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Chronic lead toxicity-induced alterations in oxygen uptake and energy metabolites of Rohu (*Labeo rohita*) fingerlings

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Abstract
In aquatic ecosystems heavy metal contamination adversely affects fish especially their physiology and metabolism. The present study explores the chronic impact of lead acetate on oxygen consumption and energy metabolites in *Labeo rohita* fingerlings. The 96-hour LC_{50} of lead acetate was determined to be 31.5 ppm, and a sublethal concentration (3.15 ppm; $1/10^{th}$ LC_{50}) was used for a 28-day exposure trial. Oxygen consumption, along with protein and carbohydrate levels was estimated from muscle tissues. Our results exhibited a significant time-dependent decline in oxygen consumption (4.78 to 2.94 mg O_2/hr/100g), protein (12.45 to 7.24 mg/g), and carbohydrate content (5.86 to 2.86 mg/g), indicating metabolic suppression and energy depletion. The observed results show differences in the physiological stress induced by chronic lead exposure, even at sublethal levels as compared to control. These findings emphasize that the oxygen uptake, protein and carbohydrate content are affected by lead acetate, posing ecotoxicological risks and negative consequences for fish health and freshwater ecosystem stability.

Keywords: Lead toxicity, *Labeo rohita*, oxygen consumption, sublethal toxicity, biochemical bio-markers. chronic exposure

Introduction
Heavy metal pollution in aquatic environments has become a major concern due to its wide-ranging toxic effects on aquatic fauna (Mahmuda *et al*. 2020; M. Sarkar, 2016) [13, 18]. Heavy metals build up in bodies of water because of natural processes and human activity. These include agricultural runoff, the erosion of landfills, port operations, and the release of industrial and household sewage.
(Ezemonye *et al*. 2019) [5]. Rapid population increase, intensive farming methods, and industrialization have brought a multitude of contaminants that alter the metabolic, physiological, and structural integrity of aquatic creatures (M. Sarkar *et al*. 2022; Shahjahan *et al*. 2019, 2021) [19, 22].
Heavy metals are one of the most worrying types of pollution since they don't break down and last a long time in the environment. People know that fish are good bioindicators of metal pollution in freshwater ecosystems (Azmat 2012) [9]. Lead (Pb) is one of the most poisonous heavy metals, found in many different forms in the environment, mostly as inorganic oxidized species (Jackson, 2005) [10]. It can enter fish through contaminated water and food, with higher accumulation typically observed in metabolically active tissues such as the gills, liver, and kidneys.
Lead exposure significantly affects fish physiology, including enzymatic activity, hormonal regulation, hematological profiles, and tissue structure (Shahjahan *et al*. 2022) [19]. Sublethal doses are recognized to reduce feeding efficiency, growth, and biochemical makeup, especially affecting proteins and carbohydrates, the main energy stores in fish (Amin *et al*. 2017) [3]. Fish age, water pH, and hardness are key factors determining the degree of lead toxicity in fish (Nussey, 2000) [14]. Oxygen uptake, a key measure of metabolic activity in aquatic organisms, is commonly employed to evaluate physiological stress (Schmidt-Nielsen 2007) [20].
This study aims to assess the chronic impacts of lead acetate exposure on oxygen consumption, protein, and carbohydrate levels in *Labeo rohita* fingerlings, offering insights into lead-induced metabolic disruptions.

Materials and Methods

Chemicals

Analytical grade lead acetate ($Pb(CH_3COO)_2 \cdot 3H_2O$; 98.5% purity) was procured from Hi-Media Chemicals Pvt. Ltd., Mumbai, and supplied by Sudarshan Scientifics, Nandgaon, District Nashik. A stock solution of 1000 ppm was prepared by dissolving the appropriate quantity of lead acetate in non-chlorinated well water and stored in an amber bottle at room temperature.

Experimental Fish Acclimatization

Healthy fingerlings of *Labeo rohita* (mean total length: 3.93±0.226 cm; mean body weight: 2.21±0.05 g) were obtained from a certified fish hatchery located in Paithan, District Chhatrapati Sambhaji Nagar. The fish were transported to the laboratory in aerated polyethylene bags and acclimatized under laboratory conditions for 15 days in a 1000-liter cement tank maintained at 34±2 °C. During acclimatization, fish were fed a commercial fish diet twice daily, and the water was renewed every 24 hours.

Post-acclimatization, the fish were transferred to 25-liter capacity aquaria (plastic troughs) for experimentation. Water quality parameters, including temperature, dissolved oxygen, pH, and alkalinity, were maintained in accordance with the guidelines recommended by the United States Environmental Protection Agency (USEPA, 1976) [27].

Acute Toxicity and LC_{50} Determination

Acute toxicity of lead acetate was assessed following standard protocols by (USEPA 1995) [27] and (OECD 2000) [15]. Fish were divided into ten groups (n=10 per group) and exposed to varying concentrations of lead acetate ranging from 10 to 65 ppm for a period of 96 hours using a static bioassay method. Mortality was recorded at 24-hour intervals. The 96-hour median lethal concentration (LC_{50}) was determined using Probit analysis (Finney 1952; Finney DJ 1970) [6, 7]. Experiments followed institutional ethical guidelines for fish handling.

Sublethal Exposure

Based on the LC_{50} value obtained, a sublethal concentration ($1/10^{th}$ of LC_{50}; 3.15 ppm) was selected for chronic exposure studies. Fish were randomly divided into two groups: a control group and an experimental group (n=10 per group). The treatment group was exposed to 3.15 ppm of lead acetate for a period of 28 days. During the exposure period, water was renewed every 24 hours, and fish were monitored for behavioral and physiological changes.

Estimation of Oxygen Consumption

Oxygen consumption was estimated using the closed chamber respirometry method for a period of 28 days. Dissolved oxygen levels were determined daily using the Winkler's titration method (Golterman and Clymo, 1969) [8]. The difference in dissolved oxygen concentrations before and after 24 hours of fish exposure in sealed chambers was used to calculate the oxygen uptake rate. Final oxygen consumption was expressed in mg O_2/h/100 g of fish body weight, following the method of (Thampi 1994).

Estimation of Total Carbohydrates

Total carbohydrate content in fish tissue was estimated following the method of (Roe 1955) [17]. Tissue samples were homogenized in 5% trichloroacetic acid (TCA) to prepare a 10% tissue homogenate. The homogenate was centrifuged at 3000 rpm for 10 minutes, and the supernatant was collected. The assay mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 620 nm using a Systronics 1305 dual-beam spectrophotometer. A blank was prepared using 1 ml of distilled water mixed with 4 ml of anthrone reagent. Results were expressed as mg carbohydrate per gram of wet tissue.

Estimation of Total Protein

Protein concentration was determined using the Lowry method (Lowry *et al*, 1951) [12]. Tissue homogenates (1%) were prepared in 10% TCA and centrifuged at 3000 rpm for 15 minutes. The resulting pellet was dissolved in 1 ml of 1N NaOH. To this, 5 ml of alkaline copper reagent was added and incubated for 10 minutes. Subsequently, 0.5 ml of Folin-Ciocalteu phenol reagent was added, and absorbance was recorded at 660 nm. Protein content was expressed in mg/g of tissue.

Statistical Analysis

Oxygen consumption, total carbohydrate and protein data were analyzed using one-way ANOVA to assess time-dependent changes, followed by Dunnett's post-hoc test to compare exposure time points to control ($p<0.05$ considered significant). Analyses were performed using (software, e.g., SPSS v26).

Results

Physico-chemical Analysis of Water

Water parameters (Temperature: 23.2±0.2 °C, pH: 7.7±0.3, alkalinity: 284±0 mg/L, dissolved oxygen: 6.5±0.4 mg/L) were maintained within recommended ranges (Table 1) throughout the experiment.

Table 1: Physico-chemical analysis of water parameters.

Parameters	Mean±SD Results	Recommended range
Temperature	23.2±0.2	18- 35ºC
pH	7.7±0.3	6.5- 8.7
Alkalinity	284±0	50- 400 mg/L
Dissolved Oxygen	6.5±0.4	5-8 mg/L.

Exposure to lead acetate (10-65 ppm) caused 12-90% mortality in *Labeo rohita* fingerlings over 96 hours, with a calculated 96-hour LC_{50} of 31.5 ppm (Table 2). Mortality increased with concentration (Probit analysis, $p<0.05$). Toxicity was evidenced by a statistically significant reduction in survival rates compared to the control group and that are similar to study of (Yaqub and Javed 2012). The increased mortality with rising lead concentrations and prolonged exposure duration may be attributed to metal accumulation in vital tissues, particularly the gills—primary sites for metal entry—resulting in tissue damage, impaired respiration, and disrupted metabolic functions (James 2003) [11].

Table 2: Study of lethal concentration of Lead acetate (96 h LC_{50}) by probit analysis on rohu fingerlings

Concentrations (ppm)	Log of concentration	% mortality	Probit value
10	1	12	3.82
20	1.301029996	18	4.08
25	1.397940009	23	4.23
30	1.477121255	28	4.42
35	1.544068044	39	4.72
45	1.653212514	46	4.9
50	1.698970004	55	5.13
55	1.740362689	67	5.44
60	1.77815125	79	5.81
65	1.812913357	90	7.33

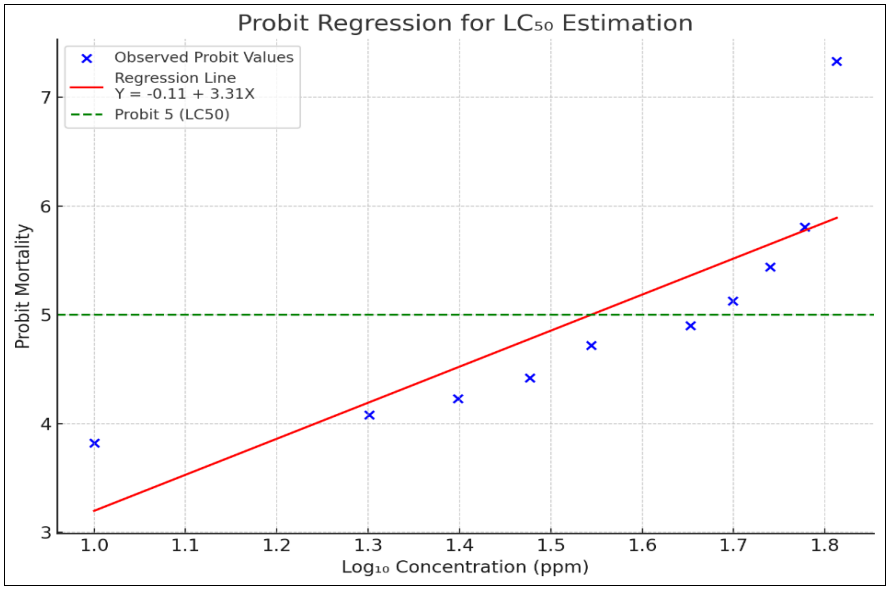
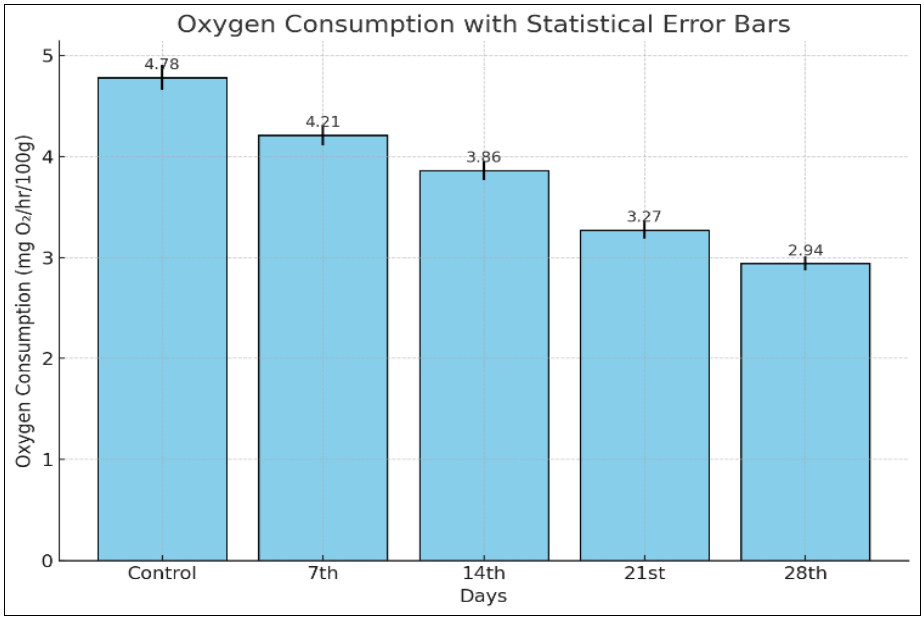


Fig 1: Probit Analysis of Lead acetate LC_{50} estimation

Table 3: Biochemical Changes (oxygen consumption, protein and carbohydrate content)

Day	Oxygen Consumption (mg O_2/hr/100g)	Protein (mg/g tissue)	Carbohydrate (mg/g tissue)
Control	4.78±0.12	12.45±0.33	5.86±0.28
7^{th}	4.21±0.10 (p = 0.042)*	11.68±0.26 (p = 0.038)*	5.14±0.22 (p = 0.035)*
14^{th}	3.86±0.09 (p = 0.003)**	10.33±0.30 (p = 0.002)**	4.39±0.17 (p = 0.001)**
21^{st}	3.27±0.08 (p<0.001)***	8.65±0.29 (p<0.001)***	3.22±0.21 (p<0.001)***
28^{th}	2.94±0.07 (p<0.001)***	7.24±0.25 (p<0.001)***	2.86±0.18 (p<0.001)***
ANOVA p-value	< 0.001***	< 0.001***	< 0.001***

Statistical notes: (*p<0.05, **p<0.01, ***p<0.001").



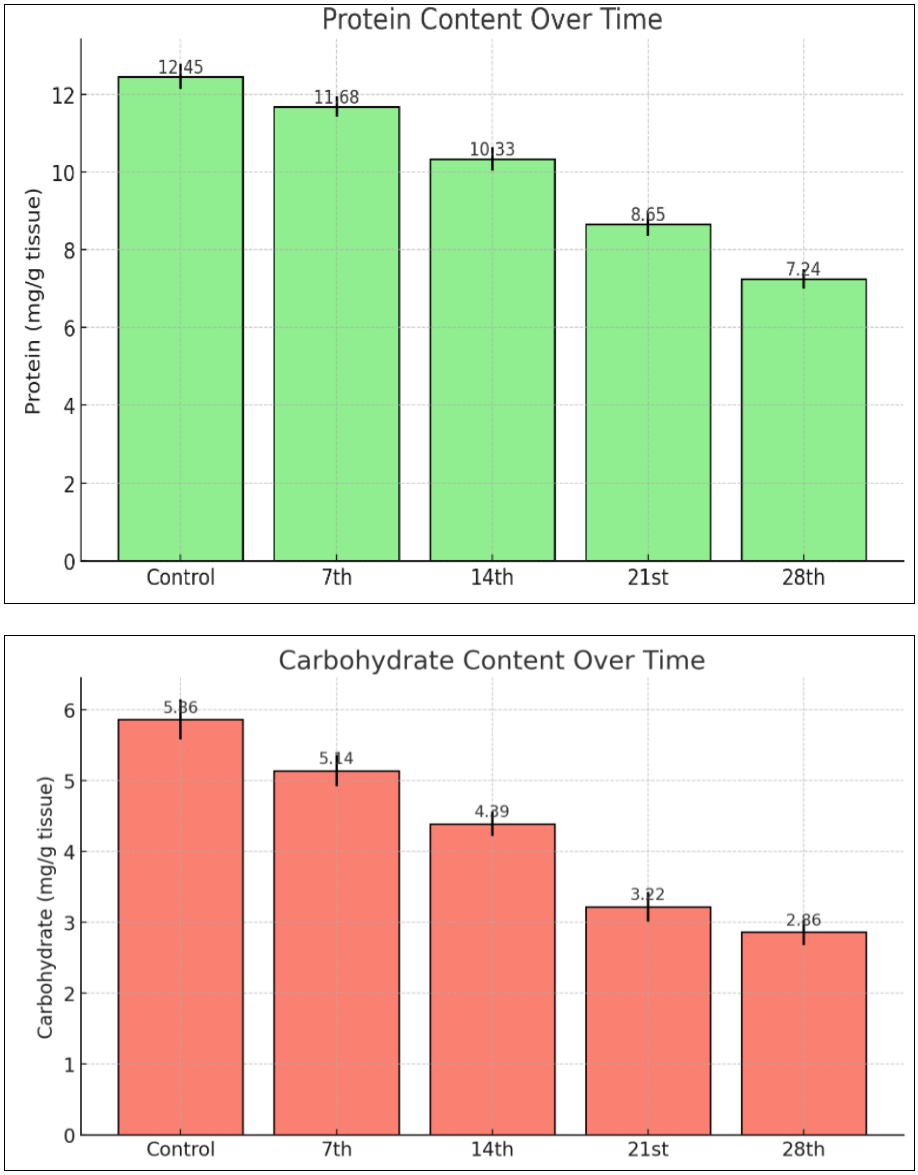


Fig 2: Biochemical changes in (a) oxygen consumption, (b) protein and (c) carbohydrate content

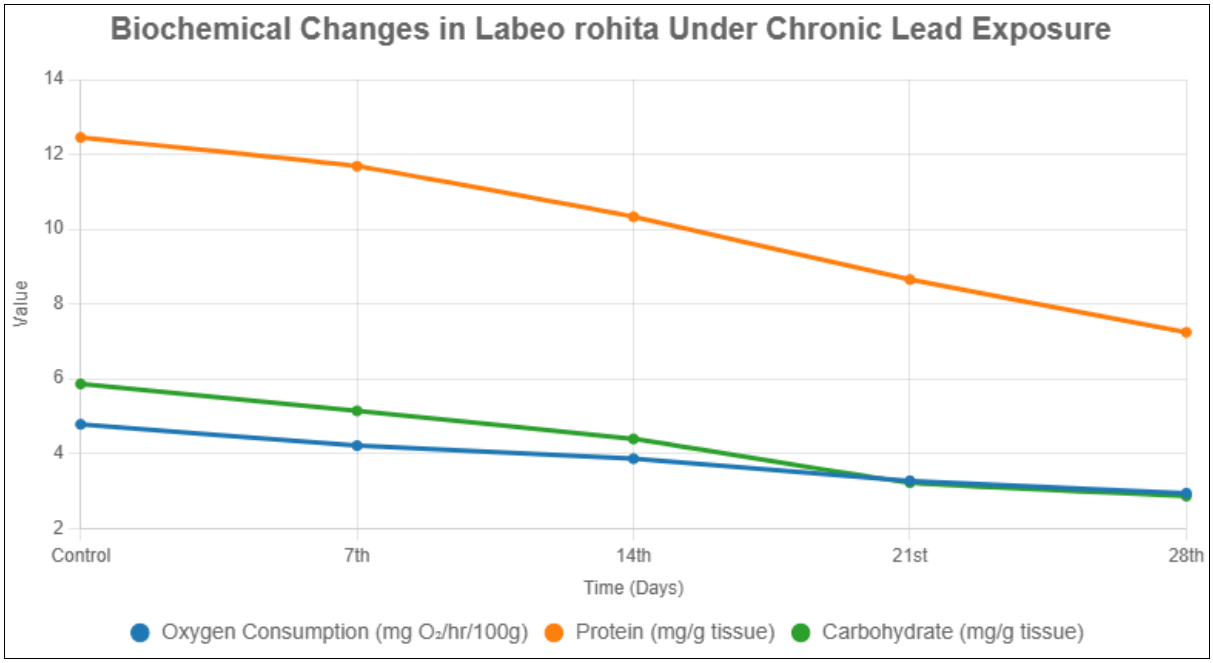


Fig 3: Combined Trends multi-line time-dependent changes in oxygen consumption, protein, and carbohydrate content.

Chronic exposure to 3.15 ppm lead acetate ($1/10^{th}$ LC_{50}) over 28 days significantly altered oxygen consumption, protein, and carbohydrate content in muscle tissues (Table 3). One-way ANOVA revealed significant time-dependent declines ($p<0.001$) for all parameters.

Oxygen consumption

Decreased from 4.78±0.12 mg O_2/hr/100g (control) to 2.94±0.07 mg O_2/hr/100g by day 28. Dunnett's test showed significant reductions by day 7 (p = 0.042), with stronger effects on days 14 (p = 0.003), 21 ($p<0.001$), and 28 ($p<0.001$) compared to control.

Protein content

Total protein decreases from 12.45±0.33 mg/g (control) to 7.24±0.25 mg/g by day 28. Significant reductions occurred by day 7 (p = 0.038), with greater differences on days 14 (p = 0.002), 21 ($p<0.001$), and 28 ($p<0.001$).

Carbohydrate content

Similarly, carbohydrate levels were reduced from 5.86±0.28 mg/g (control) to 2.86±0.18 mg/g by day 28. Significant declines were observed from day 7 (p = 0.035), increasing in magnitude on days 14 (p = 0.001), 21 ($p<0.001$), and 28 ($p<0.001$). These trends indicate progressive metabolic suppression due to lead-induced stress.

Discussion

The findings of this study reveal that prolonged exposure to sublethal levels of lead acetate induces substantial perturbations in key biochemical markers in *Labeo rohita* fingerlings, notably manifesting as diminished oxygen consumption, protein concentrations, and carbohydrate reserves. In the acute toxicity bioassay conducted herein, the 96-hour LC_{50} for lead acetate in *L. rohita* was established at 31.5 ppm, with a sublethal dose of 3.15 ppm (equivalent to $1/10^{th}$ of the LC_{50}) employed for the chronic assessment; these results are consistent with prior determinations of the 96-hour LC_{50} for lead in *L. rohita*, reported as 34.4 mg/L and 31.5 mg/L, respectively (Amin *et al*. 2017; Zulqarnain *et al*. 2024) [3]. The extent of acute toxicity is influenced by several factors, such as the dosage administered, fish age and sex, feeding habits, body size, and the length of metal exposure (Sivabalan 2022) [24]. Our findings closely align with those of (Vaseem and Banerjee 2013) [28] who reported that chronic lead exposure diminishes oxygen-carrying capacity in *Labeo rohita*. Similarly, (Ahsan Raza *et al*. 2024) [2] demonstrated in their study that lead-induced stress compromises respiratory efficiency, thereby reducing oxygen consumption rates in affected fish—a pattern highly consistent with our observations. Prolonged lead exposure triggers a marked reduction in carbohydrate metabolites, such as glycogen, alongside total protein levels in the muscle tissues of *Labeo rohita*, signaling cellular degeneration and compromised protein synthesis (Tewari, *et al* 1987; Vaseem and Banerjee 2014) [26, 29]. The decrease in carbohydrates is associated with the inability of the fish to efficiently metabolize energy under toxic stress, resulting in higher levels of lactic acid as a compensatory metabolic process (Sekhar *et al*. 2023) [21]. The results were very much in accordance with (Ahsan Raza *et al*. 2024) [2], that investigated the decrease in protein content is correlated with enhanced activities of liver enzymes, indicating tissue damage and disturbance in metabolism due to lead poisoning.

Conclusion

This investigation demonstrates that chronic exposure to sublethal concentrations of lead acetate significantly impairs the metabolic and physiological functions of *Labeo rohita* fingerlings. The observed reductions in oxygen consumption, protein, and carbohydrate levels reflect energy depletion and stress-induced metabolic disturbances. These findings underscore the vulnerability of this species to lead contamination, highlighting the critical need for regular monitoring and stricter regulation of heavy metal pollutants in aquaculture systems to safeguard fish health and ensure ecosystem sustainability.

References

1. Abdullah S, Javed M, Javid A. Studies on acute toxicity of metals to the fish *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*. Pakistan Journal of Agricultural Sciences. 2007;44(1):102-106.
2. Raza A, Hashmi SA, Hasan A, Ahmad H. Toxicity evaluation and tissue damaging effects of lead in *Labeo rohita*. Futuristic Biotechnology. 2024;4(1):53-57.
3. Amin A, Naik ATR, Priyadarshini N, Nayak H, Sree CS. Toxic effect of heavy metal lead on oxygen consumption of rohu (*Labeo rohita*) fingerlings. 2017. Available from: https://www.researchgate.net/publication/320045504
4. U.S. Environmental Protection Agency (EPA). Guidance for risk characterization. 1995;1:15.
5. Ezemonye LI, Adebayo PO, Enuneku AA, Tongo I, Ogbomida ET. Potential health risk consequences of heavy metal concentrations in surface water, shrimp (*Macrobrachium macrobrachion*) and fish (*Brycinus longipinnis*) from Benin River, Nigeria. Toxicology Reports. 2019;6:1-9.
6. Finney DJ. Probit analysis. 1952;388-390.
7. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press; 1970. p. 1-333.
8. Golterman HL, Clymo RS. Methods for chemical analysis of freshwaters. Oxford: Blackwell Scientific Publications; 1969. IBP Handbook No. 8. p. 1-180.
9. Jabeen G, Javed M, Azmat H. Assessment of heavy metals in the fish collected from the River Ravi, Pakistan. Pakistan Veterinary Journal. 2012;32(1):107-111.
10. Jackson RN, Baird D, Els S. The effect of the heavy metals lead (Pb^{2+}) and zinc (Zn^{2+}) on the brood and larval development of the burrowing crustacean *Callianassa kraussi*. Water SA. 2005;31(1):107-116.
11. James R, Sampath K, Edward DS. Copper toxicity on growth and reproductive potential in an ornamental fish, *Xiphophorus helleri*. Asian Fisheries Science. 2003;16(4):317-326.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry. 1951;193(1):265-275.
13. Mahmuda M, Hossain MA, Mahmud Y, Islam MS, Rahman M. Heavy metal contamination in tilapia, *Oreochromis niloticus* collected from different fish markets of Mymensingh district. Journal of Agriculture, Food and Environment. 2020;1(4):1-5.
14. Nussey G, Van Vuren JHJ, Du Preez HH. Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, *Labeo umbratus* (Cyprinidae), from Witbank Dam, Mpumalanga. Water SA. 2000;26(2):269-284.
15. OECD. OECD guideline for the testing of chemicals.

Paris: OECD Publishing; 2000. p. 1-11.
16. Thampi R, Rattan P, Chatterji A. Respiratory metabolism in *Oreochromis mossambicus* Peters under different environmental conditions. 1994;9-13.
17. Roe JH. The determination of sugar in blood and spinal fluid with anthrone reagent. Journal of Biological Chemistry. 1955;212(1):335-343.
18. Sarkar M, Islam JB, Akter S. Pollution and ecological risk assessment for the environmentally impacted Turag River, Bangladesh. Journal of Materials and Environmental Science. 2016;7(7):2295-2304.
19. Sarkar MM, Rohani MF, Hossain MAR, Shahjahan M. Evaluation of heavy metal contamination in some selected commercial fish feeds used in Bangladesh. Biological Trace Element Research. 2022;200(2):844-854.
20. Schmidt-Nielsen K. Animal physiology: adaptation and environment. 5th ed. Cambridge: Cambridge University Press; 2007. p. 1-611.
21. Sekhar PR, Savithri Y, Aruna Kumari D, Prasad GLN. Combined toxic effect of cypermethrin and chlorpyrifos on carbohydrate metabolism of freshwater carp fish *Labeo rohita* (Hamilton, 1822). International Journal of Zoology Studies. 2023;44(23):408-415.
22. Shahjahan M, Islam SM, Bablee AL, Siddik MAB, Fotedar R. Increase in water temperature increases acute toxicity of sumithion causing nuclear and cellular abnormalities in peripheral erythrocytes of zebrafish *Danio rerio*. Environmental Science and Pollution Research. 2019;26(36):36903-36912.
23. Shahjahan M, Islam SM, Bablee AL, Siddik MAB, Fotedar R. Sumithion usage in aquaculture: benefit or forfeit? Reviews in Aquaculture. 2021;13(4):2092-2111.
24. Sivabalan A. Cadmium toxicity studies and the effect of several biofeeds in *Labeo rohita* (Hamilton, 1822). Uttar Pradesh Journal of Zoology. 2022;43(1):5-12.
25. Taslima K, Khanam S, Rahman MM, *et al*. Impacts of heavy metals on early development, growth and reproduction of fish - a review. Toxicology Reports. 2022;9:858-868.
26. Tewari H, Gill TS, Pant J. Impact of chronic lead poisoning on the hematological and biochemical profiles of a fish, *Barbus conchonius* (Ham.). Ecotoxicology and Environmental Safety. 1987;13(1):748-752.
27. United States Environmental Protection Agency (USEPA). National interim primary drinking water regulations. Federal Register. 1976;41:28404.
28. Vaseem H, Banerjee TK. Contamination of the River Ganga and its toxic implication in the blood parameters of the major carp *Labeo rohita* (Ham.). Environmental Science and Pollution Research. 2013;20(8):5673-5681.
29. Vaseem H, Banerjee TK. Biochemical alteration in the *Labeo rohita* tissues following exposure to the effluent generated during extraction of metals from polymetallic sea nodules. Ecotoxicology and Environmental Safety. 2014;42(8):1060-1065.
30. Yaqub S, Javed M. Acute toxicity of water-borne and dietary cadmium and cobalt for fish. International Journal of Agriculture and Biology. 2012;14(2):276-280.
31. Zulqarnain S, Rasheed S, Hussain A, Saeed I. Effect of lead acetate toxicity on the histological and biochemical changes in liver and kidney of fish rohu (*Labeo rohita*). Biological and Clinical Sciences Research Journal. 2024;1:884-889.