk et al., 2021). Additionally, exposure to elevated Cr levels can result in hyperpigmentation of the skin (Al Hossain et al., 2019).

Nickel is an essential metal for living organisms; however, both an excess and a deficiency of this metal can adversely affect the fitness of aquatic vertebrates. Additionally, nickel induces oxidative stress and elevates cellular reactive oxygen species, leading to DNA and lipid damage (Sun et al., 2020). Exposure to nickel has been linked to various health concerns, including carcinogenicity, hematotoxicity, developmental and neurotoxicity, reproductive toxicity, and hepato-renal dysfunctions. Furthermore, nickel toxicity is associated to the disruption of the physiological functions of essential elements such as zinc, manganese, magnesium, and calcium (Owumi et al., 2020).

Previously, a study illustrated behavioral, histological and immunological alterations caused by Cr exposure, and its detrimental impact on fish health (Bakshi and Panigrahi, 2018). Similarly, few studies have reported that nickel exposure results in histopathological alterations, enzymatic disturbances and oxidative stress in various tissues of fish including the muscles, gills and liver (Jayaseelan et al., 2014). The combined effects of heavy metals have become an increasing issue, as co-exposure to metals like Cr and Ni can have synergistic or antagonistic consequences on fish biology. A previous study on wild and farmed tilapia demonstrated the buildup of heavy metals including Cr and Ni muscles of fish. This study highlight the concerns about food safety and human health risks due to the transfer of these metals through the food chain (Simukoko et al., 2022). The effects of chromium on protein metabolism in fish brain and muscle tissues showed decreased protein activity and elevated amino acid levels emphasizing the substantial metabolic disturbances caused by chromium contamination (Palaniappan and Muthulingam, 2016). These earlier findings highlight the urgent need to explore the chronic effects of chromium and nickel co-exposure on vital fish tissues such as the brain and muscle to gain a deeper understanding of the mechanisms of toxicity. Such investigations are essential for formulating approaches to mitigate heavy metal pollution in aquatic environments and ensuring the health of both aquatic life and the human population.

MATERIALS & METHODS

Experimental Organisms and their Management

The present experiment was carried out at the Department of Zoology and the Department of Pathology, The Islamia University of Bahawalpur (Pakistan). A total of 80 Tilapia fish, weighing between 85 to 100g body weight, were purchased from a local fish breeding facility and allowed a two-week acclimation period. During this time, they were fed a commercial pelleted fish diet consisting of 22% crude proteins and groundnut oil cake. The feed, equivalent to 2-3% of fish's body weight was administered twice daily. This research work was approved by board of studies of department of zoology.

Study Design and Treatment Protocols

After the acclimatization period, the fishes were divided into four equal groups (A-D) in glass aquarium. Each aquarium contained 100L of water and each group had 20 fish. Group A was named the as the control group. Group B was exposed to Ni (1mg/L), group C to Cr (10mg/L), and group D to a mixture of both metals for a duration of 21 days. The fishes were monitored daily for any physical or behavioral changes. The metals were administered in the form of metallic salts ($NiCl_2$, $CrCl_3$).

Sample Processing and Biochemical Analyses

For biochemical analysis the brain and muscles were collected from each fish at days 7, 14 and 21 of trial and were placed in deionized water. After that the tissues were minced to obtain a homogenate. The homogenates were centrifuged at 6000rpm for 10min, and the resulting supernatants were stored at −20˚C. The reactive oxygen species (ROS) and TBARS were measured at absorbance of 505nm and 532nm while POD, SOD, CAT and GSH were measured at absorbance of 470, 560, 240, and 412nm respectively using an ultraviolet spectrophotometer in accordance with the previous protocol (Ghazanfar et al., 2018; Alam et al., 2025).

Digestion Process and Metal Analysis in Fish

The tissues were collected at day 7, 14, and 21 and then were stored at −20˚C for estimation of heavy metals. For acid digestion, nitric acid (65%) and perchloric acid (60%) were mixed in a specific ratio to

the samples for 4-5hours. The samples were heated on a hot plate at 200˚C for approximately 30min. The samples were cooled to room temperature and then filtered. The filtered samples were diluted with distilled water for further analysis. The heavy metals were measured with help of an acetylene air flame atomic absorption spectrometer (AAS) (Naz et al., 2023).

Histopathological Assessment

For histopathological analysis, the brain and muscle tissues of the fish were collected at day 21 of study. The tissues were preserved in a 10% formalin solution followed by dehydration with ascending grades of alcohol and then cleared in xylene. The tissues were then embedded in paraffin wax, sectioned using a microtome and stained with Hematoxylin and Eosin. These sections were mounted on slides for microscopic examination (Nursanti et al., 2017).

Statistical Evaluation

The data collected in this study were expressed as mean±SE. Statistical analysis was conducted using one-way analysis of variance (ANOVA) in IBM SPSS statistics software (version 20). Differences in mean values were identified through post hoc Tukey's test with significance at $P<0.05$.

RESULTS

Histopathological Abnormalities

No mortality or behavioral and physical changes were observed in the fish of the control group. However, in fish treated with metals in mixtures exhibited increased surface breathing, tremors of fins, jerking movement and lying on one side.

In the muscles (Fig. 1) more pronounced histopathological changes were observed in co-exposure including inflammatory cell infiltrate, degeneration of muscle fibers, edema and intrafiber necrosis as compared to the individual metal and control groups. Microscopic examination of the brain (Fig. 2) of fish reared in individual metal groups such revealed mild to moderate changes (cytoplasmic vacuolization, necrosis of neurons, and congestion) while whereas severe histopathological changes were observed in the metal mixture group as shown in Table 1.

Table 1: Severity of histopathological ailments in brain and muscle of Tilapia Fish treated with different heavy metals

Histopathological lesions	Groups			
	A (Ctrl)	B (Ni)	C (Cr)	D (Ni-Cr)
Brain				
Necrosis of neurons	-	+	++	++++
Enlarged cytoplasm of vacuolization	-	++	+++	+++
Neurons with Eccentric nuclei	-	++	++	++++
Microgliosis	-	+	++	+++
Muscle				
Atrophy of Myocytes	-	+	+	+++
Degeneration of Muscle Fibers	-	++	++	+++
Edema	-	++	+	+++
Myofibrillosis	-	++	++	+++
Inflammatory Cell Infiltration	-	++	++	+++
Necrosis of Myocytes	-	+	++	++++

Normal (-), mild (+), moderate (++), Severe (+++) , very severe (++++).

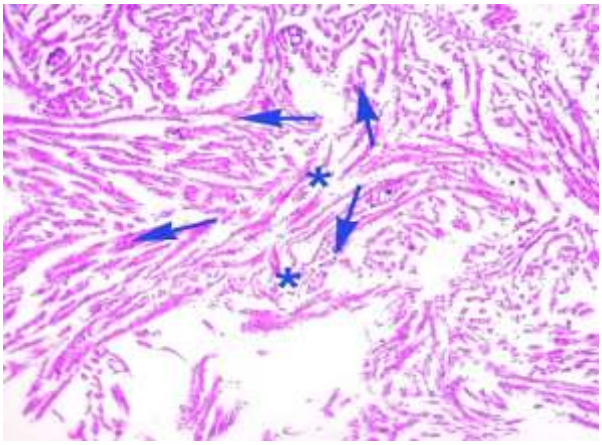

Fig. 1: Microscopic section of muscles of Tilapia Fish treated with different heavy metals (mixture metals) showing disruption and breakdown of muscles fibers (*) and necrosis of myocytes (arrows). H & E stain; 400X.

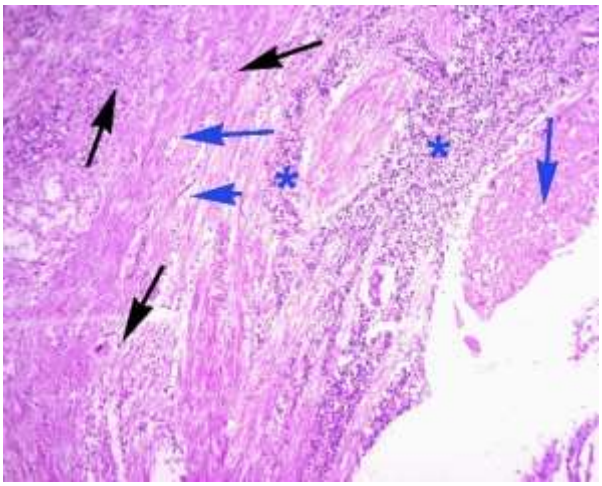

Fig. 2: Microscopic section of brain of Tilapia Fish treated with different heavy metals (mixture metals) showing atrophy and degeneration of neurons (arrows black), microgliosis (*) eccentric nuclei of neurons (arrows blue) and enlarged cytoplasm of neuron (arrow head). H & E stain; 400X.

Oxidative Stress and Antioxidant Capacity

In the muscle, the activity of ROS and TBARS increased significantly in fish reared in metal co-exposure group compared to the single metal exposure and the control group on day 21. The results on the antioxidant enzymes (SOD, POD, and CAT) showed a significant decrease on day 21 in group D compared to group A. The activity of GSH decreased significantly in fish of group D compared to group A on day 21.

In the brain, the activity of ROS and TBARS increased significantly on day 21 in group D compared to normal group A. The results regarding the antioxidant enzymes (SOD, POD and CAT showed a significant decrease in fish of metal co-exposure group compared to the single metal and the control group on day 21. The activity of GSH decreased significantly in fish of group D compared to groups A, B, and C on day 21 as shown in Table 2.

Metal Accumulation in Fish Tissues

The quantity of heavy metals like nickel and chromium was more pronounced in the brain than in the muscle tissue of fishes. Greater bioaccumulation of metals occurred in the combined metal group compared to the single metal groups as shown in Table 3.

Table 2: Oxidative and Antioxidant profile in brain and muscles of Tilapia Fish treated with different heavy metals

Parameters/Days	Groups			
	A (Ctrl)	B (Ni)	C (Cr)	D (Ni-Cr)
Brain				
ROS (Optical density)				
7	0.37±0.03	0.38±0.04	0.39±0.02	0.41±0.02
14	0.38±0.02	0.40±0.03	0.41±0.01	0.43±0.04
21	0.39±0.01	0.41±0.02	0.43±0.03	0.51±0.01*
TBARS (nmol/TBARS formed/mg protein/min)				
7	0.25±0.04	0.27±0.02	0.28±0.01	0.29±0.03
14	0.26±0.03	0.28±0.01	0.29±0.02	0.31±0.04
21	0.27±0.01	0.30±0.04	0.32±0.03	0.41±0.02*
GSH (µmol/g tissue)				
7	0.39±0.01	0.38±0.03	0.37±0.01	0.36±0.02
14	0.38±0.02	0.36±0.02	0.35±0.04	0.33±0.03
21	0.37±0.03	0.33±0.04	0.32±0.03	0.26±0.04*
Antioxidant enzymes				
SOD (units/mg protein)				
7	0.27±0.03	0.26±0.04	0.25±0.01	0.23±0.02
14	0.26±0.02	0.25±0.02	0.24±0.04	0.20±0.03*
21	0.25±0.01	0.24±0.03	0.22±0.03	0.12±0.01*
POD (units/min)				
7	0.22±0.04	0.21±0.03	0.20±0.02	0.19±0.04
14	0.21±0.03	0.20±0.02	0.18±0.03	0.15±0.03*
21	0.20±0.02	0.18±0.01	0.17±0.04	0.13±0.01*
CAT (units/min)				
7	0.29±0.03	0.28±0.04	0.27±0.03	0.26±0.01
14	0.28±0.02	0.27±0.03	0.25±0.04	0.23±0.03
21	0.27±0.01	0.25±0.02	0.22±0.01	0.14±0.02*

Values bearing asterisks in the row significantly increased or decreased as compared to the normal group.

Table 3: Metal accumulation in brain of Tilapia Fish treated with different heavy metals

Concentration/Days	Groups			
	A (Ctrl)	B (Ni)	C (Cr)	D (Cd-Ni)
Brain				
Chromium				
7	ND	-	11.36±5.84	11.87±6.80
14	ND	-	14.53±7.80	20.31±8.83
21	ND	-	19.16±10.28	29.20±12.11
Nickel				
7	ND	4.78± 0.66	-	6.49±0.76
14	ND	6.36± 1.50	-	8.54±1.59
21	ND	8.31±2.05	-	11.33±2.09

Table 4: Metal accumulation in muscles of Tilapia Fish treated with different heavy metals

Concentration/Days	Groups			
	A (Ctrl)	B (Ni)	C (Cr)	D (Cd-Ni)
Muscle				
Chromium				
7	ND	-	1.27±0.50	2.29±0.70
14	ND	-	2.08±0.82	2.58±0.94
21	ND	-	2.68±0.79	3.80±1.02
Nickel				
7	ND	0.46±0.23	-	0.73±0.28
14	ND	0.74±0.26	-	0.92±0.21
21	ND	1.15±0.56	-	1.88±0.68

The results revealed that the metal mixture increased the buildup of metals in fish tissues. Additionally, the results showed that, over time, the quantity of metals increased in fish tissues, leading to greater accumulation by day 21 as shown in Table 4.

DISCUSSION

Heavy metal (HM) pollution poses a serious threat to plants, animals, aquatic ecosystems, and public health. Because of their high stability, bioaccumulation and biomagnification qualities, HMs have become a major environmental concern in aquatic habitats. These metals enter ecosystems through both natural processes and human activities. The toxicity of heavy metals endangers the long-term viability of the aquaculture sector by impairing fish growth, reproduction, and general physiological function. Exposure to heavy metals in various forms can cause environmental risks which may impact the human health through direct poisoning or inducing negative effects (Qiao et al., 2021; Wang et al., 2024).

Excessive discharge of industrial effluents (pesticides, fungicides, herbicides and heavy metals) causes a serious threat to aquatic environment, domestic animals and public health (Hussain et al., 2018; Ghaffar et al., 2021a; Hussain et al., 2022). In aquatic animals such as fish some heavy metals can also have mutagenic (Jamil Emon et al., 2023) and carcinogenic (Kortei et al., 2020) effects. Heavy metals and other environmental effluents can result in oxidative stress, genotoxicity, structural damage, biochemical and functional problems, and deadly disease in the kidneys, liver, reproductive, respiratory, and neurological systems (Ghaffar et al., 2021b; Haseeb et al., 2022).

Contact with heavy metals like chromium and nickel disrupts the balance between the generation of ROS and the protective mechanisms leading to oxidative stress. ROS are bioactive molecules that naturally occur as byproducts of a series of processes starting with the metabolism of cellular oxygen (Nakamura and Takada, 2021; Alam et al., 2025). The conversion of electrons to oxygen on the inner mitochondrial membrane naturally produces these ROS, that engage in the cellular electrons (Bekhet and Eid, 2021; Rauf et al., 2024; Ahmad et al., 2025).

Heavy metal pollution increases ROS production through a variety of methods. Exposure to HMs has been shown to affect cellular redox status and mitochondrial electron transport, resulting in increased ROS generation (Eskander and Saleh, 2020). Furthermore, heavy metals including Cd, As, Fe, Cu, Ni, Pb, Cr, and Hg may react with nuclear proteins and DNA to produce reactive radicals that can harm cells and reduce enzyme activity through lipid peroxidation (Younas et al., 2024). Studies have demonstrated that Cr and Ni exposure induced oxidative stress by regulating antioxidant enzyme activities, resulting in significant reductions in SOD, CAT, and GSH activities in fish. The levels of TBARS and ROS were significantly higher in the fish of the metal mixture group compared to the individual metal groups. The elevation of ROS can be detrimental and lead to oxidative stress resulting in numerous disorders (Gul et al., 2024; Alam et al., 2025; Hussain et al., 2025). As previously mentioned, oxidative stress occurs when the body's antioxidant defense systems and ROS production are out of balance (Wang et al., 2020; Nakamura and Takada, 2021; Naz et al., 2025). This disruption of the oxidation-reduction (redox) equilibrium results in damage to molecules (Checa and Aran, 2020; Saeed et al., 2025). Furthermore, the generation of ROS damages DNA, lipids, and proteins within cells, which can result in cell damage and even cancer (Kanwal et al., 2024; Gul et al., 2025).

The peroxisomal enzyme catalase (CAT) breaks down

hydrogen peroxide into water and oxygen (Sznarkowska et al., 2017; Lee et al., 2023). The first line of defense against oxygen toxicity is SOD and CAT activity which may assist to keep normal physiological functions in balance by neutralizing ROS (Qu et al., 2014). GSH interacts with many environmental contaminants and metals providing fast protection against oxidative stress through the GSH redox cycle, immediately detoxifying the ROS produced by hazardous compounds (Kanak et al., 2014).

The release of industrial effluents, such as heavy metals, insecticides, fungicides and herbicides, induces oxidative stress, and disrupts antioxidant enzyme activity in fish damages their organs, causes serious harm to living organisms (Iqbal et al., 2024). A study on goldfish (*Carassius auratus*) exposed to chromium reported a notable decrease in these antioxidant enzymes, signifying oxidative stress and potential tissue damage (Velma and Tchounwou, 2010). Moreover, nickel exposure has been linked with oxidative stress in aquatic animals. Research shows that Ni accumulates in fish organs, leading to increased ROS generation and subsequent reduction of antioxidant protective mechanisms. A study on the impacts of heavy metals including nickel on fish reported changes in antioxidant enzyme activities, pointing to oxidative harm (Garai et al., 2021). Similarly, in the current study, the activity of antioxidant enzymes such as SOD, CAT, and POD was significantly reduced in the metal mixture group compared to single metal groups. The level of GSH was also significantly reduced in the combined metal group compared to the alone groups.

Heavy metals induce histopathological changes in different fish, which correlate with the distribution of these metals in various fish organs (Bibi et al., 2021; Onita et al., 2021). Histopathological changes were observed in muscles and brain due to the combined exposure to the metals. Histopathological changes observed in muscles and brain tissues in our study could be due to rapid and increased generation of oxidative stress. Previous studies have also reported that nickel and chromium induced significant histological changes in different fish organs, affecting the overall health of fish (Velma and Tchounwou, 2010; Garai et al., 2021).

Fish are considered as one of the foods at the top of the food pyramid. As a result, they are vulnerable to the biomagnification of HMs and are likely to as act as carrier for heavy metals to humans (Kumar et al., 2024). Human consumers are exposed to the detrimental effects of heavy metals (HMs) when they accumulate in seafood (Umeoguaju et al., 2022). For this reason, fish are often used as important biological markers to analyze metal levels in their environments and assess the environmental and health risks associated with human-generated waste discharge (Naz et al., 2023).

Because HMs can build up in fish bodies, they can easily enter the human body through seafood eating, leading to both short-term and long-term health problems (Bakhshalizadeh et al., 2022). HMs can enter the human body through the skin, inhalation, or digestive tract (contaminated food and drink) (Witkowska et al., 2021). Therefore, the type of HM and how it enters the body determine how quickly it is absorbed by the human body. For instance, eating tainted fish causes HMs to build up in the human body (Alam et al., 2023), which can lead to immune system breakdown, severe dysfunction, and malnourishment (Mehnaz et al., 2023). Additionally, among other organs in the human body, HMs have an impact on the kidneys, brain, nerves, liver, skin, and heart (Mitra et al., 2022).

Fish tissues, such as muscles, gills, intestines, liver, and kidneys, may accumulate large amounts of heavy metals (HMs) based on exposure time, metal concentrations, and other environmental parameters like pH, temperature, salinity, and metal hardness (Dhaneesh et al., 2012). The structures and activities of enzymes, proteins, hormones, and other chemicals can be affected or altered by these heavy metals' interactions with biological particles that include sulfur, nitrogen, oxygen, and other components. This can eventually cause harm to fish tissues and organs (Shahjahan et al., 2022). In present study, the accumulation of metals (Cr and Ni) also observed in muscles and brain. The accumulation of metals observed to be higher in co-exposure group.

Conclusion

This study demonstrates that exposure to nickel (Ni) and chromium (Cr), both individually and in combination, induces significant oxidative stress, disrupts antioxidant defense mechanisms, and causes histopathological damage in the brain and muscle tissues of *Tilapia* fish. The increase in oxidative stress markers (TBARS, ROS) and the depletion of antioxidant enzymes (SOD, CAT, POD, GSH) indicate an imbalance in redox homeostasis. Histopathological analysis revealed severe structural alterations, including neuronal degeneration, vacuolization, muscle fiber atrophy, and inflammatory responses, with the most pronounced damage observed in the Ni-Cr co-exposure group. Furthermore, metal bioaccumulation analysis showed a higher retention of Ni and Cr in the brain compared to muscle tissue, with the greatest accumulation occurring in the combined exposure group. These findings highlight the potential ecological risks associated with heavy metal contamination in aquatic environments. The study underscores the need for stringent environmental regulations and pollution control strategies to mitigate heavy metal contamination and protect aquatic life. Future research should explore the long-term effects of metal exposure and assess potential mitigation strategies to reduce toxicity in aquatic ecosystems.

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Data Availability: All the data related to this study is included and is available with the corresponding author.

Ethics Statement: This research trial was executed following the guidelines of bioethical committee of the institution regarding the welfare and use of laboratory animals.

Author's Contribution: H.G. G.M, A.G, Y.M: Investigation, Methodology. R.H: supervision, Investigation and Methodology. N.A, S.A and I. Q: writing and analysis. M.R: S.L R. Q and S.A.H: Data curation, Formal Analysis and Validation. R.I and K. A: Resources, Conceptualization, Writing – Review & Editing.

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