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Unveiling the Impact of Heavy Metal Lead on *Hypophthalmichthys molitrix*: Insights into Behavioral and Haematological Alterations

Sabahat Saeed[1], Noman Waheed[2*], Zeeshan Ahmad[1], Rukhsar Saleem[3], Adeeba Naseer[4], Neelam Afridi[5], Ahsan Waheed[1], Asifa Khan[1], Sana Iqbal[6] and Wajeeha Wajid[7]

[1]Department of Zoology, Hazara University Mansehra (21300) KPK **Pakistan**
[2]College of Animal Science and Technology, Jilin Agricultural University, Changchun, Jilin, 130118 PR **China**
[3]University of Veterinary and Animal Sciences Lahore, Punjab, **Pakistan**
[4]Department of Zoology, The Islamia University of Bahawalpur, Bahawalpur, **Pakistan**
[5]Department of Zoology, Islamia College, University Peshawar, **Pakistan**
[6]Department of Zoology, University of Sargodha, Sargodha, **Pakistan**
[7]Department of Environmental Sciences and Policy, Lahore School of Economics **Pakistan**

***Correspondence:** nomanwaheed9131@gmail.com **Received: January 27, 2024, Revised: March 09, 2024, Accepted: March 10, 2024 e-Published: March 12, 2024**

The present study has been conducted on *Hypophthalmichthys molitrix,* so that the impact of different concentrations of lead on behavior and blood haematological indicators could be investigated. For experimentation, the fish were distributed into four groups (n = 10). Three groups designated as E_1, E_2 and E_3 were exposed with 1/10th, 1/20th and 1/30th of LC_{50} of lead nitrate, and the fourth group was lead-free control (E_0). Results revealed that exposure to lead nitrate caused significant changes in behavioral and hematological parameters of *H. molitrix*. After one week of lead exposure, numerous behavioral changes were observed which include lack of body control, rapid movement, and loss of body equilibrium, upwards diving, blackness on the fins, operculum movements, unidirectional diving, surface respiration, fin shaking, and mucous discharge. Results showed a significant ($p < 0.05$) decrease in blood parameters such as hematocrit, red blood cells, hemoglobin, MCHC and platelets. In contrast, mean cell volume, mean cell hemoglobin and white blood cell values increased significantly. Those results suggest that lead has the ability to bring variations in behavior and blood parameters of *H. molitrix.*

Keywords: Heavy metals, *Hypophthalmichthys molitrix,* Haematology, Lead*,* Behavioral

INTRODUCTION

The metal which has a relatively high density and toxic at low quantity is referred as ‘heavy metal’ (Pandey et al.2014). Any toxic metal may be called heavy metal, irrespective of their atomic mass or density (Singh, 2007). Heavy metals are a member of an ill-defined subset of elements that exhibit metallic properties (Singh et al.2011). Heavy metals resources in the surroundings can come from both nature and anthropogenic actions. The primary anthropogenic sources include farming, manufacturing, transportation, mining, combustion of fuel, leftover organic matter, and waste water (Yin et al.2021). The major natural sources of heavy metals are windblown dust, volcanogenic particles, forest wildfires, vegetation, and sea salt.

The heavy metals are accumulated in living organisms when they are taken up, and stored faster than they are metabolized or excreted (Baby et al.2010). Aquatic organisms, such as fish, accumulate pollutants directly from contaminated water and indirectly via the food chain (Khayatzadeh and Abbasi, 2010). Fish may consume heavy metals by consumption of contaminated food through the alimentary canal or through the skin and gills (Authman et al.2015). After the ingestion of heavy metals by fish, carried through the circulatory system to the tissues and organs such as the liver, gills, intestine and kidneys where they form precipitations and the impacts become apparent when the concentrations in such tissues reach high levels (Zaynab et al.2022).The contamination by heavy metals of aquatic ecosystems arises from direct atmospheric absorption, geological weathering and erosion or the disposal of agricultural in nature, municipal, residential, and industrial wastes, as well as wastewater from agricultural treatment (Abdel-Warith et al.2020). Among the aquatic habitants, fish is the most susceptible to these elemental contaminants and more vulnerable to metals contamination than any other aquatic habitant (Ramesh et al.2009).

There are several heavy metals that could potentially adversely affect the ecosystem, but most pollutant/environmental heavy metals have been reported are Pb, Cd and Hg (Huseen and Mohammed, 2019). Among these, Lead is a persistent metal that has been regarded as a priority harmful chemical substance (Sfakianakis et al.2015). Lead is non-essential and harmful to animals even at low concentrations (Shah, 2006). Exposure of Pb can cause many effects depending on level and duration of Pb (Dongre et al.2013). Lead is prevalent in aquatic environment by anthropogenic activity such as industries of batteries, paint production, and leaded gasoline (Monteiro et al.2011). Lead bioaccumulation in aquatic creatures takes place through food and the water. The accumulation of Lead occurs in different organs of fish, such as the kidneys, liver, and spleen, as well as the digestive system and gills (Lee et al.2019). The exposure of lead in aquatic ecosystem adversely affects reproduction, growth, and behavior in fish (Kim et al.2015).

Fish is the key creatures that have a wide range of impacts on the lives of humans. Fish provides vitamins, amino acids, lipids, and a variety of additional products such as fish glue and fish oil, and meal from fish. Haematological parameters (T.L.C, RBCs, Hemoglobin, PCV, Platelets, MCV, MCH, MCHC) are frequently applied to decide special effects on fish disclosure to ecological factors like as aquatic containments and are valuable biomarkers for together aquaculture and nature protection (Burgos-Aceves et al.2019).

Silver carp (*H. molitrix*), originating from eastern Asia, has gained global attention as a significant food source and an invasive species (Garvey, 2012). Recognized for its exceptional filter feeding capability, this freshwater fish is distinguished by its silver coloration along its scales, from which its name derives. Within its native habitats, silver carp serves a critical ecological function by consuming plankton and preserving water quality (Jawdhari et al.2022). Despite its invasive tendencies, silver carp remains highly valued in aquaculture due to its nutritional richness and culinary versatility (Fang et al.2022). The ecological health of silver carp populations is under considerable threat due to pollutants infiltrating their habitats Industrial and agricultural pollutants, such as pesticides, heavy metals, and fertilizers. These pollutants interfere with crucial biological functions, compromising the immune system and reproductive abilities of silver carp, thereby endangering their long-term viability (Ullah et al.2019).

The current study was conducted to evaluate the toxic effect of Lead (Pb) in behavioral, as well as the haematological parameters in *H. molitrix* exposed to sublethal concentrations of Pb. Lead is the most widespread kind of pollutants in the rivers of many countries globally. This study plays a vital role in guiding efforts to preserve and sustainably utilize this precious resource, thereby ensuring the long-term health of freshwater ecosystems.

MATERIALS AND METHODS

Experimental fish

The *H. molitrixc* specimens, sizes ranging from 6 to 8 inch, were collected from River Siran, District Mansehra KPK. After permission granted by Fisheries department Mansehra, the local Fishers assisted in catching the fish with the help of holding and cast net, placed in plastic sacks with over oxygenated water, and then transported to the laboratory for future research. The captured fish specimens were shifted to the research laboratory.

ACCLIMATIZATION

In laboratory these fish were acclimatized for one week for adjustment of fish with laboratory conditions before the experiment. Fish were kept in aerated glass aquarium with dissolved oxygen, Temperature of 20-23°C and pH 7.6-7.8. Fish were daily feed on artificial pelleted commercial food during this period. After serving extra food was eliminated. During acclimatization period 70% of the water was changed every day.

EXPERIMENTAL DESIGN

Acclimatized fish were divided into four groups, at which a set of 30 fish were introduced in 3 replicates (10 fish/ replicate), containing 40L of water. One was control (E_0) and remaining three were experimental groups named as E_1, E_2 and E_3 treated with a sub lethal dosages of lead nitrate ($Pb(NO_3)_2$), such as 1/10th, 1/20th and 1/30th of LC_{50} accordingly. A semi-static system was employed, with the test solution being replaced every 24 hours. The stock solution of Lead (The molecular Mass 331.2 g/mole and CAS # 10099-74-8) had been synthesized in water that was distilled and then dilute to the required proportion. The investigation was carried out about period of 1 week.

Dosage formulation for numerous research groups

Groups	Doses of Pb	Density of stock (Per group)	Repeats
E_0	0	10	3
E_1	2.18 (1/10th of LC_{50})	10	3
E_2	4.37 (1/20th of LC_{50})	10	3
E_3	6.56 (1/30th of LC_{50})	10	3

Behavioural Investigation

During one week of experimentation, the behaviour of *H. molitrix* which subject to three different concentrations of lead nitrate was keenly observed.

BLOOD COLLECTION AND ANALYSIS

After the exposure period, no mortalities were observed in the experimental fish. Following the completion of toxicity test, five fish were randomly picked from each aquarium and anesthetized in clove-oil in order to collect blood. Blood samples (1 mL) were immediately taken from the caudal line of fish in each container using heparinated syringes and subsequently transferred to tubes containing EDTA for anticoagulant, for evaluation of the total leukocyte counts, total RBCs count, hemoglobin, platelets numbers, PCV, MCV, Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular hemoglobin (MCH), by using hematological analyzer (pocH-100ii).

DATA ANALYSIS

SPSS software version 26 has been used to perform the study's statistical analysis. The T-test was performed to determine the statistically significant difference between the exposed and control group. All of the parameter values were reported with a mean ± SD. ANOVA test was used for comparing variables between treated groups with each other. P<0.05 was considered as statistically significant.

RESULTS

Water parameters

The physiological and chemical properties of water were monitored at 24-hour intervals throughout the research. Mean ± SD values of water temperature, pH, conductivity, dissolved solids and dissolved oxygen were noted as 22.6 ± 1.20°C, 740.6 ± 7.78μMcm^{1}, 7.85 ± 0.06, 6.54 ± 0.04mg/L and 372.9 ± 4.92 ppm accordingly.

Observation of Fish Behavior

In all the treated groups, fish subjected to lead, demonstrated unusual behavior. Sign of stress, Fish jumping from aquariums, loss of body equilibrium, upward diving, darkness of fins, operculum movement, one-sided swimming, surface breathing, fin tremors, erratic swimming and mucus secretion in exposed groups by comparing with untreated group.

Haematological analysis

The findings of current research demonstrated the levels of the RBCs, PCV, hemoglobin, MCHC, and platelets reduced substantially ($p< 0.05$) with rising levels of Pb as illustrated in graphs. On the contrary, MCV and WBCs levels rose significantly ($p < 0.05$) and MCH values showed an insignificant rise in the treated groups in comparison to the untreated group. The highest possible value of white blood cells (14.67±1.52) were noted in fish subjected to 4.37 mg/L^{-1}of lead (E_2) and the least in untreated group (E_0). Furthermore, counts of RBCs reduced in the groups with higher doses of lead. The highest values of red blood cells noticed in untreated group E_0 (4.78±0.11) and least in E_3 (2.19±0.11). The levels of hemoglobin were observed to be least in E_3 (5.33±0.56) and maximum in E_0 (8.94±.05). The number of platelets in all the groups reduced as $E_0 > E_1 > E_2 > E_3$. Additionally, MCHC levels reduced considerably ($p < 0.05$) in treated groups.

Table 1: Alterations in behavior rate of lead treated and untreated fish.

Behavioural Alterations	Doses			
	E_0 (Control)	E_1 (1/10th)	E_2 (1/20th)	E_3 (1/30th)
Operculum movement	–	**+**	**+**	**+**
Rapid swimming	–	+	+	++
Surface breathing	–	+	+	+
Loss of scales	–	–	+	+
Darkening of fins	–	–	+	++
Discoloration	–	–	+	+

No change (−), Mild changes (+), Evident changes (++).

Computation of LC_{50}

Concentration	Log 10	Mortality (%)	Probit Value
10	1	20	4.16
20	1.30103	40	4.75
30	1.477121	55	5.13
40	1.60206	80	5.84
50	1.69897	90	6.28

The LC_{50} value of lead nitrate was found to be 21.87 mg/l over 96 hour's exposure.

Table 2: Haematological profiles of *H. molitrix* exposed to sublethal concentrations of lead nitrate.

Haematological Parameters	Mean E0	Mean E1	Mean E2	Mean E3
RBCs	4.78±0.11	3.98±0.148**a	3.41±0.46** b	2.19±0.11** c
Hb(g/dL)	8.94±.05	8.28±0.45	6.75±0.20**	5.33±0.56
HCT (%)	39.52±0.58	36.73±0.92*	34.73±0.90**	35.37±0.63*
MCV	82.63±0.73	92.34±3.00** a	102.95±11.38* b	161.97±8.57** c
MCH	18.68±0.32	20.81±1.58	19.98±2.03	24.39±2.89
MCHC	22.61±0.19	22.56±1.81	19.42±0.24**	15.03±1.34**
Platelets×10^3	50.66±92.11	47.33±25.80 a	43.00±30.38 b	39.66±76.76c
WBC (/uL)	8.33±2.51	10.33±1.52a	14.67±1.52*b	11.33±1.52c

Summary of (Mean ± S.D) values for Haematological indices with symbol * and ** denotes significant deviation ($p<0.05$) between the experimental groups and the control group and symbols (a,b,c) indicates significant intergroup difference.

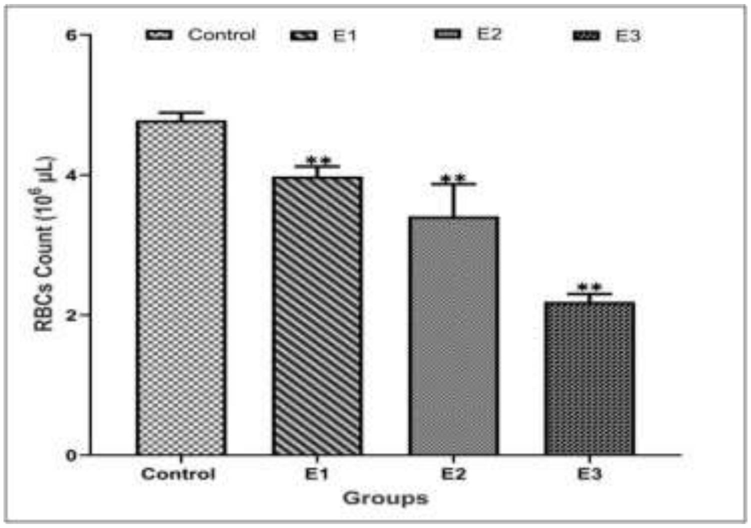

Figure 1: Graphical illustration of the RBCs of control and exposed groups of *H. molitrix.*

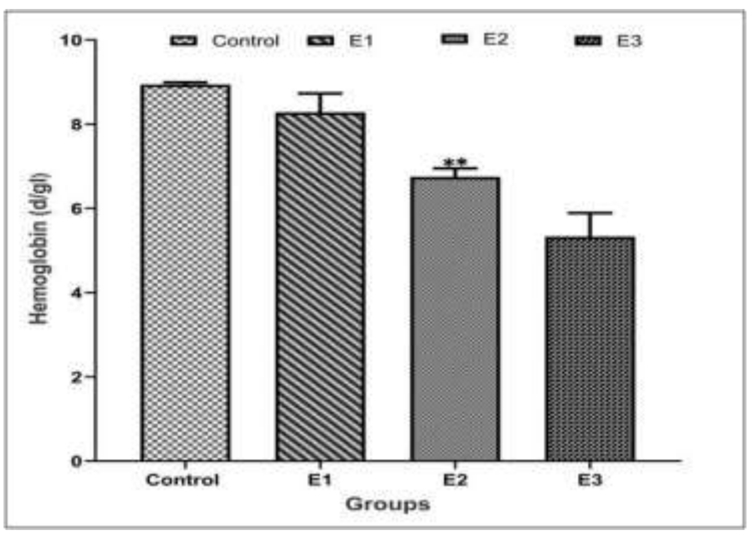

Figure 2: Graphical illustration of the Hemoglobin of control and exposed groups of *H. molitrix*.

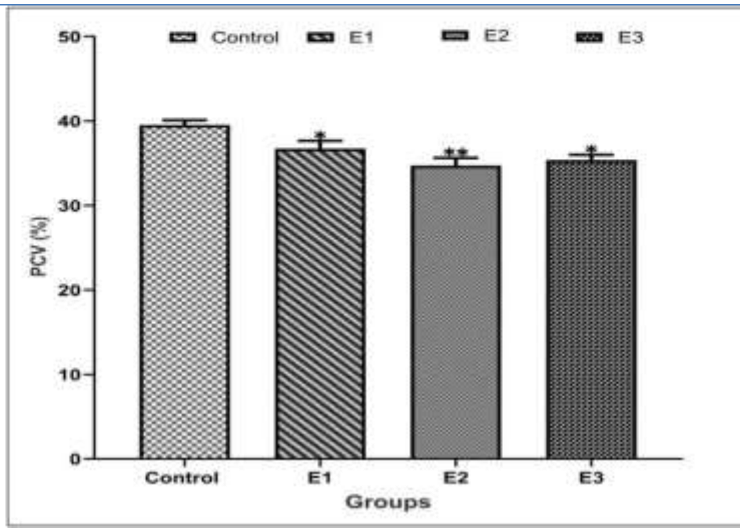

Figure 3: Graphical illustration of the PCV of control and exposed groups of *H. molitrix*.

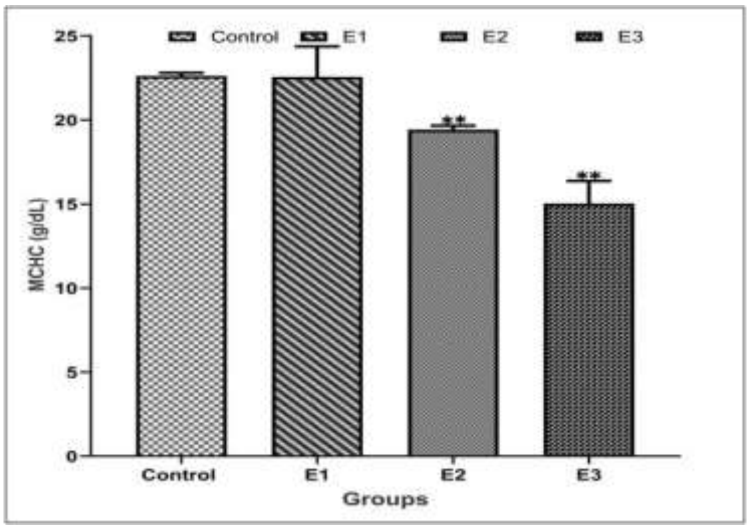

Figure 4: Graphical illustration of the MCHC of control and exposed groups of *H. molitrix*.

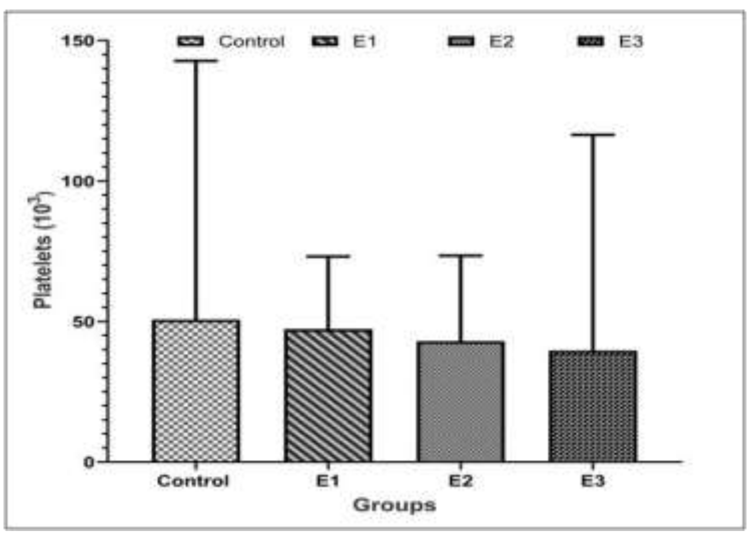

Figure 5: Graphical illustration of the Platelets of control and exposed groups of *H. molitrix.*

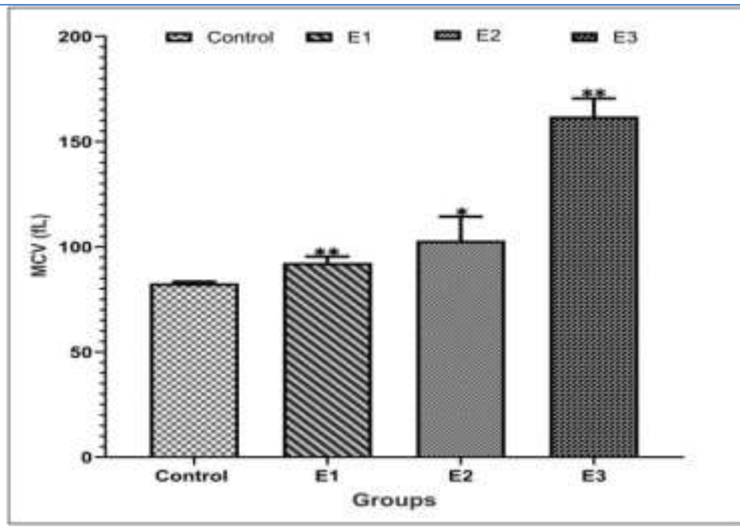

Figure 6: Graphical illustration of the MCV of control and exposed groups of *H. molitrix.*

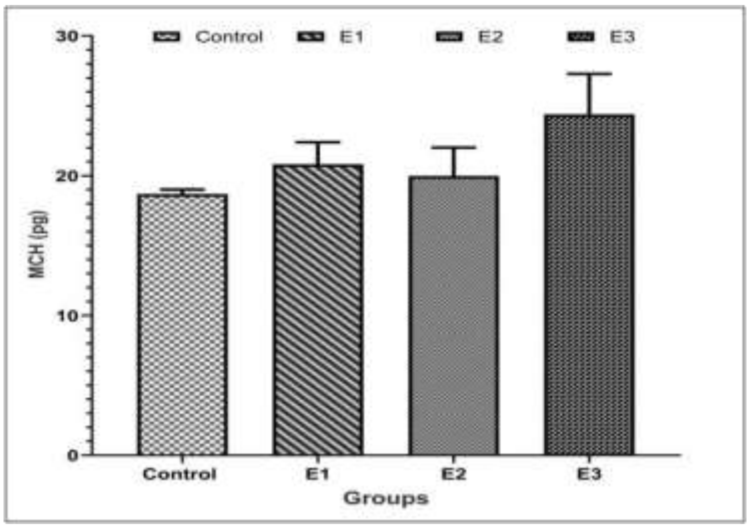

Figure 7: Graphical illustration of the MCH of control and exposed groups of *H. molitrix.*

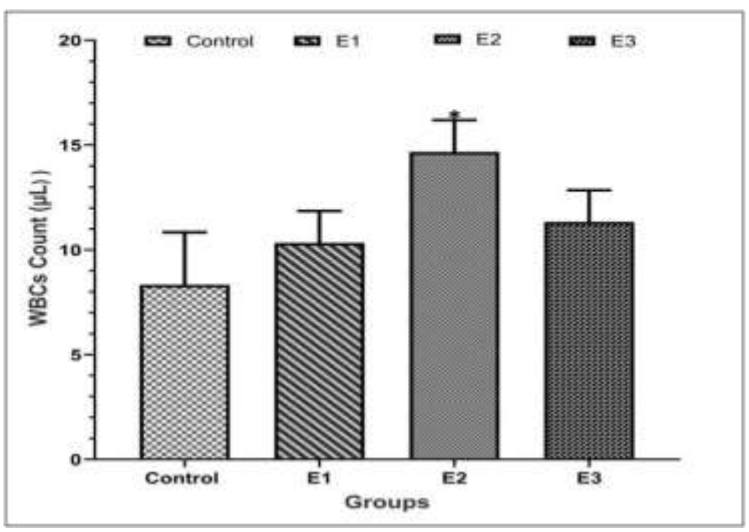

Figure 8: Graphical illustration of the WBCs of control and exposed groups of *H. molitrix*

DISCUSSION

The worldwide population is growing every day, resulting in growing industries, urbanization, and intensification of agriculture. The growing industrial sector released a variety of pollutants into the environment without restoration. These contaminants have various adverse effects on living beings (Ali et al.2021). The contaminants emitted into the surroundings include heavy metals, oxidizing agents, pesticides and chemical fertilizers. Several studies have reported alterations in fish blood markers as a result of exposure to toxins.

H. molitrix exposed to Pb heavy metal displayed abnormal behavior. In comparison with the control fish, the exposed fish emerged frequently. Enhanced swimming, anxiousness, erratic diving, and loss of posture were seen as a result of oxygen deprivation, operculum movements, laying on one side, and attempts to jump out of the harmful surroundings. Comparable behavioral characteristics have been encountered by (Vasanthi et al.2019) in fish *O. mossambicus* when exposed to mercury. Current outcomes reveal similarities with (Naz et al.2021), noted same behavioral alterations in *C. catla* subjected to cadmium and copper. Our outcomes are also supported by (Chaudhary et al.2023)revealed the toxicity of chromium in *Labeo rohita* by applying different concentrations of chromium.

In current study, the sub lethal doses of Pb caused considerable declines in the number of red blood cells, platelet count, MCHC, PCV, and hemoglobin, although MCV, and WBCs values rose substantially ($P < 0.05$). The levels of MCH also showed an insignificant increase in lead treated groups.

Our results showed a decline pattern in RBCs, PCV and Hb values. These outcomes are in lined with (Mahmoud et al.2013), noted that *C. gariepinus* exposed to 7 mg/l of Pb indicated a notable reduction in their Hb, PCV, and RBCs values. Reduction in blood markers levels are the sign of malfunction of hematopoietic tissues, defective osmoregulatory systems and enhanced destruction to red blood cells in hemoglobin-forming organs (Ghaffar et al.2021).The noticeable decline in PCV and hemoglobin levels indicated a hemodilution mechanism caused by inadequate osmoregulation or gill injury (Hedayati and Darabitabar, 2017). In the current findings, a reduction in RBCs and hemoglobin levels occurred against lead when compared with the untreated group, which is coupled with (Shah et al.2020) that sub lethal doses of chromium, lead, and copper exposure have resulted in hemolytic anemia due to break down of RBCs along with decline in blood cell counts. (Vinodhini et al.2009) subjected common carp to a mixed metal solution and observed a comparable reduction in blood parameters.

The rise in white blood cell count demonstrate damage caused by leukemia, extreme physical exertion, and tissue-related infections (Afaq et al., 2009). The rise in TLC can be associated with immune system action in response to lead-induced tissue damages. These results were in lined with outcomes obtained by (Chaudhary et al.2023)who reported that adult *L. rohita* exposed to various concentrations (11, 22, 37 mg/l) of chromium chloride for 7 days, recorded a rise in WBCs counts in treated groups when compared with control group. A comparable rise has been noted in *Garra gotyla gotyla* by (Sharma and Langer, 2014)and (Naz et al.2021) in *C. catla*.

In the current investigation, when *H. molitrix* was subjected to heavy metal lead, the number of platelets reduced in the treatment groups relative to the untreated group. The decline in number of platelets suggested a possible hemodilution mechanism that was probably brought on by gill damage or impaired osmoregulation.

The $Pb(NO_3)_2$ induced changes towards increases or reductions in MCH, MCHC, and MCV values were noted in the current investigation. In the current study, fluctuations in blood markers, such as a rise in mean cell volume (MCV) and MCH values might be attributed to lead intoxication and abnormalities in fish metabolic activity. The amount of MCV can assist determine the synthesis of RBC in erythropoiesis (Kondera et al.2019). The MCH is the proportion of hemoglobin level to RBCs count. Our findings reported a significant increase in total MCV and MCH of exposed to lead nitrate. This outcome was similar to previous research by (Kumar and Banerjee, 2016) who also reported a significant increase of MCV in *C. batrachus* exposed to arsenic. The MCHC values assessment is an indicator to determine the quantity of erythrocytes swelling (lower MCHC) or shrinking (higher MCHC) (Devi et al., 2007).This demonstrates how arsenic induces enlargement of the red blood cells. Additionally, some fish subjected to arsenic exhibited a decrease in their MCHC level(Kumar and Banerjee, 2016)and cadmium and copper (Kang et al.2005).

In the current investigation, when *H. molitrix* was subjected to particular heavy metals, the number of platelets reduced in the treatment groups relative to the untreated group. The decline in number of platelets suggested a possible hemodilution mechanism that was probably brought on by gill damage or impaired osmoregulation. Our findings are incongruous with data recorded by (Abdel-Warith et al.2020) who found that *C. gariepinus* exposed to lead showed a decline in platelet values

CONCLUSIONS

The intention of the current investigation was to evaluate that exposure of *H. molitrix* to lead nitrate at sub lethal amounts during a 7-day exposure, which demonstrates the harmful impacts on the fitness of freshwater fish. The contamination by $Pb(NO_3)_2$ may lead to considerable alterations in the physiology of *H. molitrix*, as revealed by changes in haematological and behavioral indices. Furthermore, significant alterations in blood parameters may have disrupted internal physiology. The above evidence demonstrates that contamination of the water with Pb $(NO_3)_2$ has harmful impacts on the health of fish, which is reflected in economic status, as well as human health.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/

Author contributions

All authors contributed equally.

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Institutional Review Board Statement

The study was approved by the Bioethical Committee of the Department of Zoology, Hazara University Pakistan.

Informed Consent Statement

Not applicable.

Data Availability Statement

All of the data is included in the article/Supplementary Material.

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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