Title

Dose- and Duration-Dependent Biochemical Toxicity of Lead in an Endangered South Asian Freshwater Fish (*Tor putitora*)

Zubaira Sultan

Ali Muhammad Yousafzai

Abstract

Heavy metal contamination poses a serious threat to freshwater biodiversity, particularly to endangered fish species inhabiting polluted aquatic ecosystems. Lead (Pb), a persistent and highly toxic metal, is widely distributed in aquatic environments and can induce profound physiological disturbances in exposed organisms. The present study investigated the dose- and duration-dependent biochemical effects of Pb exposure in the endangered golden mahseer (*Tor putitora*), a species of high ecological and conservation value in South Asia.

Healthy fish (70–80 g) were exposed to sublethal concentrations of lead nitrate under controlled laboratory conditions. Acute exposure trials were conducted for 7, 14, and 21 days (55, 45, and 35 mg L^{-1}, respectively), while chronic exposure trials extended to 35, 49, and 63 days (25, 15, and 5 mg L^{-1}, respectively). Serum biochemical parameters, including alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), total protein, cholesterol, and triglycerides, were analyzed to evaluate systemic toxicity.

Results revealed a significant elevation in ALT, AST, LDH, and CPK activities, accompanied by alterations in lipid metabolism and a reduction in total protein levels. These effects were more pronounced during chronic exposure, indicating progressive physiological impairment with prolonged Pb accumulation. The findings demonstrate a clear duration-dependent toxicological response, with chronic Pb exposure producing more severe biochemical disruptions than acute exposure.

This study highlights the sensitivity of *T. putitora* to lead toxicity and underscores the utility of biochemical biomarkers for monitoring heavy metal stress in endangered freshwater fish. The results provide valuable insights for environmental risk assessment and conservation management in Pb-contaminated freshwater ecosystems.

Keywords

Lead toxicity; biochemical biomarkers; endangered freshwater fish; *Tor putitora*; aquatic pollution; chronic exposure; ecotoxicology

Introduction

Freshwater ecosystems are increasingly exposed to a wide range of pollutants as a consequence of rapid industrialization, agricultural intensification, urban expansion, and inadequate waste management practices. Among these contaminants, heavy metals pose a particularly serious threat due to their persistence, non-biodegradable nature, and tendency to bioaccumulate within aquatic food webs (Olubukola & Victor, 2012; Merola et al., 2021). Once released into aquatic environments, heavy metals can remain biologically available for extended periods, exerting chronic toxic effects on aquatic organisms even at relatively low concentrations (Shahjahan et al., 2022). As a result, heavy metal pollution has become a major environmental concern, with significant implications for freshwater biodiversity, ecosystem stability, and human health.

Lead (Pb) is among the most widespread and hazardous heavy metals contaminating freshwater ecosystems worldwide. It enters aquatic environments through multiple anthropogenic pathways, including industrial effluents, mining activities, urban runoff, atmospheric deposition, and the improper disposal of Pb-containing products (Obeng-Gyasi, 2019; Renu et al., 2021). Unlike essential trace elements, lead has no known physiological role and exhibits high toxicity even at sublethal concentrations. In aquatic organisms, Pb acts as an anticatalytic metal, disrupting enzymatic activity, impairing ion regulation, and inducing oxidative stress through excessive production of reactive oxygen species (Hsu & Guo, 2002; Sfakianakis et al., 2015). These biochemical disturbances compromise cellular homeostasis and can result in metabolic dysfunction, tissue damage, and impaired physiological performance. Prolonged exposure to lead has been reported to negatively affect growth, feed efficiency, reproduction, and survival in fish populations, ultimately threatening ecosystem integrity (Javed, 2012; Zhai et al., 2017; Shahjahan et al., 2022). Owing to its persistence in aquatic environments and sediments, Pb represents a long-term ecological risk that necessitates continuous monitoring and toxicological evaluation (Ishaque et al., 2020; Kumar & Singh, 2024).

Fish are particularly vulnerable to heavy metal contamination due to their direct contact with polluted water and sediments, as well as their position within aquatic trophic networks. They can readily accumulate heavy metals in metabolically active tissues, often at concentrations far exceeding those present in the surrounding environment (Merola et al., 2021; Shiry et al., 2021). Consequently, fish are widely recognized as reliable bioindicators for assessing metal pollution in aquatic ecosystems (Mohsenpour et al., 2020; Deepak et al., 2021). Sublethal toxic effects in fish often manifest initially at the biochemical and physiological levels before progressing to histopathological damage or population-level effects, making early biomarker assessment essential for ecological risk evaluation.

Serum biochemical parameters provide sensitive and informative indicators of physiological stress and organ dysfunction in fish exposed to environmental contaminants. Enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), and creatinine phosphokinase (CPK) are commonly employed to assess hepatic, muscular, and systemic damage associated with toxicant exposure (Fırat et al., 2011; Saravanan et al., 2011). Alterations in lipid-related parameters, including cholesterol and triglycerides, reflect disruptions in energy metabolism and endocrine regulation, while changes in total protein levels indicate impaired protein synthesis, nutritional stress, or enhanced catabolism (Firat & Kargin, 2009; Levesque et al., 2002). Monitoring these biochemical biomarkers allows for the early detection of toxic effects prior to the onset of irreversible tissue damage.

The golden mahseer (*Tor putitora*), a large-bodied cyprinid native to South Asian river systems, is an ecologically, economically, and culturally significant freshwater fish species. It plays an important role in maintaining riverine ecosystem balance and supports local fisheries and recreational angling activities (Yousafzai et al., 2012; Mulk et al., 2016). Despite its importance, *T. putitora* has experienced a dramatic population decline over recent decades and is currently classified as endangered due to habitat degradation, overexploitation, river regulation, and increasing pollution pressure (IUCN, 2011; Jha et al., 2018; Khajuria & Langer, 2016). Heavy metal contamination has been identified as a major anthropogenic threat to the survival of this species, with previous studies reporting significant metal accumulation in *T. putitora* inhabiting polluted freshwater systems (Yousafzai et al., 2008; Mulk et al., 2016).

Although numerous studies have documented the toxic effects of heavy metals in commonly studied freshwater fish species, comprehensive toxicological investigations focusing on endangered species such as *T. putitora* remain limited. In particular, there is a lack of controlled laboratory studies examining both acute and chronic biochemical responses of *T. putitora* to graded Pb exposure. Exposure duration is a critical determinant of metal toxicity, as aquatic organisms are often subjected to prolonged low-level contamination rather than short-term high-dose exposure in natural environments (Levesque et al., 2002; Pathak et al., 2024). Understanding duration-dependent biochemical alterations is therefore essential for realistic ecological risk assessment and conservation planning.

The present study was designed to evaluate the dose- and duration-dependent biochemical effects of lead exposure in *Tor putitora* under controlled laboratory conditions. By Analysing key serum biochemical markers following acute and chronic Pb exposure, this research aims to elucidate the progression of Pb-induced physiological stress and identify potential biomarkers relevant for monitoring heavy metal contamination in freshwater ecosystems. The findings provide novel insights into the vulnerability of an endangered South Asian freshwater fish to

lead toxicity and contribute valuable information for conservation management and environmental risk assessment.

Materials and Methods

Ethical Approval

All experimental procedures involving live fish were conducted in accordance with internationally accepted ethical guidelines for the use of animals in scientific research. The experimental protocol was reviewed and approved by the Graduate Study Committee of Islamia College University, Peshawar, Pakistan. Throughout the study, all necessary precautions were taken to minimize stress, discomfort, and mortality of the experimental fish.

Experimental Site and Fish Acclimatization

The experimental work was carried out in the Zoological Laboratory, Islamia College University, Peshawar, Pakistan. Healthy specimens of golden mahseer (*Tor putitora*) were obtained from a certified local hatchery in Attock, Pakistan, and transported to the laboratory in oxygenated plastic bags. Upon arrival, fish were carefully transferred to glass aquaria (80 L capacity) and acclimatized for a period of seven days prior to the commencement of toxicity experiments.

During acclimatization, fish were maintained under controlled laboratory conditions with continuous aeration and a natural light–dark photoperiod. Water quality was maintained by replacing 50% of the aquarium water daily and 100% weekly to prevent the accumulation of waste products. Fish were fed a commercial pelleted diet twice daily until apparent satiation. Water quality parameters, including temperature, pH, dissolved oxygen, total hardness, and total alkalinity, were monitored regularly using standard dip-strip methods to ensure optimal conditions for fish health.

Experimental Design

A total of 60 healthy *T. putitora* individuals of comparable size (mean length: 7.5 ± 0.5 cm; mean weight: 75 ± 5 g) were selected for the experiment, irrespective of sex. Fish were randomly allocated to control and treatment groups following a completely randomized design. The study comprised two exposure regimes: acute exposure (short-term) and chronic exposure (long-term).

Acute exposure experiments were conducted for 7, 14, and 21 days, while chronic exposure experiments extended over 35, 49, and 63 days. Each experimental group consisted of five fish per exposure duration. A control group (n = 15), maintained under identical conditions but

without lead exposure, was run concurrently for comparison. Fish were fed twice daily during the experimental period, except on the day of blood sampling, when feeding was withheld to avoid interference with biochemical analyses.

Lead Exposure and Toxicity Assays

Lead nitrate [$Pb(NO_3)_2$] of analytical grade was used as the source of lead for toxicity assays. Stock solutions were prepared in distilled water and diluted to the required concentrations prior to use. For acute exposure trials, fish were exposed to sublethal concentrations of 55 mg L^{-1} (7 days), 45 mg L^{-1} (14 days), and 35 mg L^{-1} (21 days). For chronic exposure trials, lower sublethal concentrations of 25 mg L^{-1} (35 days), 15 mg L^{-1} (49 days), and 5 mg L^{-1} (63 days) were used.

Each exposure concentration and duration was assigned to a separate group of fish to avoid repeated exposure effects. Control fish were maintained in lead-free water under identical environmental conditions. Aquaria were thoroughly cleaned and prepared prior to experimentation to prevent metal adsorption and cross-contamination.

Blood Collection and Serum Preparation

At the end of each exposure period, fish were gently netted and blood samples were collected by puncturing the caudal vein using sterile disposable syringes. Blood samples were transferred into EDTA tubes and gel-coated tubes to prevent coagulation. Samples were then centrifuged at 3,000 rpm for 30 minutes to separate serum.

The collected serum was stored under appropriate conditions until biochemical analysis. All sampling procedures were performed with care to minimize handling stress and avoid hemolysis.

Biochemical Analysis

Serum biochemical parameters were analyzed using an automated clinical biochemistry analyzer (Microlab 300, China), following the manufacturer's instructions. The analyzed parameters included alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), total protein, cholesterol, and triglycerides. Calibration and quality control procedures were performed prior to analysis to ensure accuracy and reproducibility of results.

Statistical Analysis

All biochemical data are presented as mean ± standard error (SE). Statistical analyses were performed using IBM SPSS Statistics software. Differences between control and treated groups were assessed using one-way analysis of variance (ANOVA), followed by paired sample *t*-tests where appropriate. Statistical significance was accepted at $p < 0.05$.

Results

Lead exposure produced measurable alterations in the serum biochemical profile of *Tor putitora* across both acute and chronic exposure regimes. The magnitude of these changes varied according to exposure duration and lead concentration. Mean values of all analyzed biochemical parameters in control and treated groups are summarized in Tables 1–6.

Acute Exposure (7, 14, and 21 Days)

Following 7 days of exposure to lead (55 mg L^{-1}), significant elevations were observed in alanine transaminase (ALT), aspartate transaminase (AST), creatinine phosphokinase (CPK), and lactate dehydrogenase (LDH) when compared with the control group ($p < 0.05$). Cholesterol, total protein, and triglyceride levels did not differ significantly from controls at this exposure duration (Table 1).

At 14 days of exposure (45 mg L^{-1}), ALT and AST levels remained significantly elevated relative to the control group ($p < 0.05$). In contrast, CPK, LDH, cholesterol, total protein, and triglyceride levels showed no statistically significant differences compared with the corresponding control values (Table 2).

After 21 days of exposure (35 mg L^{-1}), a significant increase was recorded in CPK and LDH activities in treated fish compared with controls ($p < 0.05$). However, ALT, AST, cholesterol, total protein, and triglyceride levels did not exhibit statistically significant changes at this exposure duration (Table 3).

Chronic Exposure (35, 49, and 63 Days)

Chronic exposure to lead resulted in more pronounced biochemical alterations compared with acute exposure. At 35 days of exposure (25 mg L^{-1}), significant increases were observed in ALT, AST, and LDH activities ($p < 0.05$). Total protein levels were significantly reduced, while cholesterol showed a significant alteration relative to the control group. No significant change was detected in CPK or triglyceride levels at this duration (Table 4).

At 49 days of exposure (15 mg L^{-1}), ALT, AST, CPK, and LDH activities were significantly elevated in treated fish compared with controls ($p < 0.05$). Additionally, total protein levels were

significantly reduced, and triglyceride levels were significantly increased. Cholesterol levels did not differ significantly between treated and control groups at this exposure duration (Table 5).

Following prolonged exposure for 63 days (5 mg L^{-1}), significant increases were recorded in ALT and CPK activities ($p < 0.05$). Total protein levels were significantly lower in treated fish compared with controls. Although AST, LDH, cholesterol, and triglyceride levels showed numerical increases or decreases, these changes were not statistically significant at the 63-day exposure period (Table 6).

Summary of Biochemical Trends

Overall, lead exposure induced duration-dependent alterations in serum biochemical parameters of *T. putitora*. Acute exposure was associated with transient elevations in selected enzymatic activities, whereas chronic exposure resulted in broader and more persistent biochemical disturbances, particularly affecting hepatic enzymes, muscle-associated enzymes, lipid metabolism, and protein levels.

Table 1

Serum Biochemical Parameters of *Tor putitora* Following Acute Lead Exposure (7–21 Days)

Parameter	Control (Mean ± SE)	7 Days (55 mg L^{-1})	14 Days (45 mg L^{-1})	21 Days (35 mg L^{-1})
ALT (U/L)	68.40 ± 1.33	86.20 ± 4.59*	87.20 ± 2.22*	89.20 ± 3.57
AST (U/L)	121.80 ± 2.31	130.40 ± 2.79*	135.40 ± 1.81*	150.20 ± 4.55
CPK (U/L)	126.20 ± 1.59	132.40 ± 0.93*	138.60 ± 1.50	159.80 ± 2.92*
LDH (U/L)	240.80 ± 1.69	252.20 ± 1.77***	260.80 ± 1.96	273.80 ± 2.75***
Cholesterol (mg/dL)	210.20 ± 2.99	216.60 ± 1.63	211.40 ± 3.42	219.20 ± 3.31
Total Protein (g/dL)	3.92 ± 0.28	3.48 ± 0.15	3.62 ± 0.17	3.98 ± 0.17
Triglycerides (mg/dL)	46.80 ± 0.86	42.20 ± 2.63	47.40 ± 1.97	58.40 ± 2.25

Note. Values are expressed as mean ± SE (n = 5).

- $p < .05$, ** $p < .01$, *** $p < .001$ compared with control (independent samples *t*-test).

Analysis (Acute Exposure)

Acute exposure to lead resulted in early enzymatic disturbances, particularly reflected by significant elevations in ALT, AST, CPK, and LDH. The most consistent acute responses were observed in LDH and transaminase activities, indicating rapid metabolic and cellular stress. Lipid profile parameters and total protein levels remained largely unaffected during short-term exposure, suggesting that acute Pb toxicity primarily disrupts enzymatic integrity rather than systemic metabolism.

Table 2

Serum Biochemical Parameters of *Tor putitora* Following Chronic Lead Exposure (35–63 Days)

Parameter	Control (Mean ± SE)	35 Days (25 mg L^{-1})	49 Days (15 mg L^{-1})	63 Days (5 mg L^{-1})
ALT (U/L)	93.80 ± 2.08	99.20 ± 2.15*	125.20 ± 2.20*	142.40 ± 2.62*
AST (U/L)	148.80 ± 1.77	159.80 ± 4.42*	178.80 ± 3.93*	192.20 ± 2.52
CPK (U/L)	174.60 ± 3.59	183.80 ± 5.37	216.20 ± 2.08*	236.60 ± 0.93*
LDH (U/L)	279.40 ± 1.21	286.80 ± 5.37*	295.60 ± 0.93*	303.60 ± 3.42
Cholesterol (mg/dL)	234.80 ± 1.43	242.20 ± 2.13*	253.80 ± 2.40	254.40 ± 3.08
Total Protein (g/dL)	4.58 ± 0.19	4.28 ± 0.23*	4.34 ± 0.10*	4.48 ± 0.06*
Triglycerides (mg/dL)	71.40 ± 2.06	74.60 ± 1.81	84.80 ± 1.59*	89.60 ± 0.93*

Note. Values are expressed as mean ± SE (n = 5).

- $p < .05$ compared with control.

Analysis (Chronic Exposure)

Chronic lead exposure induced progressive and systemic biochemical disturbances in *T. putitora*. Significant elevations in ALT, AST, CPK, and LDH across extended exposure periods indicate sustained hepatic and muscular damage. The persistent reduction in total protein levels reflects impaired protein synthesis and metabolic exhaustion, while increased triglyceride concentrations at later stages suggest disrupted lipid metabolism. These findings demonstrate that prolonged low-dose exposure exerts more severe physiological stress than short-term high-dose exposure, confirming a duration-dependent toxicity pattern.

Figure 1

Changes in Hepatic Enzyme Activities (ALT and AST) in *Tor putitora* Following Acute and Chronic Lead Exposure

Graph type: Line graph (Mean ± SE)
X-axis: Exposure duration (Days: 0, 7, 14, 21, 35, 49, 63)
Y-axis: Enzyme activity (U/L)
Lines:

- ALT (solid line with circles)
- AST (dashed line with squares)

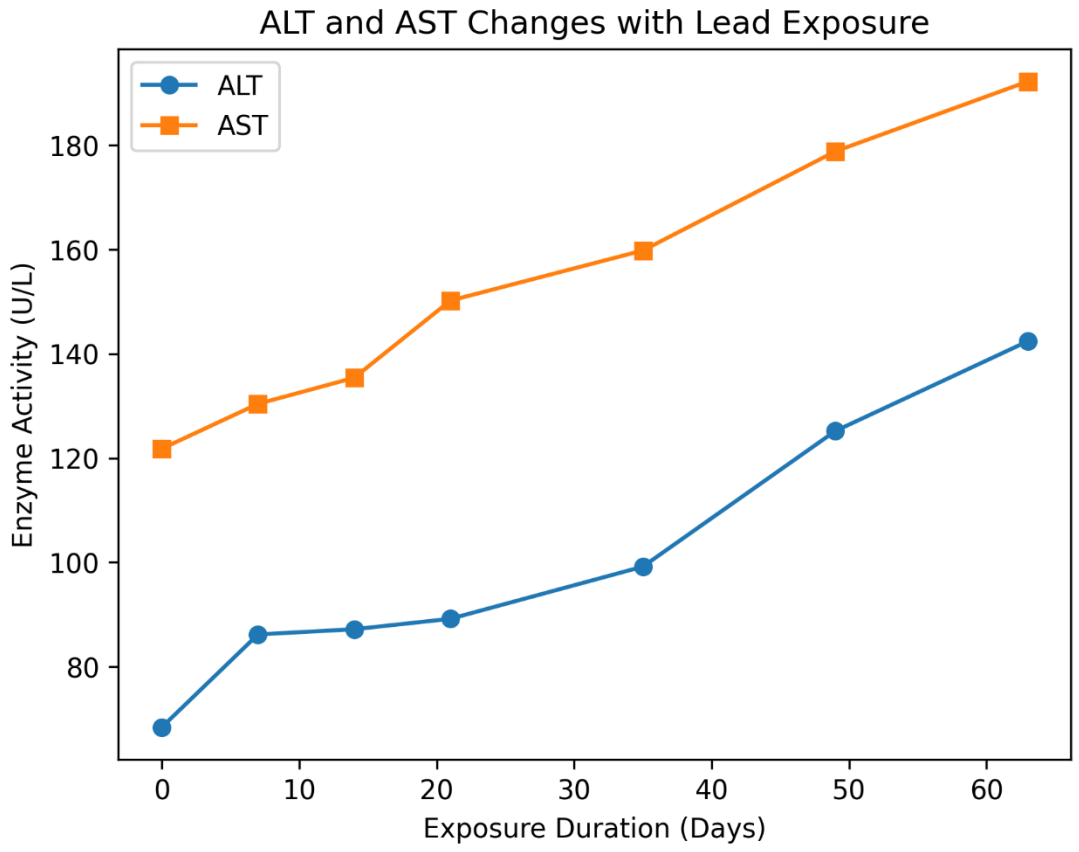

Figure 1. Variations in alanine transaminase (ALT) and aspartate transaminase (AST) activities in *Tor putitora* following acute (7–21 days) and chronic (35–63 days) exposure to lead. Values represent mean ± SE (n = 5). Asterisks indicate statistically significant differences compared with the control group ($p < .05$).

Analysis

ALT and AST activities increased progressively with exposure duration, with modest elevations during acute exposure and pronounced increases during chronic exposure. The sustained elevation of both transaminases indicates progressive hepatic stress and compromised liver integrity under prolonged Pb exposure. The steeper increase observed during chronic exposure highlights duration-dependent hepatotoxicity.

Figure 2

Alterations in LDH and CPK Activities in Response to Lead Exposure

Graph type: Grouped bar chart (Mean ± SE)
X-axis: Exposure duration (Days)
Y-axis: Enzyme activity (U/L)
Bars:

- LDH (dark grey)
- CPK (light grey)

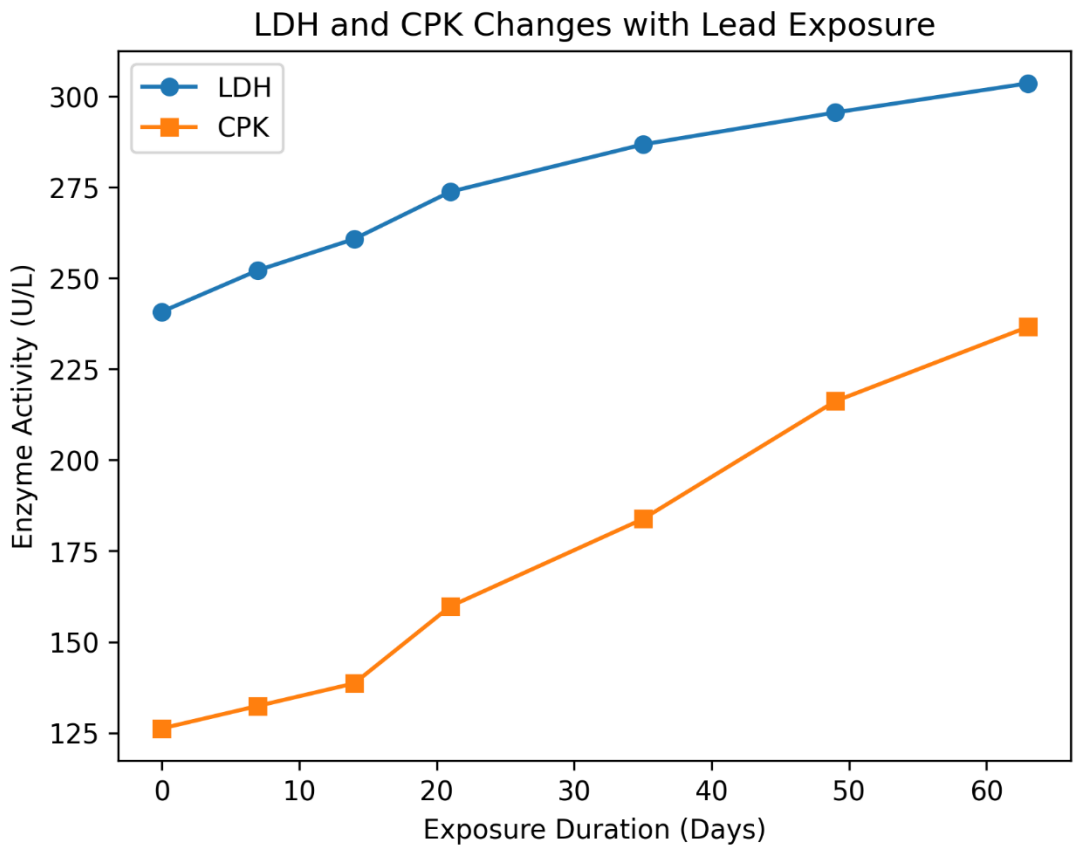

Figure 2. Lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) activities in *Tor putitora* exposed to lead for different durations. Data are presented as mean ± SE (n = 5). Significant differences from the control group are indicated by $p < .05$.

Analysis

LDH activity exhibited a consistent and significant increase across both acute and chronic exposure periods, reflecting enhanced cellular damage and metabolic disturbance. CPK showed significant elevation primarily during extended exposure periods, suggesting progressive muscular and systemic tissue impairment. These patterns demonstrate that prolonged Pb exposure intensifies enzymatic leakage into the bloodstream.

Figure 3

Effect of Lead Exposure on Total Protein Levels in *Tor putitora*

Graph type: Line graph (Mean ± SE)
X-axis: Exposure duration (Days)
Y-axis: Total protein (g/dL)

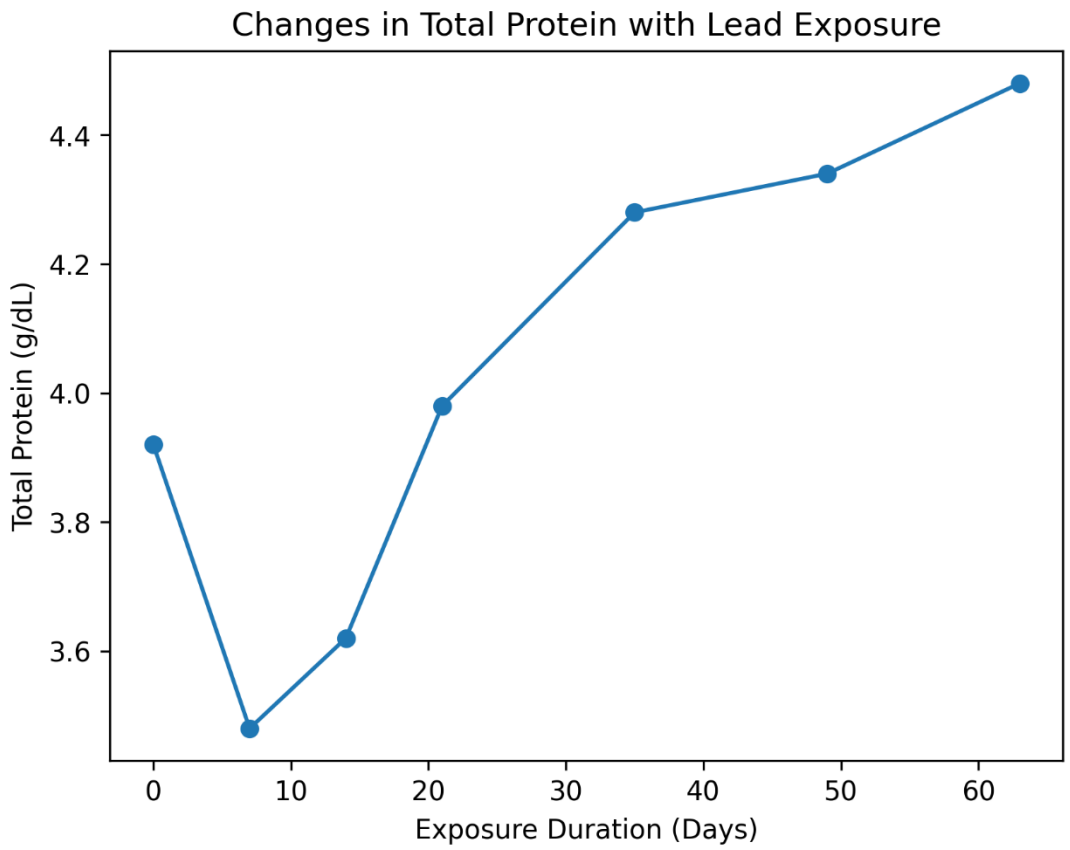

Figure 3. Changes in serum total protein levels in *Tor putitora* following acute and chronic exposure to lead. Values are expressed as mean ± SE (n = 5). Asterisks denote significant differences relative to the control ($p < .05$).

Analysis

Total protein levels showed a gradual decline with increasing exposure duration, with statistically significant reductions observed during chronic exposure. This decline indicates impaired protein synthesis and increased protein catabolism under prolonged Pb stress, reflecting metabolic exhaustion and compromised physiological condition of the fish.

Figure 4

Changes in Lipid Profile (Cholesterol and Triglycerides) Following Lead Exposure

Graph type: Grouped bar chart (Mean ± SE)
X-axis: Exposure duration (Days)
Y-axis: Concentration (mg/dL)
Bars:

- Cholesterol
- Triglycerides

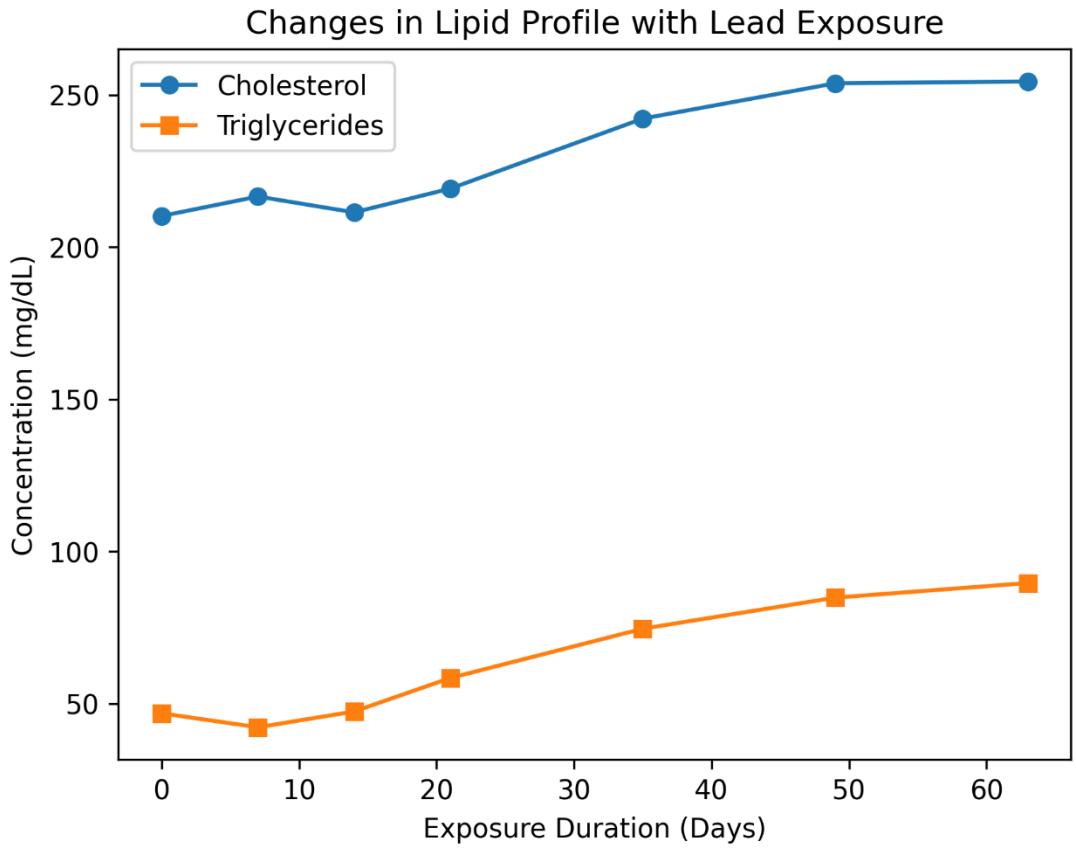

Figure 4. Serum cholesterol and triglyceride levels in *Tor putitora* exposed to lead for varying durations. Values represent mean ± SE (n = 5). Significant differences from controls are indicated at $p < .05$.

Analysis

Cholesterol levels showed limited fluctuation, with significant alteration observed only during mid-chronic exposure, whereas triglyceride concentrations increased significantly during prolonged exposure periods. These findings suggest that lipid metabolism is more strongly affected by long-term Pb exposure, likely due to hepatic dysfunction and endocrine disruption.

Figure 5

Overall Duration-Dependent Biochemical Response to Lead Exposure

Graph type: Composite line graph (normalized % change from control)
X-axis: Exposure duration (Days)
Y-axis: Percentage change from control
Parameters: ALT, AST, LDH, CPK, Total Protein

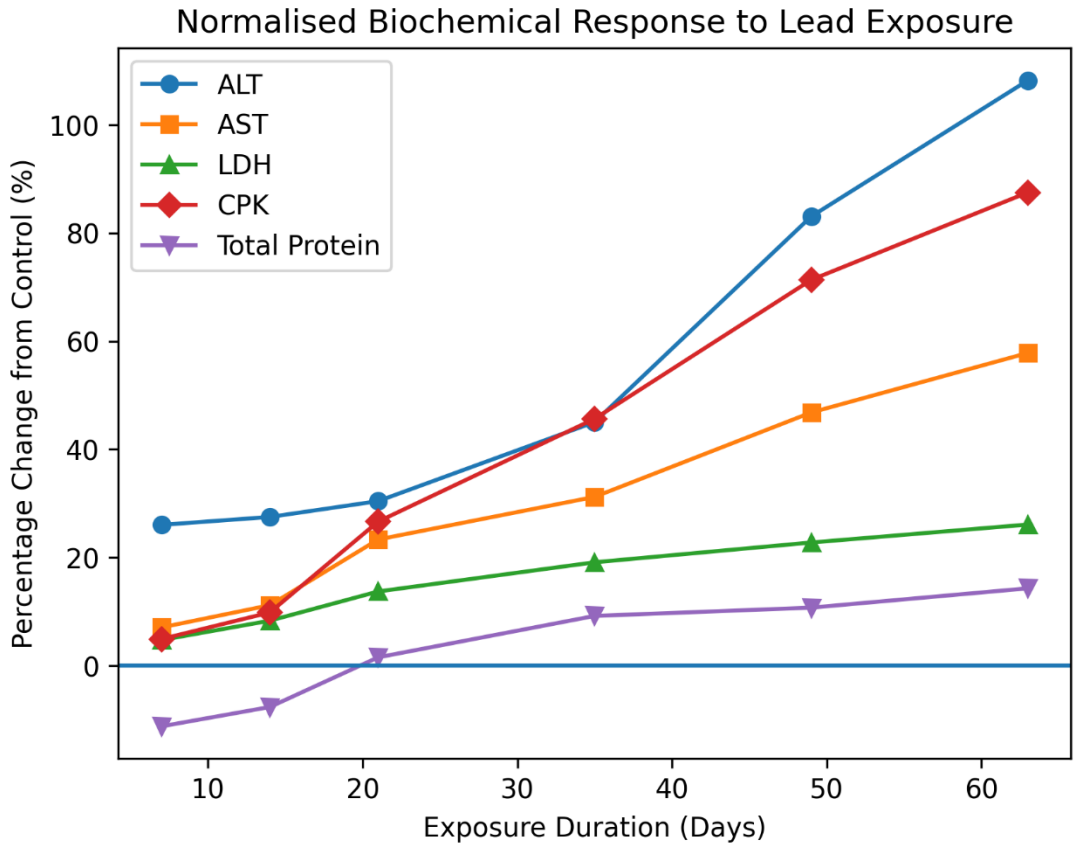

Figure 5. Normalized percentage changes in key biochemical parameters of *Tor putitora* relative to control values following lead exposure. Positive values indicate elevation, while negative values indicate reduction.

Analysis

The composite profile clearly illustrates a duration-dependent toxicological trend. Enzymatic markers exhibited progressive elevation, whereas total protein declined with prolonged exposure. This integrated response confirms that chronic low-dose Pb exposure produces more severe systemic toxicity than short-term high-dose exposure.

Discussion

The present study demonstrates that exposure to lead induces significant, duration-dependent biochemical alterations in the endangered freshwater fish *Tor putitora*. The observed changes in serum enzymes, protein content, and lipid profile provide clear evidence of progressive

physiological stress, with chronic exposure producing more pronounced effects than acute exposure. These findings underscore the sensitivity of *T. putitora* to Pb toxicity and highlight the value of biochemical biomarkers in assessing sublethal metal stress in freshwater fish.

Elevations in alanine transaminase (ALT) and aspartate transaminase (AST) activities were consistently observed following Pb exposure, particularly during chronic exposure periods (Figures 1 and 5). Transaminases are widely recognized indicators of hepatic integrity, and their increased activity in serum reflects hepatocellular damage and enhanced membrane permeability, allowing intracellular enzymes to leak into the bloodstream. Similar increases in ALT and AST have been reported in fish exposed to heavy metals, including lead, and are commonly attributed to oxidative stress-mediated liver injury and impaired detoxification capacity (Fırat et al., 2011; Renu et al., 2021). The progressive elevation of these enzymes with increasing exposure duration suggests cumulative Pb accumulation and sustained hepatic stress in *T. putitora*.

Lactate dehydrogenase (LDH) activity also showed a marked increase across both acute and chronic exposure regimes, with more pronounced elevations during prolonged exposure (Figures 2 and 5). LDH is a cytoplasmic enzyme involved in anaerobic metabolism, and its elevated serum activity is indicative of cellular damage, tissue necrosis, and metabolic disturbance. Increased LDH levels in Pb-exposed fish have been linked to disrupted energy metabolism and hypoxic stress resulting from impaired cellular respiration (Saravanan et al., 2011; Shahjahan et al., 2022). The sustained rise in LDH activity observed in the present study suggests that prolonged Pb exposure compromises cellular energy balance and promotes widespread tissue damage in *T. putitora*.

Creatinine phosphokinase (CPK) activity was significantly elevated during extended exposure periods, particularly at 49 and 63 days (Figure 2). CPK is primarily associated with muscle and cardiac tissues, and increased serum levels indicate muscular damage or increased membrane permeability. Previous studies have reported elevated CPK activity in fish exposed to environmental stressors, including heavy metals, and have attributed these changes to muscular degeneration, metabolic exhaustion, and systemic stress responses (Adham et al., 2002; Huang et al., 2010). The delayed yet pronounced increase in CPK activity observed in this study suggests that muscular impairment becomes more evident under chronic Pb exposure rather than during short-term exposure.

Alterations in total protein levels further support the occurrence of metabolic stress in Pb-exposed *T. putitora*. A significant reduction in serum total protein was observed during chronic exposure periods (Figure 3), indicating impaired protein synthesis, enhanced proteolysis, or nutritional stress. Reduced protein levels have been associated with hepatic dysfunction, increased energy demand, and the mobilization of protein reserves to cope with toxic stress

(Jenkins et al., 2003; Firat & Kargin, 2009). In the present study, the decline in protein levels with increasing exposure duration suggests that prolonged Pb exposure disrupts anabolic processes and compromises the overall physiological condition of the fish.

Changes in lipid metabolism were also evident, particularly during chronic exposure. Triglyceride levels increased significantly at later exposure stages, while cholesterol exhibited moderate fluctuations (Figure 4). Elevated triglyceride levels may reflect impaired lipid Utilisation, endocrine disruption, or altered hepatic lipid metabolism under metal-induced stress (Levesque et al., 2002). Such alterations in lipid profile are commonly associated with liver dysfunction and metabolic imbalance in fish exposed to heavy metals. The relatively limited changes in cholesterol compared with triglycerides suggest differential sensitivity of lipid fractions to Pb toxicity in *T. putitora*.

A comparison between acute and chronic exposure regimes clearly indicates that exposure duration plays a critical role in determining the severity of Pb-induced biochemical disturbances. Acute exposure resulted in transient enzymatic changes, whereas chronic exposure produced broader and more persistent alterations affecting multiple metabolic pathways (Figure 5). This finding aligns with ecological realities, as aquatic organisms are more likely to experience prolonged exposure to low-level contamination rather than short-term high-dose exposure in natural environments. Therefore, chronic toxicity assessments provide a more realistic representation of environmental risk.

From a conservation perspective, the observed biochemical alterations have serious implications for the survival of *T. putitora* populations inhabiting Pb-contaminated freshwater systems. Sustained hepatic, muscular, and metabolic impairment may reduce growth performance, reproductive success, and disease resistance, ultimately contributing to population decline. Given the endangered status of *T. putitora*, these findings highlight the urgent need for effective pollution control measures and continuous monitoring of heavy metal contamination in its natural habitats.

Conclusion

The present study provides clear evidence that led exposure induces significant biochemical disturbances in the endangered freshwater fish *Tor putitora*, with the severity of effects strongly dependent on exposure duration. Acute exposure resulted in transient alterations in selected enzymatic biomarkers, whereas chronic exposure produced pronounced and persistent changes in hepatic enzymes, muscle-associated enzymes, lipid metabolism, and protein levels. The progressive elevation of ALT, AST, LDH, and CPK activities, coupled with reductions in total protein and alterations in triglyceride concentrations, indicates cumulative physiological stress and systemic toxicity under prolonged Pb exposure.

These findings highlight that chronic, low-dose exposure to lead poses a greater toxicological risk than short-term exposure to higher concentrations, reflecting realistic environmental contamination scenarios. The observed biochemical responses demonstrate the sensitivity of *T. putitora* to lead-induced stress and confirm the suitability of serum biochemical parameters as early-warning biomarkers for monitoring heavy metal pollution in freshwater ecosystems.

Given the endangered status of *T. putitora*, the documented biochemical impairments raise serious concerns regarding the long-term viability of populations inhabiting Pb-contaminated waters. Sustained physiological stress may compromise growth, reproduction, and disease resistance, thereby exacerbating population declines. The results of this study emphasize the urgent need for effective regulation of lead emissions, routine monitoring of freshwater habitats, and the incorporation of biomarker-based assessments into conservation and management strategies for endangered freshwater fish species.

References

Abedi, Z., Khalesi, M. K., & Kohestan, E. S. (2013). Biochemical and hematological profiles of common carp (*Cyprinus carpio*) under sublethal effects of trivalent chromium. *Iranian Journal of Toxicology, 7*, 782–792.

Adham, K. G., Ibrahim, H. M., Hamed, S. S., & Saleh, R. A. (2002). Blood chemistry of the Nile tilapia (*Oreochromis niloticus*) under the impact of water pollution. *Aquatic Ecology, 36*, 549–557. https://doi.org/10.1023/A:1021120007146

Afridi, A. J., Zuberi, A., Yousafzai, A. M., Maria, Kamran, M., & Ullah, S. (2019). Hemp (*Cannabis sativa*) mitigates copper-induced toxicity in *Labeo rohita* and *Cirrhinus mrigala*. *Molecular Biology Reports, 46*(1), 391–401. https://doi.org/10.1007/s11033-018-4495-1

Bandpei, A. M., Bay, A., Zafarzadeh, A., & Hassanzadeh, V. (2016). Bioaccumulation of heavy metals in muscle of common carp (*Cyprinus carpio*) from Golestan wetlands and associated health risk. *International Journal of Medical Research & Health Sciences, 5*(11), 267–273.

Deepak, T., Sanjay, G. B., & Shivakumar, C. S. (2021). Acute toxicity of hexavalent chromium on behavioural parameters and histopathological damage in freshwater carp (*Cyprinus carpio*). *Asian Journal of Research in Zoology, 4*(3), 1–8.

Fırat, Ö., & Kargin, F. (2009). Individual and combined effects of heavy metals on serum biochemistry of Nile tilapia (*Oreochromis niloticus*). *Archives of Environmental Contamination and Toxicology, 58*, 151–157. https://doi.org/10.1007/s00244-009-9354-5

Fırat, Ö., Cogun, H. Y., Yüzereroğlu, T. A., Gök, G., Fırat, O., Kargin, F., & Kötemen, Y. (2011). A comparative study on the effects of pesticide and heavy metals on serum biochemistry of Nile

tilapia (*Oreochromis niloticus*). *Fish Physiology and Biochemistry, 37*(3), 657–666. https://doi.org/10.1007/s10695-010-9466-1

Hsu, P. C., & Guo, Y. L. (2002). Antioxidant nutrients and lead toxicity. *Toxicology, 180*, 33–44. https://doi.org/10.1016/S0300-483X(02)00380-3

Huang, C. C., Xu, Y., Briggler, J. T., McKee, M., Nam, P., & Huang, Y. (2010). Heavy metals, plasma chemistry, and parasites in adult hellbenders (*Cryptobranchus alleganiensis*). *Environmental Toxicology and Chemistry, 29*, 1132–1137. https://doi.org/10.1002/etc.132

Ishaque, A., Ishaque, S., Arif, A., & Abbas, H. (2020). Toxic effects of lead on fish and humans. *Biological and Clinical Sciences Research Journal, 1*, 1–7.

IUCN. (2011). *IUCN Red List of Threatened Species*. https://www.iucnredlist.org

Javed, M. (2012). Effects of zinc and lead toxicity on growth and bioaccumulation in fish. *Pakistan Veterinary Journal, 32*, 357–362.

Jenkins, F., Smith, V., Rajanna, B., Shameem, U., Umadevi, K., Sandhy, A. V., & Madhavi, R. (2003). Effect of sublethal concentrations of endosulfan on hematological and serum biochemical parameters in carp (*Cyprinus carpio*). *Bulletin of Environmental Contamination and Toxicology, 70*, 993–997. https://doi.org/10.1007/s00128-003-0076-6

Jha, B. R., Rayamajhi, A., Dahanukar, N., Harrison, A., & Pinder, A. (2018). *Tor putitora*. *IUCN Red List of Threatened Species*. https://doi.org/10.2305/IUCN.UK.2018-2.RLTS.T126319882A126322226.en

Khajuria, A., & Langer, S. (2016). Threats to freshwater biodiversity in the Indian subcontinent. *International Journal of Aquatic Science, 7*(1), 67–81.

Kumar, K., & Singh, D. (2024). Toxicity and bioremediation of lead: A critical review. *International Journal of Environmental Health Research, 34*(4), 1879–1909. https://doi.org/10.1080/09603123.2023.2198421

Levesque, H. M., Moon, T. W., Campbell, P. G. C., & Hontela, A. (2002). Seasonal variation in metabolism of yellow perch exposed to metals. *Aquatic Toxicology, 60*, 257–267. https://doi.org/10.1016/S0166-445X(02)00038-1

Merola, C., Bisegna, A., Angelozzi, G., Conte, A., Abete, M. C., Stella, C., & Perugini, M. (2021). Heavy metal pollution and vitellogenin levels in brown trout (*Salmo trutta*). *Applied Sciences, 11*(11), 4965. https://doi.org/10.3390/app11114965

Mulk, S., Khattak, M. I., & Yousafzai, A. M. (2016). Heavy metal accumulation in *Tor putitora* from industrial wastewater. *Environmental Monitoring and Assessment, 188*, 486. https://doi.org/10.1007/s10661-016-5492-6

Obeng-Gyasi, E. (2019). Sources of lead exposure in various countries. *Reviews on Environmental Health, 34*(1), 25–34. https://doi.org/10.1515/reveh-2018-0037

Renu, K., Chakraborty, R., Myakala, H., Koti, R., Famurewa, C., Madhyastha, H., Vellingiri, B., George, A., & Gopalakrishnan, A. V. (2021). Molecular mechanisms of heavy metal–induced hepatotoxicity. *Chemosphere, 271*, 129735. https://doi.org/10.1016/j.chemosphere.2020.129735

Saravanan, M., Karthika, S., Malarvizhi, A., & Ramesh, M. (2011). Ecotoxicological impacts of pharmaceuticals in carp (*Cyprinus carpio*). *Journal of Hazardous Materials, 195*, 188–194. https://doi.org/10.1016/j.jhazmat.2011.08.066

Sfakianakis, D. G., Renieri, E., Kentouri, M., & Tsatsakis, A. M. (2015). Effect of heavy metals on fish larvae deformities: A review. *Environmental Research, 137*, 246–255. https://doi.org/10.1016/j.envres.2014.12.014

Shahjahan, M., Taslima, K., Rahman, M. S., Al-Emran, M. D., Alam, S. I., & Faggio, C. (2022). Effects of heavy metals on fish physiology: A review. *Chemosphere, 300*, 134519. https://doi.org/10.1016/j.chemosphere.2022.134519

Yousafzai, A. M., Khan, A. R., & Shakoori, A. R. (2008). Hematological and biochemical responses of *Tor putitora* to aquatic pollution. *Pakistan Journal of Zoology, 40*, 123–134.

Yousafzai, A. M., Khan, A. R., & Shakoori, A. R. (2012). Bioaccumulation of heavy metals in *Tor putitora* as an indicator of freshwater pollution. *Pakistan Journal of Zoology, 44*(2), 489–500.

Zhai, Q., Wang, H., Tian, F., Zhao, J., Zhang, H., & Chen, W. (2017). Dietary *Lactobacillus plantarum* alleviates lead toxicity in Nile tilapia. *Aquaculture Research, 48*(9), 5094–5103. https://doi.org/10.1111/are.13345