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COMPARATIVE BIOACCUMULATION AND INDUCED TOXICITY OF LEAD ACETATE AND NICKEL CHLORIDE IN GRASS CARP (*Ctenopharyngodon idella*)

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ABSTRACT

This study investigated the comparative bioaccumulation and induced toxicity of lead (Pb) and nickel (Ni) in grass carp (*Ctenopharyngodon idella).* Healthy fish (7-8 cm; 60-70 g) were acclimatized for 7 days in 80-L glass aquaria and then divided into control and treatment groups (n = 3 fish/group for each exposure concentration). The treatment groups were exposed to varying concentrations of lead acetate (Pb acetate) and nickel chloride ($NiCl_2$) over specified durations. For acute toxicity assays, fish were exposed to $NiCl_2$ at concentrations of 6.0 mg/L for 24 hours, 5.0 mg/L for 48 hours, and 4.6 mg/L for 72 hours, while Pb acetate exposure concentrations were 5.0 mg/L for 24 hours, 4.8 mg/L for 48 hours, and 4.6 mg/L for 72 hours. In contrast, chronic exposure involved lower concentrations of $NiCl_2$ (4.0 mg/L for 10 days, 3.5 mg/L for 20 days, 3.0 mg/L for 30 days, 2.5 mg/L for 45 days, and 2.0 mg/L for 60 days) and Pb acetate (3.5 mg/L for 10 days, 2.5 mg/L for 20 days, 2.0 mg/L for 30 days, 1.5 mg/L for 45 days, and 1.0 mg/L for 60 days). The results showed significant hematological and biochemical changes, including decreased hemoglobin (4.96±0.876 g/dL) and white blood cell count (94.43±30.291 cells/µL) in nickel-exposed fish. Biochemical assessments revealed increased glucose levels, fluctuating urea and creatinine profiles, and decreased Ca2+ serum levels. Tissue-specific bioaccumulation was observed, with considerable accumulation in liver tissues. The findings highlight the potential risks associated with heavy metal concentrations in aquatic ecosystems and underscore the need for monitoring and regulating heavy metal levels to protect aquatic life and ensure food security.

Keywords: Heavy metals; lead acetate; Nickel chloride; Bioaccumulation; Grass carp

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INTRODUCTION

Anthropogenic activities, including industrialization, urbanization, agriculture, and pollution, have been identified as primary sources of aquatic pollution, posing significant threats to aquatic biota (Bhavsar *et al.,* 2016; Emenike *et al.,* 2021). The irregular and uncontrolled exposure of waterbody systems to pollutants has been found to be detrimental to aquatic organisms, particularly fish (Kakakhel *et al.,* 2023; Narwal *et al.,* 2024a). Environmental pollution poses considerable risks, and heavy metals (HMs) entering aquatic systems through natural or anthropogenic means can be transferred into food chains, affecting human health (Emenike *et al.,* 2021).

HMs, defined as metals with a specific gravity exceeding 5 g/cm³ (Kumar and Singh, 2024), occur naturally in trace amounts in water but are often toxic even at low concentrations (Sharma and Agrawal, 2005; Gwaltney-Brant, 2013). HMs cannot be easily degraded in nature and may remain in the environment for a long time, passing through the food chain (Narwal *et al.,* 2024b) and affecting human health (Cole *et al.,* 2009).

Notably, lead and nickel are two of the most hazardous environmental pollutants affecting aquatic ecosystems, primarily originating from industrial activities and anthropogenic sources (Obeng-Gyasi, 2019). Lead, classified as an anticatalytic metal, is highly toxic and can induce oxidative stress in aquatic organisms by interfering with enzyme systems, ion regulation, and disrupting regulatory systems (Ishaque *et al.,* 2020; Renu *et al.,* 2021). These toxic metals enter aquatic systems through industrial discharge, urban runoff, and atmospheric deposition, accumulating in water bodies and posing severe risks to aquatic life (Obeng-Gyasi, 2019).

Lead toxicity has far-reaching consequences, including impaired reproduction and growth in fish, as well as adverse effects on the health of amphibians and invertebrates, ultimately destabilizing the delicate balance of aquatic ecosystems (Renu *et al.,* 2021). The persistence of lead in sediments and water bodies underscores its potential for long-term ecological harm, necessitating rigorous monitoring and remediation efforts to mitigate its impacts on aquatic biodiversity and ecosystem health (Ishaque *et al.,* 2020; Kumar and Singh, 2024). The primary sources of nickel pollution in aquatic

systems are industrial discharges, mining operations, and atmospheric deposition, which facilitate the bioaccumulation of nickel in aquatic organisms and subsequent manifestation of toxic effects (Begum *et al.,* 2022). Bioaccumulation, characterized by the net accumulation of chemical substances in living organisms due to an imbalance between uptake and excretion rates, is a critical factor in assessing the ecotoxicological risks associated with nickel exposure (Shah *et al.,* 2020; Zhang, 2024). The adverse effects of nickel on aquatic ecosystems, including growth inhibition, reproductive impairment, and biochemical changes, highlight the importance of implementing effective measures to mitigate nickel pollution and protect aquatic biota (De Luca *et al.,* 2007; Begum *et al.,* 2022)

The existing literature suggests that most studies have focused on assessing the impact of heavy metals (HW) under water pollution conditions, primarily without investigating dose-response relationships for acute and chronic toxicity (Jomova *et al.,* 2025). Further, this study mainly focused on lead and nickel as both have low permissible limits that are 0.01 mg/L for lead, 0.07 mg/L for nickel in drinking water. The industrial activities like textile, leather, and metal industries significantly contribute to lead and nickel pollution. The accumulation of these two HM in fish can cause oxidative stress, DNA damage, and mortality. Therefore, this study investigated the toxicological effects of lead and nickel on hematological and biochemical parameters in freshwater grass carp (*Ctenopharyngodon idella*), a model cyprinid species for behavioral and toxicological research. This study also examined the bioaccumulation of these two HM in various tissues and organs of *C. idella* under laboratory conditions, providing insights into the mechanisms of toxicity and the potential use of this species as a bioindicator for aquatic pollution.

MATERIALS AND METHODS

Experimental Site and Sample Collection: The experimental study was conducted in the laboratory of the Zoological Garden, Islamia College, Peshawar, Pakistan. Healthy grass carp (*C. idella*) specimens were procured from a local hatchery in Mardan, Pakistan, and transported to the laboratory in oxygenated plastic bags. Upon arrival, the fish underwent a 7-day acclimatization period in glass aquaria (80 L capacity) filled with tap water. The aquaria were equipped with aerators, artificial lighting, and water removal pipes to ensure optimal water quality. To maintain water quality, 50% of the water was replaced daily and 100% weekly, following established protocols (Kakakhel *et al.,* 2021). During acclimatization, the fish were fed commercial fish food to minimize stress and promote adaptation to the laboratory environment. Water quality parameters, including pH, temperature, total hardness, dissolved oxygen, and total alkalinity, were monitored using the dip strip method (Stoskopf, 2015; Khan *et al.,* 2016). After acclimatization and water analysis, nickel and lead toxicity assays were conducted.

Experimental Design: Healthy grass carp (*C. idella*), approximately 4 months old, with a uniform mean size of 7.5 ± 0.5 cm and mean weight 65 ± 5 g, were selected for experimentation, without regard to sex. The fish were randomly assigned to eight experimental groups (excluding control for each heavy metal), each consisting of three individuals, and divided into two experimental batches (acute and chronic exposure) according to completely randomized design layout. The study comprised both short-term acute exposures (24, 48, and 72 hours) and long-term chronic exposures (10, 20, 30, 45, and 60 days), with a normal light-dark cycle maintained throughout. A control group, devoid of any toxicant, was run in parallel. To prevent metal residue absorption, the test chambers were thoroughly cleaned prior to use. Fish were fed twice daily (9:00 AM and 9:00 PM), except for the day of sample collection, when food was withheld to minimize potential confounding variables in subsequent analyses.

Heavy Metals and Toxicity Assays: Lead acetate (Pb acetate) and nickel chloride ($NiCl_2$) were procured from local suppliers and used to investigate their toxicological effects on grass carp. For acute toxicity tests, 27 fish were divided into three groups: Group 1 (Control, n=9) received no treatment, Group 2 was exposed to $NiCl_2$ at concentrations of 6.0 mg/L (24 hours, n=3), 5.0 mg/L (48 hours, n=3), and 4.6 mg/L (72 hours, n=3), and Group 3 was exposed to Pb acetate at concentrations of 5.0 mg/L (24 hours, n=3), 4.8 mg/L (48 hours, n=3) and 4.6 mg/L (72 hours, n=3).

For chronic toxicity assays, 45 fish were divided into three groups: Group 1 (Control, n=15) received no treatment, Group 2 was exposed to $NiCl_2$ at concentrations of 4.0 mg/L (10 days), 3.5 mg/L (20 days), 3.0 mg/L (30 days), 2.5 mg/L (45 days), and 2.0 mg/L (60 days), and Group 3 was exposed to Pb acetate at concentrations of 3.5 mg/L (10 days), 2.5 mg/L (20 days), 2.0 mg/L (30 days), 1.5 mg/L (45 days), and 1.0 mg/L (60 days). Each concentration and/or duration used separate fish (n=3).

Blood Collection for Hematological and Biochemical Parameters Determination: Blood samples were collected from the caudal vein of 10 fish in each group using a syringe without anticoagulant for serum preparation. The samples were allowed to clot at room temperature for 30 minutes and then centrifuged at 3,000 rpm for 30 minutes to obtain serum. For hematological analysis, blood samples were collected in sterilized plastic tubes coated with EDTA and analyzed using a hematological analyzer (Mindray Auto Hematology Analyzer BC 5150, China) following the manufacturer's

protocols. The hematological parameters evaluated included white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets.

The biochemical analysis encompassed the determination of random blood glucose (RBG), alanine transaminase (ALT), urea, creatinine (Creat), and serum calcium (Ca) levels. Commercially available colorimetric assay kits were utilized for these assessments, including the glucose assay kit (GOD-POD method; Elabscience, Cat. No. E-BC-K234-S) and the creatinine assay kit (Jaffe method; LSBio, Cat. No. LS-K207-500). The manufacturer's instructions were strictly followed to ensure the accuracy, precision, and reliability of the results.

Tissue Analysis for Bioaccumulation of Lead Acetate and Nickel Chloride: Following each experimental duration, fish were measured for length using a measuring tape and weight using a balance, respectively. Thereafter, fish were washed thoroughly with distilled water, and excised for harvesting various organs (muscles, gills, intestines, and liver). Various organs (muscles, gills, intestines, and liver) were harvested, washed with distilled water, the samples were dried in an oven at 80-90°C. Subsequently, 1 g of each organ was subjected to acid digestion by following the method of Van Loon (1980), and Du Preez and Steyn (1992), modified by Yousafzai and Shakoori (2006). The digestion process involved adding 10 ml of nitric acid (55%) and 5 ml of perchloric acid (70%) to each sample, followed by overnight incubation at room temperature. The next day, an additional amount of 5 ml of nitric acid and 4 ml of perchloric acid were added and the samples were heated on a hot plate at 200-250°C until a clear solution formed. The appearance of dense white fumes indicated the completion of the digestion process.

After digestion, the samples were cooled and diluted with 10 ml of distilled water in the digestion flasks and filtered through Whatman filter paper. The concentrations of nickel and lead in the tissue samples were determined using an Atomic Absorption Spectrophotometer (Spectra AA-6300, Shimadzu, Japan).

Statistical Analysis: The data on hematological and biochemical parameters were analyzed by one-way ANOVA following completely randomized design. The Duncan Multiple Range Test was used to compare differences among control and treatment groups at each exposure duration. For bioaccumulation data, Student's T-test was applied to compare metal accumulation in treated and non-treated groups. A p-value ≤ 0.05 was considered statistically significant. The data analysis was done by using SPSS software (version 21).

RESULTS

Hematological and Biochemical Parameters: The hematological and biochemical data are presented in Tables 1 and 2, respectively. The results revealed significant differences ($P \leq 0.05$) in some hematological parameters such as WBC, HCT, MCV, MCHC, and PLT etc. across treatment groups compared to the control group, varying with exposure duration. During acute exposure (24 hours), Pb acetate (5.0 mg/L) significantly increased white blood cell (WBC) count, hematocrit (HCT), and mean corpuscular volume (MCV), while decreasing mean corpuscular hemoglobin concentration (MCHC) ($p \leq 0.05$). In contrast, $NiCl_2$ (6.0 mg/L) exposure elevated MCV but reduced platelet (PLT) count. After 72 hours of acute exposure, Pb acetate (4.6 mg/L) increased MCH and MCV, while decreasing PLT count. Nickel chloride (4.6 mg/L) exposure resulted in elevated WBC count and HCT, alongside reduced MCHC count. In chronic exposure, $NiCl_2$ (4 mg/L) for 10 days increased WBC count and HCT while reducing PLT count ($p \leq 0.05$). Conversely, 60-day chronic exposure to Pb acetate (1.0 mg/L) decreased HCT, whereas $NiCl_2$ (2.0 mg/L) had no significant effects compared to the control group ($P > 0.05$).

The effects of $NiCl_2$ and Pb acetate exposure on various biochemical parameters, including random blood glucose (RBG), alanine transaminase (ALT), urea, creatinine, and serum calcium, indicated a significant difference ($P \leq 0.00$) in the exposed groups compared to the control groups across both acute (24 and 72 hours) and chronic (10 and 60 days) exposure periods (Table 2). Acute exposure (24 hours) to Pb acetate (5.0 mg/L) resulted in a significant increase in glucose levels ($p \leq 0.05$) and a concomitant decrease in ALT activity. In contrast, $NiCl_2$ exposure (6.0 mg/L) elevated creatinine levels and moderately increased glucose levels. Following 72 hours of acute exposure, Pb acetate (4.6 mg/L) significantly reduced ALT activity ($p \leq 0.05$), whereas $NiCl_2$ (4.6 mg/L) increased glucose levels. During chronic exposure, Pb acetate (3.5 mg/L) for 10 days caused a marked increase in glucose levels ($p \leq 0.05$) and a pronounced reduction in ALT activity ($p \leq 0.01$). Similarly, $NiCl_2$ (4 mg/L) decreased ALT activity ($p \leq 0.05$). After 60 days of chronic exposure, Pb acetate (1.0 mg/L) significantly decreased blood glucose levels ($p \leq 0.05$) and increased creatinine levels. In contrast, $NiCl_2$ (2.0 mg/L) reduced both ALT activity and serum calcium levels ($p \leq 0.05$ for both).

Bioaccumulation: The bioaccumulation of lead (Pb acetate) and nickel ($NiCl_2$) in various organs of *C. idella*, including muscles, gills, intestine, and liver, was investigated under acute (24 and 72 hours) and chronic (10 and 60 days) exposure conditions. Significant metal accumulation of lead (Pb acetate) and nickel ($NiCl_2$) in

Table 1. Changes in the hematological parameters of *Ctenopharyngodon idella* fish exposed to different concentrations of heavy metal during acute and chronic exposure.

Treatments	Hematological Parameters of *Ctenopharyngodon Idella*							
	WBC (*10^3/µL)	Hb (g/dL)	RBC (*10^6/µL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (*10^3/µL)
Acute Exposure (24 hours)								
Control	61.72±1.99^{b}	10.26±0.584	2.490±0.130	19.60±1.548^{c}	78.33±2.603^{b}	41.26±0.233	52.67±1.351^{a}	107.66±8.838^{a}
Lead acetate (5.0 mg/L)	195.60±13.804^{a}	7.13±0.887	2.31±0.052	33.33±1.162^{a}	144.36±7.183^{a}	30.73±3.183	21.63±3.433^{c}	32.00±22.120^{b}
Nickel Chloride (6.0 mg/L)	117.38±35.420^{b}	10.76±1.072	1.95±0.186	28.31±1.983^{b}	145.70±6.206^{a}	57.33±11.985	38.83±6.476^{b}	18.33±3.666^{b}
Acute Exposure (72 hours)								
Control	98.50±5.819^{c}	9.366±0.317	2.41±0.106	18.64±1.111^{c}	80.66±3.480^{b}	40.96±0.796^{c}	53.16±0.938^{a}	82.00±3.055^{a}
Lead acetate (4.6 mg/L)	123.83±32.787^{b}	11.00±1.209	2.07±0.270	30.34±1.510^{b}	149.53±13.051^{a}	56.26±13.870^{a}	33.80±8.245^{b}	12.00±3.055^{c}
Nickel Chloride (4.6mg/L)	205.10±15.421^{a}	8.60±1.665	2.50±0.138	37.20±1.852^{a}	117.80±37.857^{b}	34.16±5.777^{b}	22.96±4.682^{c}	16.00± 6.000^{b}
Chronic Exposure (10 days)								
Control	123.50±4.810^{b}	9.23±0.660^{a}	2.40±0.165	19.90±1.620^{b}	143.30±0.880	40.80±0.800	25.40±0.890	96.30±3.700^{a}
Lead acetate (3.5 mg/L)	108.61±58.280^{b}	9.30±1.410^{a}	2.38±0.720	27.30±7.900^{b}	125.10±25.600	48.90±17.500	40.80±11.700	79.00±12.500^{a}
Nickel Chloride (4 mg/L)	213.30±2.140^{a}	6.56±0.660^{b}	2.32±0.110	35.50±0.970^{a}	152.30±8.410	28.40±1.430	18.50±0.340	31.30±9.060^{b}
Chronic Exposure (60 days)								
Control	122.66±12.600	9.36±1.046^{a}	2.30±0.251^{a}	20.56±1.047^{a}	155.80±2.640	39.63±0.491^{a}	25.40±0.702	18.00±17.776
Lead acetate (1.0 mg/L)	94.43±30.291	4.96±0.876^{b}	1.34±0.069^{b}	18.10±0.873^{b}	158.46±9.353	36.43±4.830^{ab}	22.80±1.732	14.60±3.700
Nickel Chloride (2.0 mg/L)	82.33±2.027	6.13±0.290^{b}	1.86±0.033^{b}	18.70±1.073^{ab}	161.20±7.419	35.10±0.585^{b}	22.33±1.333	18.66±4.333

^{abc} The mean values in a column with common superscripts differ significantly (P≤0.05).

Abbreviations: WBC; White blood cell count, HCT: hematocrit, MCV: Mean corpuscular volume, Hb: hemoglobin, RBC; Red blood cells, HCT; Hematocrit, MCH; Mean corpuscular hemoglobin (MCH), MCHV; Mean corpuscular hemoglobin concentration, PLT: Platelet count.

Table 2. Changes in the Biochemical Parameters of *Ctenopharyngodon idella* fish exposed to different concentrations of heavy metals during acute and chronic exposure.

	Biochemical Parameters of *Ctenopharyngodon Idella*				
	Glucose (mg/dL)	Alanine Transaminase **ALT (mcg/L)**	**Urea (mg/dL)**	**Creatinine (mg/dL)**	**Serum Calcium (mg/dL)**
Acute Exposure (24 hours)					
Control	97.33±6.741^{c}	40.33±5.487	12.33±1.201^{a}	0.13±0.033^{c}	9.30±0.435^{a}
Lead acetate (5.0 mg/L)	236.33±28.614^{a}	21.33±1.201	5.66±1.201^{c}	0.16±0.066^{b}	8.26±0.202^{b}
Nickel Chloride (6.0 mg/L)	153.66±12.250^{b}	23.33±3.333	8.66±0.881^{b}	0.26±0.120^{a}	8.43±0.466^{b}
Acute Exposure (72 hours)					
Control	88.66±8.293^{b}	21.00±1.154^{a}	10.00±1.732^{a}	0.23±0.088^{b}	8.66±0.290
Lead acetate (4.6 mg/L)	96.00±10.535^{b}	13.33±0.881^{b}	10.00±1.732^{a}	0.33±0.066^{a}	8.03±0.523
Nickel Chloride (4.6mg/L)	115.66±6.173^{a}	12.00±2.081^{b}	12.33±2.848^{b}	0.20±0.057^{b}	7.83±0.352
Chronic Exposure (10 days)					
Control	93.30±7.440^{c}	53.00±1.150^{a}	18.40±4.190^{a}	0.10±0.057^{c}	9.06±0.330^{a}
Lead acetate (3.5 mg/L)	147.00±7.440^{a}	24.30±2.180^{b}	8.86±2.460^{c}	0.16±0.033^{b}	8.50±0.300^{b}
Nickel Chloride (4 mg/L)	123.00±1.520^{b}	22.30±3.170^{b}	11.30±3.260^{b}	0.26±0.080^{a}	8.66±0.460 ^{b}
		Chronic Exposure (60 days)			
Control	82.33±7.440^{b}	36.66±1.452^{a}	21.33±7.125^{a}	0.33±0.033^{b}	9.36±0.272^{a}
Lead acetate (1.0 mg/L)	74.66±1.450^{c}	18.33±1.452^{b}	11.00±1.000^{b}	0.63±0.176^{a}	8.43±0.088 ^{b}
Nickel Chloride (2.0 mg/L)	99.00±12.280^{a}	11.40±4.910^{c}	11.33±1.760^{b}	0.50±0.260^{a}	7.70±0.416^{c}

^{abc} The mean values in a column with common superscripts differ significantly (P≤0.01).

Table 3. Bioaccumulation of lead acetate (µg/g) and nickel chloride (µg/g) in various organs of *Ctenopharyngodon idella* fish during acute and chronic exposure.

	Organs[1]			
	Muscles	**Gills**	**Intestine**	**Liver**
Acute Exposure (24 hours)				
Lead acetate (5.0 mg/L)	0.26±0.263*	0.55±0.172*	0.55±0.118*	0.71±0.135*
Nickel Chloride (6.0 mg/L)	0.09±0.048	0.06±0.048	0.18±.086*	0.03±0.039
Acute Exposure (72 hours)				
Lead acetate (4.6 mg/L)	0.82±0.191*	1.00±0.190*	3.19±1.729**	1.13±0.151*
Nickel Chloride (4.6mg/L)	0.15±0.016*	0.19±0.009**	0.65±0.210*	0.16±0.032*
Chronic Exposure (10 days)				
Lead acetate (3.5 mg/L)	1.67±0.530*	4.67±1.960**	2.49±0.630*	2.00±0.850*
Nickel Chloride (4 mg/L)	0.04±0.030	0.30±0.029*	0.96±0.081**	0.15±0.150
Chronic Exposure (60 days)				
Lead acetate (1.0 mg/L)	5.36±0.411*	5.14±0.557*	5.33±0.171*	6.34±0.740**
Nickel Chloride (2.0 mg/L)	2.28±0.375*	2.41±0.478*	3.72±0.388*	3.00±0.339*

[1]Data presented as Mean ± SE (n=3) and analyzed by Student's T-test comparing with the control; *Significant (p≤0.05), ** Significant (p≤0.01)

various organs of *C. idella*, including muscles, gills, intestine, and liver, was observed under acute (24 and 72 hours) and chronic (10 and 60 days) exposure conditions in the treated groups, with distinct organ-specific and exposure duration-dependent trends (Table 3). During .acute exposure (24 hours), Pb acetate (5.0 mg/L) resulted in notable bioaccumulation in the gills, intestine ($p \leq 0.05$), and liver ($p \leq 0.05$), whereas $NiCl_2$ (6.0 mg/L) showed comparatively lower accumulation, with prominent levels in the intestine. Following 72 hours of acute exposure, Pb acetate (4.6 mg/L) induced significant ($p \leq 0.05$) bioaccumulation in muscles, gills, and liver ($p \leq 0.05$), with exceptionally high levels in the intestine. In contrast, $NiCl_2$ (4.6 mg/L) significantly ($p \leq 0.05$) increased metal levels in intestine, muscles, gills, and liver ($p \leq 0.05$). Under chronic exposure conditions, Pb acetate (3.5 mg/L) for 10 days led to substantial accumulation across all organs, while $NiCl_2$ triggered significant intestinal bioaccumulation ($p \leq 0.05$). After 60 days of chronic exposure, Pb acetate (1.0 mg/L) resulted in marked accumulation in all organs. Notably, $NiCl_2$ (2.0 mg/L) caused significant bioaccumulation in all organs, including muscles, gills, intestine, and liver ($P \leq 0.05$).

DISCUSSION

The present study evaluated the impact of Pb acetate and $NiCl_2$ on hematological and biochemical parameters in *C. Idella.* The observed significant increase in white blood cell (WBC) count following acute exposure to both metals suggests they act as potent environmental toxicants affecting leukocyte dynamics. Conversely, the non-significant decrease in WBC counts after chronic exposure indicates potential immunosuppression or adaptive exhaustion. The constitutive WBC levels in control groups reflect baseline immune competence under unstressed conditions. Chronic metal exposure likely induced leukopenia through multifactorial mechanisms including hematopoietic suppression, oxidative stress, glucocorticoid-mediated immunosuppression or resource reallocation (Pottinger, 2008; Witeska *et al.,* 2023).

Regarding hemoglobin (Hb), the observed decreases, particularly exacerbated under chronic exposure, suggest impaired synthesis and potential hemolytic anemia (Ahmed *et al.,* 2020; Witeska *et al.,* 2023). The profound decrease in red blood cell (RBC) counts during chronic exposure further supports hematological disruption. These alterations align with established adverse effects of metal exposure on fish hematology (Ray, 2016; Mahi *et al.,* 2022; Witeska *et al.,* 2023).

The significant increase in hematocrit under acute conditions contributes to metal-induced stress responses. The reduced impact chronically may relate to developing anemia or fluid retention (Ahmed *et al.,* 2022). The significantly increased MCV values indicate potential macrocytic anemia or hypoxia adaptation (Lataretu *et al.,* 2013). Furthermore, the decreased MCH and MCHC reflect impaired hemoglobin synthesis and hypochromic anemia (Islam *et al.,* 2020; Shahjahan *et al.,* 2022; Ahmed *et al.,* 2022). The significant reduction in platelet counts across exposures suggests a compensatory response to vascular damage (Petitjean *et al.,* 2019; Witeska *et al.,* 2023). Collectively, these changes highlight the adverse effects of heavy metal exposure on fish blood parameters and immune function.

The observed hyperglycemia across exposures indicates sustained stress-induced metabolic disruption (Pottinger, 2008; Jun-Hwan *et al.,* 2017; Renu *et al.,* 2021; Witeska *et al.,* 2023). The significantly decreased ALT levels suggest either liver recovery or reduced hepatocyte turnover (Reema Rose *et al.,* 2023; Sudhabose *et al.,* 2024). Alterations in urea levels reflect varying renal stress and potential adaptation processes (Pottinger, 2008; Olivier *et al.,* 2005; Paolo *et al.,* 2017). Creatinine levels increased with both exposures, reflecting progressive renal dysfunction and oxidative stress (Olivier *et al.,* 2005; Antonio *et al.,* 2014; Paolo *et al.,* 2017).

Calcium, a vital element primarily stored in bones, plays a crucial role in maintaining body structural integrity and various physiological processes (Witeska *et al.,* 2023). Exposure to metals disrupted calcium homeostasis, as evidenced by decreased serum calcium levels, indicating impaired bone metabolism (Goldstein, 1990; Ray, 2016; Jun-Hwan *et al.,* 2017; Shahjahan *et al.,* 2022). The pattern of calcium levels observed may reflect a transient compensatory response by the fish, such as mobilization of calcium from bone stores or hormonal regulation involving parathyroid hormone and calcitonin, which temporarily stabilizes calcium levels. However, prolonged exposure likely overwhelms these mechanisms, leading to sustained hypocalcemia due to impaired calcium absorption, renal dysfunction, or disrupted bone metabolism. These findings are consistent with previous studies reporting time-dependent effects of heavy metals on calcium regulation in fish (Atli and Canli, 2011; Renu *et al.,* 2021; Shahjahan *et al.,* 2022). This disruption is likely related to altered calcium transport and hormonal regulation under metal exposure (Renu *et al.,* 2021; Reema Rose *et al.,* 2023).

Strong bioaccumulation occurred across tissues, with accumulation patterns showing distinct organ-specificity and duration dependence. The liver consistently accumulated high levels, aligning with its role in detoxification (Shahjahan *et al.,* 2022; Moiseenko and Gashkina, 2020). Significant muscle accumulation, particularly increasing over time, indicates prolonged retention with implications for trophic transfer. Nickel exhibited distinct uptake patterns compared to lead, with

notable accumulation in gills and intestine. These findings support the liver as a primary accumulation site (Al-Balawi *et al.,* 2013; Güldiren and Tekin-Ozan, 2018; Moiseenko and Gashkina, 2020). Given the prevalence of heavy metals from sources like industrial discharge (Wang, 2025), understanding their bioaccumulation in consumed fish species like grass carp is crucial for assessing ecological and human health risks. This study provides valuable insights into the toxicological impacts of lead and nickel, highlighting the importance of understanding bioaccumulation patterns and physiological responses, while emphasizing implications for food safety.

Conclusions: Exposure to lead acetate and nickel chloride induced significant hematological and biochemical alterations in *C. idella*. Both metals caused significant bioaccumulation in various organs, with organ-specific and exposure duration-dependent trends. The study highlights the potential risks of lead and nickel exposure to aquatic life. The observed changes may serve as biomarkers for metal exposure in aquatic organisms.

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Authors' contribution: AMKY designed the study. MK executed the overall experimental work and interpreted the data. AMKY critically revised the manuscript for important intellectual contents and approved the final version.

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