



Effect of some Heavy Metals (Pb, Hg, Cd and Cu) on Physiological and Immunological Parameters in the Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Aquaculture is a vital pillar of global food security, particularly for Egypt, as it is the principal fish-producing sector. Heavy metal contamination in aquatic environments represents one of the most significant environmental hazards. A 90-day feeding experiment was conducted to evaluate the effects of sublethal concentrations of heavy metals: Cu (0.125 ppm), Hg (0.005 ppm), Pb (1.5 ppm), and Cd (1 ppm) on the Nile tilapia. The investigation focused on evaluating growth performance, somatic indices, metal accumulation in tissues, and key physiological parameters. 144 fish (initial weight, 17 ± 0.4 g) were divided at random in triplicate into 12 glass aquaria (90 L each), placed into four treatment groups for heavy metals and control. Fish received a commercial feed containing 30% crude protein, administered at 3% of their body weight per day. Heavy metal exposure resulted in dramatic decreases ($P \leq 0.05$) in the mean weight gain, specific growth rate, and percentage survival compared to control. Both feed conversion ratio and protein efficiency ratio were markedly reduced, indicating a decline in feed utilization efficiency. Residue analysis revealed the greatest accumulation of heavy metals in the kidney and liver, with lesser amounts detected in the gills and muscles, highlighting the liver's role in detoxification. Significant changes ($P \leq 0.05$) in hepato-, spleen-, and kidney-somatic indices are indicative of organ injury. Moreover, biochemical examination revealed a decrease in total protein and globulin concentration. In addition, increased serum activities of hepatic enzymes (ALT and AST) indicate hepatocellular damage. Hemogram profile examination established that hemoglobin concentration, packed cell volume, and counts of erythrocytes and leukocytes were significantly higher ($P \leq 0.05$) in control fish compared to those exposed to heavy metals. Results indicate the acute physiological distress and hematobiochemical change resulting from metal exposure, and highlight their bioaccumulation and toxicological effects in freshwater habitats.

INTRODUCTION

Fish play a crucial role in global food security since they provide a more affordable and superior-quality source of animal protein relative to other livestock products (Boyd et

al., 2022; Afonso *et al.*, 2024). Beyond their high protein content, fish offer an excellent supply of minerals, vitamins, and omega-3, thereby enhancing dietary quality and diversity (Phogat *et al.*, 2022).

Although aquaculture has been practiced in Egypt for millennia, the adoption of modern technologies to enhance production has occurred predominantly in recent decades (Schar *et al.*, 2020; Ashry *et al.*, 2024). Over recent decades, Egypt has emerged as the foremost aquaculture producer in Africa, generating approximately 1.8 million metric tons annually, which accounts for nearly 74% of the continent's total aquaculture yield (Jamil Emon *et al.*, 2023; Hussein *et al.*, 2024; Arafa *et al.*, 2025).

This rapid expansion has been propelled by the implementation of extruded feeds, water recirculation systems, and enhanced farm management practices, collectively boosting overall aquaculture production (Pennells *et al.*, 2025). With vast aquatic resources, including the Nile River and numerous lakes, Egypt possesses enormous yet underutilized potential for further expansion (Ashour *et al.*, 2024; Ribeiro *et al.*, 2024). Currently, the country accounts for 68% of Africa's total aquaculture volume and 56% of its economic value, ranking seventh globally in production (Walakira *et al.*, 2023; Hasan, 2025).

Egypt is the undisputed center of the Nile tilapia aquaculture (*Oreochromis niloticus*) in Africa, accounting for approximately 84% of the continent's farmed production (1.08 million metric tons) in 2019 and ranking as the world's third-largest producer globally (FAO, 2021). This species forms the backbone of the nation's fisheries sector (GAFRD, 2022), constituting over 60% of Egypt's total fish production (El-Sayed & Fitzsimmons, 2023). Nevertheless, the long-term viability of this vital sector faces challenges due to contamination by heavy metals. These persistent and bioaccumulative contaminants present considerable ecological and public health hazards (Ali *et al.*, 2021; Edo *et al.*, 2024). Exacerbated by accelerated industrial development and agricultural runoff, metals such as chromium (Cr), arsenic (As), mercury (Hg), cadmium (Cd), lead (Pb), copper (Cu), and nickel (Ni) are increasingly introduced into aquatic environments (Shukla *et al.*, 2022; Daripa *et al.*, 2023); once introduced, they bioaccumulate in fish tissues, disrupting physiological homeostasis (Metwally *et al.*, 2023; Singh & Sharma, 2024).

Exposure to heavy metals triggers oxidative stress, causes tissue injury, and leads to alterations in hematological and biochemical parameters (Munir *et al.*, 2021; Ahmed *et al.*, 2022; Wang *et al.*, 2024). Among these metals, Pb, Hg, Cd, and Cr are recognized as the most toxic elements, severely impairing fish health and growth performance (Abdel-Tawwab & Wafeek, 2010; Garai *et al.*, 2021). In *O. niloticus*, the gills, liver, and kidneys are primary targets for heavy metal toxicity (Pb, Cd, Zn, Cu), with studies documenting that exposure induces pronounced hepatic damage, growth impairment, and histopathological alterations in these organs (Fatima *et al.*, 2024; Zahran *et al.*, 2025).

WHO and the FAO have emphasized the necessity to monitor key metals such as Hg, Cd, Pb, arsenic (As), Cu, Zn, iron (Fe), and tin (Sn) due to their environmental persistence and potential for bioaccumulation (Zhao *et al.*, 2020; Mamdouh *et al.*, 2021). Their increased concentration in aquatic ecosystems poses a danger to the fish, the balance in the ecosystem, and consumers of the fish because of biomagnification along the food chain (Sharma *et al.*, 2025). Consequently, the present study is designed to evaluate the toxic impacts of sublethal concentrations of copper, mercury, lead, and cadmium on growth performance, feed utilization, biochemical composition, hematological parameters, and organ-specific residue accumulation in the Nile tilapia.

MATERIALS AND METHODS

Experimental design and treatments

A total of 144 Nile tilapia fingerlings, with a mean initial body weight of 17.92 ± 0.01 g, were sourced from El-Reyad Fish Farm, earthen ponds, and subsequently transferred to the Aquaculture Research Unit, Sakha, Kafr El-Sheikh Governorate, Agriculture Research Center, Egypt. The fish were held in twelve glass aquaria ($80 \times 50 \times 60$ cm; 90L capacity each), continuously aerated using air pumps and supplied with dechlorinated tap water to ensure optimal rearing conditions. To ensure optimal water quality, one-third of the total water volume was replaced daily.

Sublethal exposure levels were determined based on a preliminary LC_{50} assay following (Weil, 1952). The selected concentrations were 0.125, 0.005, 1.5, and 1.0 ppm for Cu, Hg, Pb, and Cd, respectively. Fish were randomly allocated into four treatment groups corresponding to these metal exposures, each with three replicate aquaria (12 fish per aquarium). This study was designed to evaluate the impact of these sublethal concentrations on growth performance, feed efficiency, body composition, accumulation of heavy metals in different organs, and relative organ weights. Details of the experimental design and treatment distribution are presented in Fig. (1).

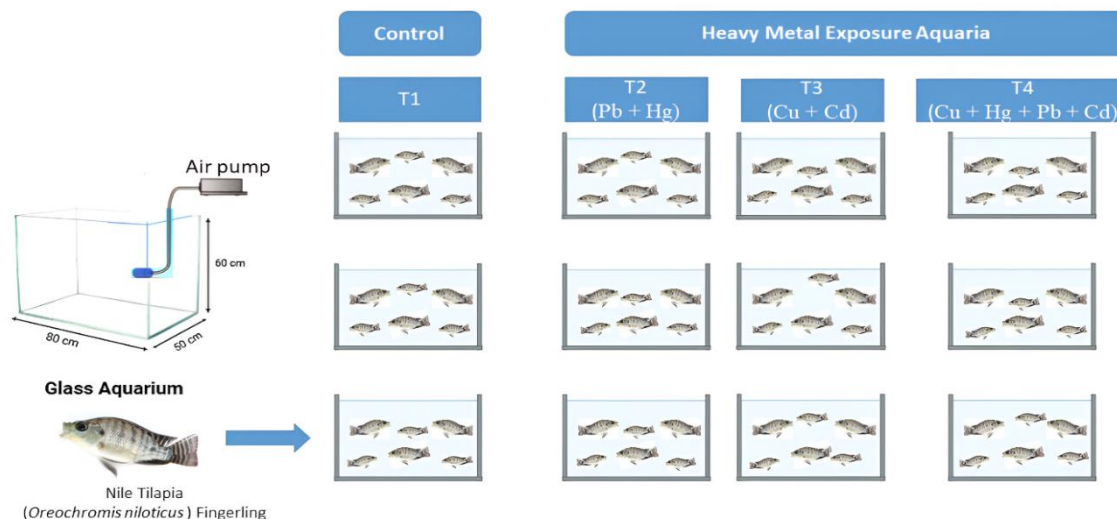


Fig. 1. Schematic representation of the experimental setup and treatment allocation, illustrating the aquaria utilized for heavy metal exposure. The factorial design included 4 treatments $\times$ 3 replicates, with 12 fish per tank (90 L water; Cu = 0.125 ppm, Hg = 0.005 ppm, Pb = 1.5 ppm, Cd = 1.0 ppm)

Fish acclimation and diet

For acclimation, fish were held for one month in glass aquaria and fed a basal diet at a rate of 3% of their body weight per day. Following acclimation, fish were randomly allocated into 12 aquaria. Fish were distributed at a density of 2.27g per liter in each tank, with three replicate aquaria allocated to each treatment group.

A 30% crude protein basal diet was composed of the following locally sourced commodities, such as fish and soybean meal, wheat bran, yellow corn, sunflower oil, as well as a mineral–vitamin mixture. These contents were sourced from the regional suppliers, with the exception of the mineral–vitamin premix, which was sourced from VICTOIR Co. (Cairo, Egypt). The fish received the experimental diets twice daily (8:00 and 15:00) at a feeding rate of 3% of their body weight. Feed amounts were adjusted weekly in accordance with body weight changes. The feeding trial continued for 90 days.

The basal diet's ingredients and chemical profile are detailed in Table (1), including dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), and ash, all determined following the standard procedures outlined by **Thiex *et al.* (2012)**. Water quality was assessed weekly throughout the trial from samples collected from each aquarium. Upon conclusion of the trial, a sampling of representative specimens was taken from all experimental unit, and subsequently subjected to oven-drying at 60°C for 48h, pulverization with an electric mill, and archival at 4°C for subsequent analysis. Total tissue residues of heavy metals were analyzed using an Atomic Absorption Spectrophotometer, adhering to the methodological protocol established by **Abdel Hakim *et al.* (2016)**.

Table 1. Formulation and proximate analysis of the experimental basal diet (% DM)

Item	%
Fish meal (63% CP)	16
Soybean meal (42% CP)	38
Yellow corn (9% CP)	31
Wheat bran (12.5% CP)	10
Sunflower oil	4
Vitamin and mineral premix ¹	1
Total	100
<i>Proximate composition: DM Basis (%)</i>	
DM	90
CP	30
CL	6.50
CF	4.50
Ash	8
NFE ²	51
GE ³	4.38

¹ Premix (per kg diet): Vitamins (IU or mg): (1,200,000), (300,000), (700), (500; 200; 670; 600; 300; 3,000), (500), (450) for A, D₃, E, B-group (1; 2; 5; 6; 7; 9; 12), K₃, and C, respectively. Amino Acid/Other (mg): NAM, 3,000 mg; Met, 3,000 mg; CCL, 1,000 mg. Minerals (as sulfates, mg): (3,000; 3,000; 10,000), (180), (300) for sulfates (Mg, Cu, Fe, Zn, and Co), respectively. Amino Acid: Methionine, 3,000 mg. DM: dry matter, CP: crude protein, CL: crude lipid, CF: crude fiber.

² Nitrogen-free extract (NFE) , calculated by difference $100 - [\% (CP + CL + ash + CF)]$.

³ Gross energy (GE, kcal/g DM) calculated by applying the standard conversion factors of 5.6 (protein), 9.44 (fat), and 4.1 (carbohydrate), following the **NRC (2012)** guidelines.

Water quality and heavy metal analysis

Water samples were collected weekly from each aquarium to determine physicochemical parameters, such as temperature, dissolved oxygen (DO), total dissolved solids (TDS) and pH, and all were performed as outlined by **Rice et al. (2012)** using a waterproof portable HI9829 multi-parameter probe (HANNA Instruments, Woonsocket, RI, USA). Routine monitoring of ammonia (NH₃) levels was performed with a portable spectrophotometer 2000 (HACH Co.; Loveland, USA). The concentrations of heavy metals in both aquarium water and fish tissues were quantified using an Atomic Absorption Spectrophotometer (Varian AA240FS), following the procedures outlined by **Capar (1977)**.

Growth patterns, feed utilization and fish survival rates

To determine growth tendency, feed efficiency, and survival rate, all fish were sampled from each aquarium at the end of the trial. The assessment was conducted according to the equations described by **El-Sharkawy et al. (2023)**, as follows:

Weight gain (WG; g/fish) = Final body weight (FBW, g) – Initial body weight (IBW, g)

$$\text{Daily-weight gain (DWG; g fish}^{-1} \text{ day}^{-1}) = \frac{WG \text{ (g)}}{Time \text{ (days)}}$$

$$\text{Relative-growth rate (RGR\%)} = \frac{FBW \text{ (g)} - IBW \text{ (g)}}{IBW \text{ (g)}} \times 100$$

$$\text{Specific-growth rate (SGR, \% day}^{-1}) = \frac{\ln FBW - \ln IBW}{Time \text{ (days)}} \times 100$$

Feed utilization efficiency was calculated employing the formula described by **Alias *et al.* (2023)**.

$$\text{Feed-conversion ratio (FCR)} = \frac{\text{Total feed consumption (g)}}{WG \text{ (g)}}$$

$$\text{Protein-efficiency ratio (PER)} = \frac{WG \text{ (g)}}{\text{Protein intake (g)}}$$

$$\text{Survival (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

Somatic indices

At the termination of the experiment, the liver, spleen, and kidneys were removed and weighed individually (**Subbotkin & Subbotkina, 2023**). The somatic indices were then calculated according to **Li and Wang (2007)** using the following equations:

$$\text{Hepato-somatic index (HSI, \%)} = \frac{\text{Liver weight}}{\text{Fish weight}} \times 100$$

$$\text{Spleen-somatic index (SSI, \%)} = \frac{\text{Spleen weight}}{\text{Fish weight}} \times 100$$

$$\text{Kidney-somatic index (KSI, \%)} = \frac{\text{Kidney weight}}{\text{Fish weight}} \times 100$$

Hematological and serum biochemical analyses

Upon termination of the trial, all fish were weighed, and 4–5 individuals per aquarium were randomly selected for blood collection. Blood samples were treated with an anticoagulant and promptly analyzed to determine erythrocyte (RBC) and leukocyte

(WBC) counts following **Houston (1990)**. Differential leukocyte counts were conducted on Giemsa-stained blood smears. Packed cell volume (PCV, %) was determined using the microhaematocrit technique, while hemoglobin (Hb) concentration was measured spectrophotometrically at 540nm according to **Blaxhall and Daisley (1973)** (Model RA 1000, Technicon Corporation, USA). The remaining blood was centrifuged at 3500 rpm for 15min to obtain plasma, which was analyzed for total protein and albumin concentrations following **Doumas *et al.* (1981)** using a spectrophotometer (JENWAY 6051 Colorimeter). Globulin concentration was calculated by subtracting albumin from total protein values.

Statistical analysis

The statistical analysis was conducted using SAS statistical program software version 9.1 (**SAS, 2002**). To confirm the assumption of homoscedasticity, data homogeneity was evaluated via the Shapiro-Wilk test. All datasets were confirmed to be normally pattern. Consequently, the results are expressed as the arithmetic mean $\pm$ standard deviation (Mean $\pm$ SD). Data were analyzed to identify significant differences among treatments for each measured parameter, with statistical significance set at $P \leq 0.05$. The data were subjected to a one-way ANOVA, and where significant differences were found, they were further analyzed using the least significant difference (LSD) test, following the procedure of **Gomez and Gomez (1984)**.

RESULTS AND DISCUSSION

Water quality parameters

The primary physicochemical parameters of the experimental water are presented in Fig. (1). All recorded variables throughout the experimental period remained within the optimal range for the Nile tilapia culture, indicating that the observed responses were predominantly attributed to heavy metal exposure rather than to water quality fluctuations, consistent with **Metwally *et al.* (2023)**. Water temperature ranged between 26.1 and 26.5°C across treatments, falling within the optimal thermal range (20– 35°C) reported for tilapia culture (**El-Sayed & Fitzsimmons, 2023**). DO levels significantly declined ($P \leq 0.05$) in the heavy metal-treated groups compared to the control, although they remained within the acceptable range (4.1– 5.5mg/ L) for tilapia growth under continuous aeration, consistent with **Ashour *et al.* (2024)**. The pH values (7.8–8.2) did not differ significantly among treatments and were within the ideal range for tilapia culture (**Abdel Hakim *et al.*, 2016**). However, NH₃ levels were significantly elevated ($P \leq 0.05$) in tanks exposed to copper and cadmium (T₃), reflecting potential metabolic or microbial activity under sublethal metal stress (**Schar *et al.*, 2020**).

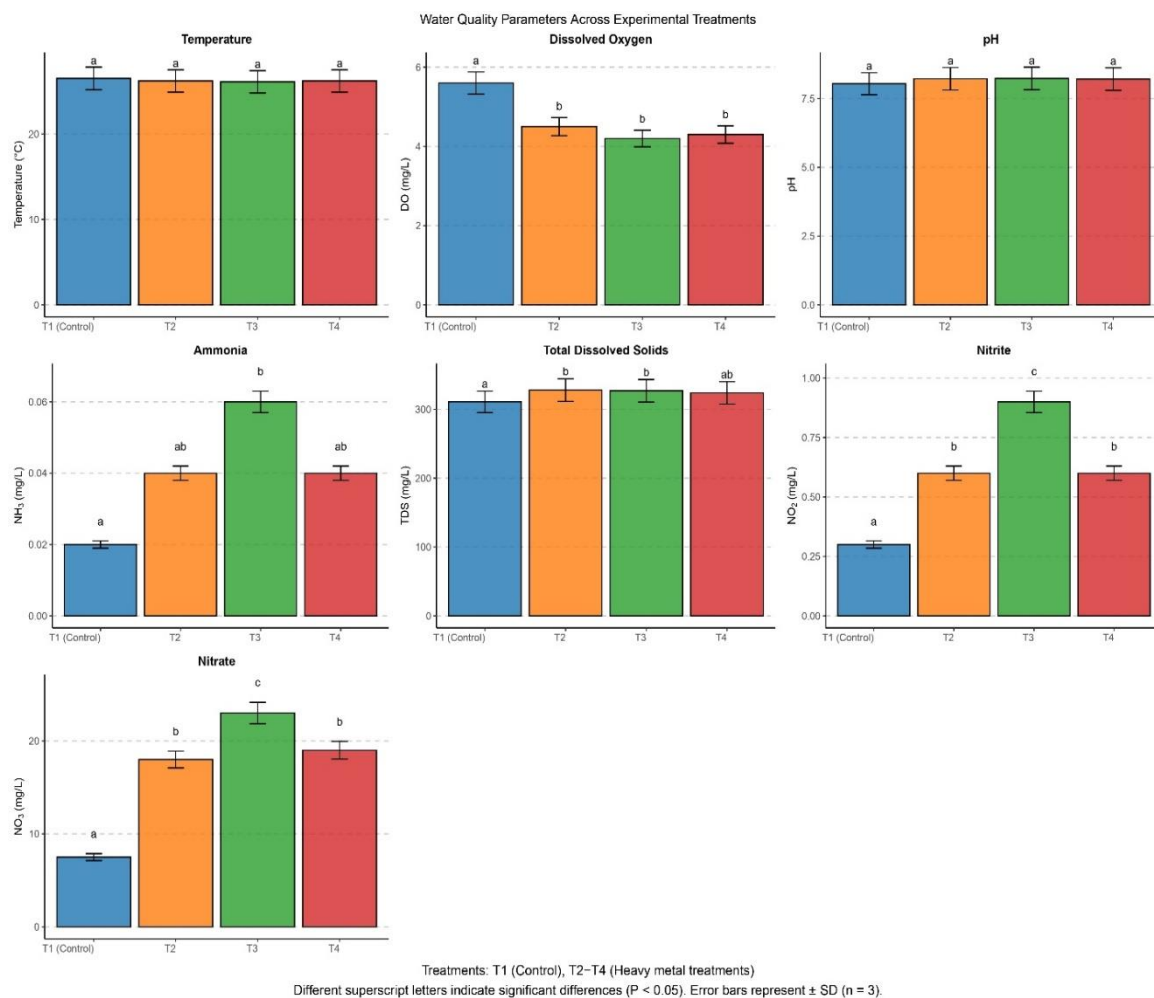


Fig. 2. Physico-chemical parameters of water under different heavy metal treatments

A significant rise ($P \leq 0.05$) in both nitrite and nitrate levels was also observed following heavy metal exposure, with the T₃ group displaying the maximum accumulation. The range of TDS was between 311 and 328mg/ L, although statistically significant differences did occur among treatments ($P \leq 0.05$). All obtained values of these parameters were, however, within the safety limits for freshwater aquaculture use as reported by **Rice *et al.* (2012)**, and thus provided assurance that the general water quality remained within suitable limits for tilapia culture over the course of the experiment (**Wang *et al.*, 2024**). It is more probable, therefore, that the recorded statistical differences in dissolved oxygen, ammonia, nitrite, and nitrate represent metal-induced physiological stress in the fish and changes in the function of microbial assemblages, rather than overall deterioration of water quality (**Metwally *et al.*, 2023**).

Zootechnical performance and feed utilization

As presented in Table (2), the zootechnical performance was significantly impaired ($P \leq 0.05$) by exposure to examined heavy metals, with all growth-related parameters showing a notable decline. A pronounced decline in growth performance was observed with increasing heavy metal exposure, as reflected in the mean total weight gain values: 23.13g. fish⁻¹ (T₁), 8.99g. fish⁻¹ (T₂), 8.74g. fish⁻¹ (T₃), and 8.55g. fish⁻¹ (T₄). Both DWG and SGR followed a similar trend, being significantly higher ($P \leq 0.05$) in the control group compared with all heavy-metal-treated groups. Despite the absence of significant differences ($P \geq 0.05$) between the treated groups, a marked decline in survival was evident, culminating in the lowest percentage in the T₄ group, which was exposed to the full heavy metal mixture. These findings align with previous studies reporting the growth-suppressive effects of heavy metals on *O. niloticus*. In the Nile tilapia, CuSO₄ exposure at a concentration of 0.25 ppm was shown to significantly impair growth, manifesting as decrease body weight and length (Fatima *et al.*, 2024). Similarly, Kumar *et al.* (2024) and Wang *et al.* (2024) reported that exposure to copper and cadmium at concentrations between 1.1 and 29.5ppb markedly decreased growth rate and survival in tilapia. Notably, these findings corroborate earlier reports that sublethal heavy metal concentrations suppress appetite, inhibit nutrient assimilation, and reduce energy conversion efficiency in the Nile tilapia (Garai *et al.*, 2021; Fatima *et al.*, 2024; Zahran *et al.*, 2025).

Table 2. Growth and feed efficiency indices of *O. niloticus* fingerlings exposed to heavy-metal treatments during the 90-day experimental period

Indices	T ₁	Heavy metal treatments		
		T ₂	T ₃	T ₄
IBW (g)	17.92 ± 0.01 ^a	17.77 ± 0.04 ^a	17.89 ± 0.01 ^a	17.80 ± 0.08 ^a
FBW (g)	41.05 ± 0.22 ^a	26.76 ± 0.00 ^b	26.63 ± 0.01 ^b	26.36 ± 0.03 ^b
WG (g)	23.13 ± 0.21 ^a	8.99 ± 0.04 ^b	8.74 ± 0.02 ^b	8.55 ± 0.04 ^b
DWG	0.25 ± 0.00 ^a	0.09 ± 0.00 ^b	0.09 ± 0.00 ^b	0.09 ± 0.00 ^b
SGR	0.92 ± 0.00 ^a	0.45 ± 0.00 ^b	0.44 ± 0.00 ^b	0.43 ± 0.00 ^b
RGR	129.09 ± 1.09 ^a	50.59 ± 0.34 ^b	48.85 ± 0.16 ^b	48.06 ± 0.50 ^b
FCR	2.21 ± 0.00 ^b	4.32 ± 0.02 ^a	4.44 ± 0.01 ^a	4.50 ± 0.03 ^a
PER	1.55 ± 0.00 ^a	0.79 ± 0.00 ^b	0.77 ± 0.00 ^b	0.76 ± 0.00 ^b
SR	100 ± 0.00 ^a	79.16 ± 2.40 ^b	70.83 ± 4.17 ^c	62.49 ± 2.40 ^d
TFI	51.35 ± 0.54 ^a	38.94 ± 0.00 ^b	38.87 ± 0.02 ^b	38.58 ± 0.08 ^b
PI	14.87 ± 0.15 ^a	11.28 ± 0.00 ^b	11.25 ± 0.00 ^b	11.17 ± 0.02 ^b

Results are shown as mean ± standard deviation (Mean ± SD). Within a row, mean values bearing distinct superscript letters (a-d) are statistically different at $P \leq 0.05$ based on the LSD test.

T₁: control group (no heavy-metal exposure), T₂: fish group exposed to a combined of Pb and Hg, T₃: fish group exposed to a combined of Cu + Cd, T₄: fish group exposed to a combined of Cu + Hg + Pb + Cd.

IBW: initial-body weight, FBW: final-body weight, WG: weight gain, DWG: daily weight gain, SGR: specific-growth rate, RGR: relative-growth rate, FCR: feed-conversion ratio, PER: protein-efficiency ratio, SR: survival rate. FI: feed intake, and PI: protein intake.

In Table (2), the key indices of feed efficiency are summarized. These metrics were evaluated to determine the effects of heavy metal exposure on nutritional efficiency. All

feeding indicators outcome were significantly lower ($P \leq 0.05$) in metal-exposed fish compared to the control group. This consistent reduction in both consumption and metabolic conversion indicates that heavy metal toxicity adversely affects feeding behavior and impairs core metabolic function (Ahmed *et al.*, 2022; Wang *et al.*, 2024).

Similarly, Ahmed *et al.* (2022), reported that Cd exposure negatively influenced the growth response and biochemical makeup of *O. niloticus* due to defective liver metabolism and oxidative injury. Comparable results were obtained by Abdel-Tawab and Wafeek (2010), who found that cadmium (Cd) exposure adversely affected the performance and biochemical profile of *O. niloticus* even when fed selenium-supplemented diets.

Concurrently, findings of Wang *et al.* (2024) highlighted increased feed conversion ratios as well as inefficient feed use in fish fed sublethal doses of heavy metals, indicating inhibited metabolism. Lead toxicity, its effective impairment of physiological and biochemical functions in aquatic animals, has been well-proven by Garai *et al.* (2021). According to Boyd *et al.* (2022) and Singh and Sharma (2024), osmoregulation as well as enzymatic functions in fish are affected due to heavy metal toxicity, in turn leading to inefficient use of feed in addition to a decrease in metabolic rates.

Heavy-metal residues and somatic indices

As presented in Table (3), the bioaccumulation factors of Cu, Hg, Pb, and Cd in *O. niloticus* after a period of exposure of 90 days indicated that a distinct pattern of bioaccumulation in various tissues existed. Background values of Cu in various tissues of fish in the control treatment (T_1) measured $30.75 \pm 0.89 \mu\text{g/g}$ in the liver tissue, with a secondary accumulation of $22.90 \pm 1.61 \mu\text{g/g}$ in the kidneys, followed by the gills ($3.15 \pm 0.25 \mu\text{g/g}$), and the least in the muscles ($1.75 \pm 0.25 \mu\text{g/g}$). As one expected, Hg, Pb, and Cd in the control treatment were found to be under the limits of detection in the various tissues studied.

There is a notable accumulation pattern of heavy metals in distinct organs. A high accumulation of most of the metals was observed in the liver and kidney in each treatment group, thus confirming once again the importance of these sites in the process of detoxification and sequestration of metals. For example, in T_4 , a high level of Cu ($207.35 \mu\text{g/g}$) in the liver and a sudden increase in Pb ($942.75 \mu\text{g/g}$) in the kidney have been observed. Conversely, the lowest values in each treatment group were manifested in the muscles, thus indicating a negligible bioaccumulation factor of the muscles.

Table 3. Heavy-metal residues in fish tissues and somatic indices of *O. niloticus* fingerlings exposed to heavy-metal treatments during the 90-day experimental period

Indices	T ₁	Heavy metal treatments							
		T ₂		T ₃		T ₄			
Metal	Cu*	Pb	Hg	Cu	Cd	Cu	Hg	Pb	Cd
<i>Organ (µg/g)</i>									
Liver	30.75 ± 0.89 ^c	55.15 ± 2.22 ^b	17.45 ± 2.51 ^b	259.50 ± 17.20 ^a	224.15 ± 6.72 ^a	207.35 ± 3.72 ^b	22.00 ± 1.78 ^c	13.00 ± 4.44 ^a	0.29 ± 0.01 ^b
Kidney	22.90 ± 1.61 ^c	63.60 ± 4.90 ^b	25.60 ± 1.15 ^b	456.60 ± 14.14 ^a	52.40 ± 17.72 ^b	435.60 ± 10.62 ^b	430.60 ± 2.02 ^a	942.75 ± 3.72 ^a	25.80 ± 4.27 ^c
Gills	3.15 ± 0.25 ^c	42.65 ± 0.31 ^b	16.05 ± 1.93 ^b	13.55 ± 1.93 ^b	145.30 ± 4.09 ^a	10.40 ± 2.13 ^c	12.60 ± 2.42 ^c	20.10 ± 1.90 ^c	128.00 ± 2.48 ^b
Muscle	1.75 ± 0.25 ^b	0.74 ± 0.06 ^b	1.15 ± 0.20 ^b	4.25 ± 0.49 ^a	2.86 ± 0.72 ^a	4.30 ± 0.75 ^a	2.25 ± 0.72 ^b	0.76 ± 0.06 ^a	0.79 ± 0.06 ^b
<i>somatic indices (%)</i>									
HIS	0.0198 ± 0.00 ^d	0.0654 ± 0.00 ^b		0.0550 ± 0.00 ^c			0.0865 ± 0.00 ^a		
SSI	0.0032 ± 0.00 ^c	0.0043 ± 0.00 ^b		0.0046 ± 0.00 ^a			0.0045 ± 0.00 ^a		
KSI	0.0029 ± 0.00 ^d	0.0036 ± 0.00 ^c		0.0038 ± 0.00 ^b			0.0041 ± 0.00 ^a		

Values are presented as mean ± standard deviation (M ± SD). For each metal within the same tissue row, significant differences ($P \leq 0.05$; LSD test) between means are indicated by different superscript letters. ND: Not Detected, signifying that the value was lower than the method's Limit of Detection (LOD).

T₁ = control group (no heavy-metal exposure); T₂ = fish exposed to Pb + Cds; T₃ = fish exposed to Cu + Cd; T₄ = fish exposed to Cu + Hg + Pb + Cd.

*Heavy metals analyzed: Cu, Hg, Pb, and Cd. Hg, Pb, and Cd were not detected in any tissues of the control group (T₁). HSI: Hepatosomatic index; SSI: Splenosomatic index; KSI: Kidney somatic index.

In general, the bioaccumulation pattern approximates that of previous studies in *O. niloticus* and teleosts, where the liver and kidneys represent primary sites of metal sequestration as a process of metallothionein induction (Boyd *et al.*, 2022; Fatima *et al.*, 2024; Zahran *et al.*, 2025). It is important to point out that the relatively low accumulation of metals in muscles supports the idea that, despite a reduction in the degree of tissue contamination in edible portions, the risk of exposure remains (Ali *et al.*, 2021; Sharma *et al.*, 2025).

The treatment of heavy metals also brought about marked ($P \leq 0.05$) adverse effects on the somatic indices (SIs) of the respective organ, such as the hepatosomatic index (HSI), spleen somatic index (SSI), and kidney somatic index (KSI). This indicated that exposure to heavy metals leads to inhibited overall growth of the body as well as physiological stresses in the form of increased values of SIs, which cause enlargement of internal body organs. This can be ascribed to histopathology, such as cellular hyperplasia, hypertrophy,

or edema due to increased vascularization or fluid accumulation (Garai *et al.*, 2021; Zahran *et al.*, 2025). Similar physiological alterations in tilapia as well as in freshwater fish under sublethal stress of metals have been observed (Abdel-Tawwab & Wafeek, 2010; Munir *et al.*, 2021). High values of HSI, specially in copper treatment, indicated increased overall metabolic activity of the liver as a compensatory response toward detoxification (Daripa *et al.*, 2023; Metwally *et al.*, 2023).

Hemogram profile and serum biochemical indicators

The results presented in Table (4) demonstrate that combined exposure to heavy metals significantly altered key serum biochemical parameters in *Oreochromis niloticus*, indicating pronounced metabolic disruption and hepatotoxicity.

Regarding biochemical responses, protein profile analysis revealed that total protein (Tp), albumin, and globulin concentrations were significantly ($P \leq 0.05$) higher in the control group (T₁) compared with all heavy metal-exposed groups (T₂–T₄). The observed reduction in serum proteins among the exposed groups suggests impaired protein synthesis and hepatic dysfunction caused by heavy metal accumulation. These results align with previous observations where sublethal exposure to Cu and Cd led to marked declines in total protein and albumin in *O. niloticus* (Abdel-Tawwab & Wafeek, 2010; Munir *et al.*, 2021; Ahmed *et al.*, 2022). The inhibitory effect of Cd on serum proteins is commonly linked to oxidative stress and hepatocellular damage, reflecting disruption of hepatic enzyme systems and metabolic homeostasis (Wang *et al.*, 2024). However, liver enzymes activities (ALT and AST) increased significantly ($P \leq 0.05$) in the groups exposed to heavy metals, as compared to those in the control group. High activities of liver enzymes are indicators of membrane damage due to sublethal toxicity of heavy metals. These findings are consistent with previous studies that indicated exposure to Cd and Cu leads to elevation of hepatocellular enzymes activity as a result of initial effects of hepatic stress and initiation of detoxification processes (Fatima *et al.*, 2024; Zahran *et al.*, 2025). High activities of ALT and AST also support that exposure to heavy metals affects liver functions and causes oxidative stress in tilapia, hence supporting the use of liver enzymes activities as sensitive indicators of liver toxicity in tilapia (Singh & Sharma, 2024; Metwally *et al.*, 2023).

Table 4. Hematological parameters and serum biochemical indicators of *O. niloticus* fingerlings exposed to heavy-metal treatments during the 90-day experimental period

Indices	T ₁	Heavy metal treatments		
		T ₂	T ₃	T ₄
<i>Hemogram</i>				
RBCs (mil/cm ³)	2.07 ± 0.04 ^a	1.05 ± 0.02 ^c	1.57 ± 0.04 ^b	1.00 ± 0.05 ^c
WBCs (Th./cm ³)	48.50 ± 0.86 ^d	82.50 ± 1.44 ^c	131.50 ± 0.86 ^b	197.50 ± 1.44 ^a
PCV (%)	27.00 ± 0.57 ^a	16.50 ± 0.28 ^d	20.50 ± 0.28 ^b	19.00 ± 0.57 ^c
Hemoglobin (g/dL)	6.50 ± 0.05 ^a	5.65 ± 0.02 ^c	5.85 ± 0.02 ^b	5.70 ± 0.05 ^c
<i>Serum biochemical</i>				

Tp (g/dL)		5.14 ± 0.08 ^a	3.45 ± 0.08 ^b	2.95 ± 0.02 ^c	2.85 ± 0.02 ^c
Serum Albumin (g/dL)		1.88 ± 0.12 ^a	1.45 ± 0.02 ^b	1.25 ± 0.02 ^c	1.25 ± 0.02 ^c
Globulin (g/dL)		3.26 ± 0.04 ^a	2.00 ± 0.05 ^b	1.70 ± 0.00 ^c	1.60 ± 0.05 ^c
ALT (u/L)		54.50 ± 3.17 ^b	103.00 ± 3.46 ^a	99.50 ± 0.86 ^a	102.00 ± 3.46 ^a
AST (u/L)		29.50 ± 0.86 ^c	44.50 ± 0.28 ^b	45.00 ± 1.73 ^b	49.00 ± 0.57 ^a

Results are shown as mean ± standard deviation (Mean ± SD). Within a row, mean values bearing distinct superscript letters (a-d) are statistically different at $P \leq 0.05$ based on the LSD test.

T₁ = control group (no heavy-metal exposure), T₂ = fish exposed to Pb + Cds, T₃ = fish exposed to Cu + Cd, T₄ = fish exposed to Cu + Hg + Pb + Cd.

RBCs: Red blood cells, WBCs: White blood cells, PCV: Packed cell volume, Tp: Total protein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

The hematotoxic effects of heavy metals affected hematohomeostasis severely. The RBC count, hemoglobin (Hb), and packed cell volume (PCV) of fish in the control group were significantly ($P \leq 0.05$) higher than those in the metal-exposed fish groups (T₂–T₄). This reduction in hemato parameters signifies the reduction in erythropoiesis and oxygen-carrying capacities, as observed in anemia triggered by the long-term effects of metal stress. However, a marked elevation in white blood cell (WBC) counts was observed in the fish of all exposed groups, signifying stimulated immunity as well as inflammation. This indicates that the hemato toxicity of the various combinations of heavy metals is identical.

These results agree with previous findings that exposure to Cd, Pb, Cu, and Hg results in a decrease in Hb and PCV values as well as an elevation in the counts of white blood cells in freshwater fish (Garai *et al.*, 2021; Zahran *et al.*, 2025). These hematotoxic effects in fish are a result of bioaccumulation and generation of ROS by Cd, Pb, Cu, and Hg that accumulate in high proportions in the liver and kidneys of fish, making them ideal targets of toxicity (Ali *et al.*, 2021; Shukla *et al.*, 2022; Daripa *et al.*, 2023). Cadmium specifically causes damage to liver and renal cells, a process induced by high copper levels that trigger ROS generation (Ahmed *et al.*, 2022; Wang *et al.*, 2024). Lead causes severe toxicity in freshwater fish as it is a nonbiodegradable element that affects hematology as well as feeding efficiency in fish (Abdel-Tawwab & Wafeek, 2010; Garai *et al.*, 2021). Hepatic and renal accumulation of Hg causes slow growth and reproduction in fish (Metwally *et al.*, 2023; Sharma *et al.*, 2025). The hematological and biochemical changes documented in this experiment collectively demonstrate that exposure to sublethal levels of heavy metals has hepatotoxic, hematotoxic, and immunosuppressive effects on *O. niloticus*. These factors can therefore be used as sensitive diagnostic tools to assess physiological as well as pathological stresses caused by metals in fish.

CONCLUSION

This study conclusively demonstrates that sublethal exposure to heavy metals (Cu, Hg, Pb, Cd) induces severe multi-organ damage in *Oreochromis niloticus*. The result was

due to the toxicity effects of growth suppression, impaired feed conversion, and metabolic disorders, primarily caused by serious hematological changes and organ damage. Notably, the hierarchical level of heavy metal bioaccumulation was prominent in the liver and kidney, followed by gills and muscles, thus establishing the central role of these organs in the sequestration and detoxification process, which ultimately fails. This point was established by the histopathological stress, indicated by the increased somatic body ratio and marked hepatocellular injury, characterized by impaired protein synthesis and elevated levels of transaminase activity. Notably, heavy metal pollution thus presents a dual concern threatening aquaculture production processes, as indicated by inhibited growth rates and mortality rates, as well as possible health hazards associated with bioaccumulation that pose risks to human health via fish consumption for human nutrition.

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