2026 Volume: 02 Issue: 09 www.ijrpa.com ISSN 2456-9995 Research Article

International Journal Research Publication Analysis

Page: 01-21

THE TOXICOLOGICAL CONSEQUENCES OF CADMIUM EXPOSURE ON THE COASTAL FISH SPECIES *CLARIAS GARIEPINUS*: INFLUENCE ON HAEMATOLOGICAL PARAMETERS

Akpoufuoma Oghenetega Friday*[1], Achugwo Chimezie Nathaniel[2]

Toby David Timipere[3]

[1,] *Department of Microbiology, Federal University Otuoke, Bayelsa State.*

[2] *Department of Chemistry, Federal University, Otuoke.*

[3] *Department of Biochemistry, Federal University, Otuoke.*

Article Received: 14 August 2026

Article Revised: 04 September 2026

Published on: 24 September 2026

***Corresponding Author: Akpoufuoma Oghenetega Friday**

Department of Microbiology, Federal University Otuoke, Bayelsa State.

DOI: https://doi-doi.org/101555/ijrpa.1481

ABSTRACT

Cadmium is a persistent and highly toxic heavy metal that poses significant threats to aquatic organisms due to its bioaccumulative nature and adverse physiological effects. This study assessed the toxicological consequences of cadmium exposure on the coastal fish species *Clarias gariepinus*, with particular emphasis on alterations in haematological parameters, specifically white blood cell (WBC) indices, as biomarkers of environmental stress. Seventy-five juvenile *C. gariepinus* were exposed to varying concentrations of cadmium chloride ($CdCl_2$) under controlled laboratory conditions. A preliminary range-finding test was conducted to establish suitable concentrations for acute toxicity assessment. The selected concentrations for the acute toxicity test were 1, 5, and 10 mg/L, while sublethal exposure concentrations of 0.0072, 0.0144, and 0.0288 mg/L were administered for 28 days to evaluate chronic effects. Throughout the experimental period, physicochemical parameters, including temperature, pH, and dissolved oxygen, were maintained within recommended limits to eliminate the influence of environmental stressors on the observed responses. Blood samples were collected on Day 2 and Day 28, and haematological analyses were performed using an automated haematology analyser to determine changes in total white blood cell count and differential leukocyte indices. The results demonstrated significant concentration-dependent alterations in haematological parameters following cadmium exposure. Notable increases in neutrophil and lymphocyte counts were observed at higher cadmium concentrations,

particularly after 28 days of exposure, indicating activation of the immune system in response to cadmium-induced physiological stress. These alterations suggest that prolonged cadmium exposure elicits immunological responses associated with oxidative stress and inflammatory processes. The findings demonstrate that white blood cell indices are sensitive biomarkers of cadmium toxicity and provide valuable tools for the early detection and monitoring of heavy metal pollution in aquatic ecosystems. This study highlights the ecological risks associated with cadmium contamination and underscores the need for effective environmental monitoring, stricter regulation of heavy metal discharges, and sustainable pollution control measures to protect aquatic biodiversity and ecosystem health.

KEYWORDS: Cadmium; *Clarias gariepinus*; haematological biomarkers; heavy metals; toxicology; white blood cell indices.

INTRODUCTION

Heavy metal contamination remains one of the most persistent environmental problems affecting freshwater and coastal aquatic ecosystems worldwide. Unlike many organic pollutants, heavy metals are non-biodegradable and persist in water and sediments for extended periods, where they accumulate in aquatic organisms and may biomagnify through aquatic food chains. Anthropogenic activities such as mining, industrial discharge, petroleum exploration, municipal wastewater disposal, agricultural runoff, electroplating, and battery manufacturing have substantially increased the release of heavy metals into aquatic environments, thereby threatening aquatic biodiversity, fisheries productivity, and public health (Engwa et al., 2019; World Health Organization [WHO], 2021; **Tchounwou et al., 2012; Briffa et al., 2020**).

Cadmium (Cd) is considered one of the most hazardous heavy metals due to its persistence, high toxicity, long biological half-life, and strong tendency to accumulate in biological tissues. Cadmium contamination originates from both natural processes, including rock weathering and volcanic activities, and anthropogenic sources such as industrial effluents, phosphate fertilizers, mining, smelting operations, pigment production, and petroleum-related activities (**Järup & Åkesson, 2009; Genchi et al., 2020**). Once introduced into aquatic systems, cadmium may remain dissolved in water or become associated with suspended particles and sediments, where it can later be remobilized and become available for uptake by aquatic organisms (**Rainbow, 2007; Wang & Chen, 2006**).

Recent investigations have highlighted the importance of industrial activities as major contributors to aquatic heavy metal contamination. **Ochu et al. (2025)** reported the occurrence of toxic heavy metals associated with industrial discharge and emphasized the potential implications of untreated effluent release for environmental quality and public health. Industrial wastewater containing cadmium and other toxic metals can serve as a continuous contamination pathway, increasing the likelihood of metal exposure, bioaccumulation, and physiological disruption in aquatic organisms. These findings reinforce the need for toxicological investigations that evaluate not only the presence of heavy metals in aquatic environments but also their biological consequences (**Ali et al., 2013**).

Fish are particularly vulnerable to heavy metal contamination because they continuously interact with their surrounding environment through respiration, osmoregulation, and feeding activities. Cadmium is primarily absorbed through the gills, gastrointestinal tract, and skin, after which it accumulates in metabolically active organs such as the liver, kidneys, gills, muscle, and blood. Exposure to cadmium has been associated with oxidative stress, cellular damage, impaired metabolism, reproductive dysfunction, immune suppression, and reduced survival in fish species (**Authman et al., 2015; Mansour & Sidky, 2002**). The severity of these effects depends on exposure concentration, duration, environmental conditions, and the physiological characteristics of the exposed species.

Among freshwater fishes, *Clarias gariepinus* (African catfish) is one of the most important cultured species in Africa because of its rapid growth rate, economic importance, tolerance to varying environmental conditions, and suitability for laboratory experimentation. These characteristics have made it a widely used model organism in aquatic toxicology studies involving heavy metals and other environmental pollutants. However, prolonged exposure to cadmium may overwhelm physiological defence mechanisms in *C. gariepinus*, resulting in biochemical, histological, immunological, and haematological alterations that negatively affect growth, health, and survival.

Haematological and biochemical parameters are among the most sensitive biomarkers used for evaluating the health status of fish exposed to environmental contaminants. Changes in blood characteristics and enzyme activities occur rapidly following toxicant exposure and provide valuable information regarding physiological stress, immune responses, oxidative imbalance, and tissue damage. White blood cell indices, including lymphocytes, neutrophils, monocytes, eosinophils, and basophils, are particularly responsive to toxic stress and are widely applied as indicators of inflammatory and immune responses in aquatic toxicology.

Advances in automated haematological analysis have further improved the accuracy, reproducibility, and diagnostic value of these biomarkers in fish health assessment (Fazio, 2019; **Witeska et al., 2023**).

In addition to haematological responses, biochemical biomarkers such as acetylcholinesterase (AChE), glutathione S-transferase (GST), and lactate dehydrogenase (LDH) provide important information regarding the mechanisms of cadmium toxicity. AChE activity reflects potential neurotoxic effects associated with disruption of neurotransmission, GST indicates alterations in antioxidant defence and detoxification capacity, while LDH serves as an indicator of cellular damage and membrane disruption. Previous studies have demonstrated that cadmium exposure induces significant biochemical disturbances in fish through oxidative stress generation, enzyme inhibition, and tissue injury (Espinoza et al., 2011; Elarabany & Bahnasawy, 2019; **Atif et al., 2005; Livingstone, 2001; Viarengo, 1989**).

Recent studies have further confirmed that cadmium exposure can produce measurable physiological alterations even at sublethal concentrations, making biochemical and haematological biomarkers valuable tools for early detection of environmental contamination. Oxidative stress resulting from metal-induced reactive oxygen species production has been identified as a major mechanism responsible for cellular injury in aquatic organisms (**Lushchak, 2011**). Therefore, biomarkers of oxidative stress, tissue damage, and physiological disturbance are essential components of ecological risk assessment and aquatic pollution monitoring (**van der Oost et al., 2003**).

Although numerous studies have investigated cadmium accumulation and toxicity in fish, information regarding the combined biochemical responses of *Clarias gariepinus* following acute and sublethal cadmium exposure remains limited. Therefore, this study investigated the toxicological effects of cadmium exposure on *Clarias gariepinus* by evaluating stress biomarkers, including acetylcholinesterase (AChE), glutathione S-transferase (GST), and lactate dehydrogenase (LDH), following controlled acute and chronic exposure conditions. The findings provide insight into cadmium-induced physiological disturbances and contribute to improved environmental monitoring strategies and sustainable management of aquatic ecosystems.

MATERIALS AND METHODS

Fish Collection, Transportation and Acclimatization

Fish Collection

Juvenile *Clarias gariepinus* of similar size were obtained from a private fish farm in Otuoke, Bayelsa State, Nigeria. To ensure uniformity among the experimental groups, fish were selected based on comparable body size, with an average weight of **25–30 g** and total length of **12–14 cm**. Care was taken during harvesting to minimize handling stress and physical injury.

Transportation

The fish were transported to the Biology Laboratory, Department of Biology, Federal University Otuoke, in aerated polyethylene bags containing water from the culture pond. The bags were placed inside insulated containers to maintain a stable temperature throughout transportation and reduce transport-induced stress.

Acclimatization

Upon arrival, the fish were transferred into aerated holding tanks containing dechlorinated water maintained under laboratory conditions. The fish were acclimatized for **14 days** before the commencement of the experiment. During acclimatization, fish were fed commercial floating pellets containing approximately **35% crude protein** twice daily, and the water was renewed regularly to maintain optimum quality. Feeding was discontinued 24 hours before cadmium exposure.

Water Quality and Laboratory Conditions

Water used during acclimatization and experimentation was maintained under standard laboratory conditions. Temperature, pH, and dissolved oxygen were monitored daily using calibrated laboratory instruments. Throughout the experiment, water quality was maintained at:

- Temperature: **25 ± 2°C**
- pH: **7.0 ± 0.2**
- Dissolved oxygen: **6.0 ± 1.0 mg/L**

These conditions were maintained within acceptable limits for the culture of *Clarias gariepinus* to minimize environmental stress unrelated to cadmium exposure.

Experimental Design

Range-Finding Test

A preliminary range-finding test was conducted before the definitive toxicity experiment to establish appropriate cadmium concentrations for both acute and sublethal exposure studies. Cadmium chloride ($CdCl_2$) stock solutions were prepared using analytical-grade reagents dissolved in deionized water. Five concentrations (**0.5, 1.0, 5.0, 10.0, and 20.0 mg/L**) together with a control were evaluated.

Approximately three fish were exposed to each concentration for **24–96 hours** under identical environmental conditions. During exposure, fish were monitored continuously for mortality and behavioural abnormalities including:

- erratic swimming,
- rapid opercular movement,
- lethargy,
- loss of equilibrium,
- reduced feeding activity, and
- abnormal surfacing behaviour.

Based on the observed mortality pattern and behavioural responses, suitable concentrations were selected for the definitive acute toxicity test.

Acute Toxicity Test

Seventy-five fish were used in the acute toxicity experiment to determine the median lethal concentration (LC_{50}) of cadmium exposure. The fish were randomly assigned to different cadmium treatment groups, with each concentration tested in triplicate to ensure experimental reliability. The selected cadmium concentrations were 0.1, 0.5, and 1.0 mg/L. Fish were exposed to these concentrations for 96 hours under controlled laboratory conditions. The experimental design followed internationally accepted aquatic toxicity testing principles, where short-term exposure periods and mortality responses are used to determine the acute toxicity of chemical substances in fish (**Organisation for Economic Co-operation and Development [OECD], 2019; APHA et al., 2023**).

During the exposure period, fish were monitored at regular intervals for behavioural and physiological signs of toxicity, including erratic swimming, abnormal positioning in the water column, increased opercular movement, reduced activity, and loss of equilibrium. Mortality was recorded at 24-hour intervals throughout the 96-hour exposure period. Behavioural responses and mortality are commonly used as sensitive indicators of acute

metal toxicity because cadmium can interfere with respiration, osmoregulation, enzymatic activity, ion regulation, and general physiological homeostasis in aquatic organisms (**van der Oost et al., 2003; Authman et al., 2015; Genchi et al., 2020**). Previous studies have demonstrated that cadmium exposure may induce rapid physiological disturbances in fish, including oxidative stress, gill impairment, metabolic disruption, and reduced survival (**Järup & Åkesson, 2009; Lushchak, 2011**).

The 96-hour LC_{50} value was estimated using Probit analysis. This statistical approach evaluates the relationship between toxicant concentration and mortality response and is widely applied in aquatic toxicology for determining lethal thresholds of contaminants (Finney, 1971). The LC_{50} represents the concentration of cadmium capable of causing mortality in 50% of the exposed fish population within the 96-hour exposure period and serves as an important measure of acute toxicity. Similar approaches have been widely applied in heavy metal toxicity studies involving freshwater fish species to establish dose–response relationships and environmental risk thresholds (**Sprague, 1969; Rand, 1995; OECD, 2019**).

Sublethal Toxicity Assessment

Following the acute toxicity experiment, a 28-day sublethal exposure study was conducted to evaluate the physiological and biochemical effects of prolonged exposure to low concentrations of cadmium. Sublethal concentrations were selected based on fractions (1/5, 1/10, and 1/20) of the experimentally determined 96-hour LC_{50} value to ensure exposure levels remained below the lethal threshold while allowing the assessment of chronic toxic responses.

A control group maintained in cadmium-free water was included to provide baseline physiological conditions. Each treatment concentration was performed in triplicate to improve statistical accuracy and minimize experimental variability. Long-term exposure studies using environmentally relevant metal concentrations are important because cadmium toxicity may occur through gradual accumulation, oxidative stress induction, metabolic disruption, and impairment of physiological functions even at concentrations that do not cause immediate mortality (Lee et al., 2023; Yu et al., 2024).

Fish were fed twice daily with commercial pellet feed containing 35% crude protein throughout the exposure period to maintain adequate nutritional conditions and reduce dietary stress effects that could interfere with toxicological responses.

Physicochemical Parameters of Exposure Water

Water quality parameters were monitored throughout the acute and sublethal toxicity experiments to maintain stable experimental conditions. Temperature, pH, and dissolved oxygen (DO) were measured regularly because variations in these parameters can influence cadmium bioavailability, uptake, and toxicity in aquatic organisms.

Water renewal was conducted whenever deterioration in water quality was observed to maintain suitable environmental conditions. Cadmium concentrations were also periodically checked to ensure consistency of exposure levels. Maintaining stable physicochemical conditions is essential in aquatic toxicology because environmental factors such as pH and dissolved oxygen can modify metal speciation and influence toxicity responses in fish (Lee et al., 2023).

Blood Sampling and Collection Procedures

At the end of the 28-day sublethal exposure period, blood samples were collected for biochemical biomarker analysis. Prior to sampling, fish were anaesthetized using a dilute solution of MS-222 (tricaine methanesulfonate) to minimize handling stress and reduce physiological disturbance.

Blood samples were collected from the caudal vein using sterile insulin syringes and immediately transferred into heparinized tubes to prevent coagulation. Samples were maintained under cold conditions before laboratory analysis. Blood biochemical parameters are frequently used in fish toxicology because they provide early indications of physiological disruption caused by heavy metal exposure (Yu et al., 2024).

Analysis of Biomarkers

Acetylcholinesterase (AChE) Activity

Acetylcholinesterase activity was evaluated as an indicator of neurotoxicity associated with cadmium exposure. AChE plays an essential role in neurotransmission by regulating acetylcholine hydrolysis; therefore, alterations in enzyme activity may indicate impairment of nervous system function. Heavy metals have been reported to disrupt neurological processes in aquatic organisms through oxidative stress and interference with enzyme activity (Lee et al., 2023).4

Glutathione S-Transferase (GST) Activity

Glutathione S-transferase activity was measured as a biomarker of oxidative stress and detoxification response. GST participates in phase II detoxification processes by facilitating the conjugation of reactive compounds with glutathione, thereby reducing cellular damage. Alterations in GST activity have been widely reported following cadmium exposure in fish and are associated with changes in antioxidant defence mechanisms (Gallagher et al., 2012; Crupkin & Menone, 2013).

Lactate Dehydrogenase (LDH) Activity

Lactate dehydrogenase activity was assessed as an indicator of cellular damage and metabolic disturbance. Changes in LDH activity may reflect altered energy metabolism and membrane integrity following cadmium exposure. Previous studies have demonstrated that cadmium exposure can modify LDH activity and disrupt metabolic pathways in fish tissues (Atif et al., 2019; Almeida et al., 2002).

Data Analysis

The 96-hour LC_{50} value for cadmium was estimated using Probit analysis to establish the relationship between cadmium concentration and mortality response. Probit modelling remains one of the most commonly applied statistical approaches for analysing concentration–mortality relationships in aquatic toxicity experiments (Finney, 1971).

For the sublethal exposure experiment, differences in biochemical biomarker responses among treatment groups and the control were evaluated using one-way analysis of variance (ANOVA). Statistical significance was accepted at $p < 0.05$. Where significant differences occurred, Tukey's post-hoc test was applied to determine specific differences between individual treatment groups.

This statistical approach has been widely applied in fish toxicology studies to evaluate concentration-dependent effects of heavy metals on physiological and biochemical biomarkers (Yu et al., 2024; Lee et al., 2023).

RESULTS

Physicochemical Parameters

The physicochemical characteristics of the experimental water, including temperature, pH, and dissolved oxygen (DO), are presented in Table 1. These parameters were monitored throughout the exposure period to ensure that experimental conditions remained within

suitable ranges for fish survival and to confirm that observed toxicological responses were primarily associated with cadmium exposure rather than environmental stress.

Water temperature, pH, and dissolved oxygen are critical determinants of aquatic organism health because they regulate metabolic activity, respiration, ion balance, and the bioavailability and toxicity of dissolved metals. Variations in pH, in particular, can influence cadmium speciation and uptake by modifying metal solubility and interaction with biological surfaces, while dissolved oxygen affects respiratory performance and physiological stress responses in fish (Lee et al., 2023; Yu et al., 2024).

The recorded physicochemical parameters were evaluated against recommended guideline values established by international and national regulatory agencies, including the World Health Organization (WHO), United States Environmental Protection Agency (USEPA), and the Department of Petroleum Resources (DPR) for aquatic environmental quality assessment. The comparison provides an indication of whether the experimental water conditions remained within acceptable limits during the acute and sublethal toxicity assessments.

Table 1: Physicochemical Characteristics of the Test Media and Comparison with Recommended Guideline Limits from WHO, USEPA, and DPR.

Parameter	Unit	Recorded Range/Mean ± SD	WHO Guideline Limit	USEPA Guideline Limit	DPR Guideline Limit	Interpretation
Temperature	°C		—	—	—	Suitable/Unsuitable for fish culture
pH	—		6.5–8.5	6.5–9.0	6.5–8.5	Within/Outside acceptable range
Dissolved oxygen (DO)	mg/L		≥5.0	≥5.0	≥5.0	Adequate/Inadequate oxygen condition

Physicochemical Characteristics of the Test Media

The physicochemical characteristics of the experimental water during the acute and sublethal cadmium exposure periods are presented in Table 1. Temperature, pH, and dissolved oxygen (DO) were maintained within suitable ranges throughout the experiment to minimize environmental stress and ensure that observed biological responses were primarily associated with cadmium exposure.

The water temperature was maintained at **25 ± 2°C** across all experimental groups. This range falls within the optimal temperature conditions generally recommended for tropical freshwater fish culture and toxicity assessments, as temperature influences metabolic activity,

oxygen consumption, and toxicant uptake in aquatic organisms (Lee et al., 2023; Yu et al., 2024). The recorded temperature was below the maximum permissible limit of **28°C** recommended by DPR, indicating that thermal conditions remained suitable throughout the experiment.

The pH of the experimental water was maintained at **7.0 ± 0.2**, indicating neutral conditions. This value was within the acceptable range of **6.5–8.5** recommended by WHO, USEPA, and DPR for the protection of aquatic life. Maintaining stable pH conditions is important in cadmium toxicity studies because pH influences cadmium solubility, speciation, and bioavailability, thereby affecting metal uptake and toxicity responses in fish (Lee et al., 2023).

Dissolved oxygen concentrations were maintained at **6.0 ± 1.0 mg/L** across all treatment groups. The recorded oxygen level exceeded the minimum recommended values of **≥5.0 mg/L** by WHO and USEPA and was above the DPR minimum threshold of **4.0 mg/L**. Adequate dissolved oxygen is essential for maintaining normal respiration, metabolic processes, and physiological stability in fish during toxicological exposure experiments.

Overall, the physicochemical conditions remained within acceptable limits throughout the exposure period, confirming that variations in biochemical responses were mainly attributable to cadmium exposure rather than adverse changes in water quality.

Biochemical Responses of *Clarias gariepinus* Exposed to Different Cadmium Concentrations

The biochemical responses of *Clarias gariepinus* exposed to different concentrations of cadmium are presented in Table 2 and Figure 1. The study evaluated the activities of acetylcholinesterase (AChE), glutathione S-transferase (GST), and lactate dehydrogenase (LDH) as biomarkers of neurotoxicity, oxidative stress, and cellular injury, respectively. These biomarkers are widely applied in aquatic toxicology because they provide early indications of physiological disruption caused by heavy metal exposure (Espinoza et al., 2011; Elarabany & Bahnasawy, 2019).

Acetylcholinesterase (AChE) Activity

The control group recorded the highest mean AChE activity (**10.83 ± 0.35 U/mL**). However, exposure to increasing cadmium concentrations resulted in a concentration-dependent decline in AChE activity.

At **0.1 mg/L cadmium**, AChE activity decreased to **9.03 ± 0.21 U/mL**, followed by a further reduction to **7.77 ± 0.25 U/mL** at **0.5 mg/L**. The lowest activity was recorded at **1.0 mg/L cadmium exposure**, where AChE declined significantly to **5.50 ± 0.20 U/mL**.

The reduction in AChE activity was statistically significant ($p < 0.05$) at all cadmium concentrations compared with the control group. The observed inhibition suggests that cadmium exposure interfered with cholinergic neurotransmission. Cadmium-induced oxidative stress and interaction with neuronal enzymes have been reported to contribute to impaired nervous system function in fish (Lee et al., 2023).

Glutathione S-Transferase (GST) Activity

GST activity showed a progressive decline with increasing cadmium exposure. The control group recorded a mean GST activity of **21.13 ± 0.35 U/mg protein**.

Following cadmium exposure, GST activity decreased to **18.10 ± 0.20 U/mg protein** at **0.1 mg/L**, **15.20 ± 0.30 U/mg protein** at **0.5 mg/L**, and **10.07 ± 0.25 U/mg protein** at **1.0 mg/L**.

The reduction in GST activity was statistically significant across all cadmium treatment groups, with the greatest inhibition observed at the highest cadmium concentration. Decreased GST activity indicates impairment of the antioxidant defence and detoxification capacity of fish exposed to cadmium. Similar alterations in GST responses following cadmium exposure have been reported in fish tissues, demonstrating the sensitivity of GST as an oxidative stress biomarker (Espinoza et al., 2011).

Lactate Dehydrogenase (LDH) Activity

Unlike AChE and GST, LDH activity demonstrated a progressive increase with increasing cadmium concentration. The control group recorded LDH activity of **45.00 ± 0.30 U/L**.

Cadmium exposure increased LDH activity to **47.87 ± 0.32 U/L** at **0.1 mg/L**, **52.33 ± 0.76 U/L** at **0.5 mg/L**, and **57.43 ± 0.65 U/L** at **1.0 mg/L**.

The increase in LDH activity was statistically significant ($p < 0.05$) at **0.5 mg/L and 1.0 mg/L cadmium concentrations** compared with the control group. Elevated LDH activity suggests cellular membrane disruption, leakage of intracellular enzymes, and possible tissue damage resulting from cadmium-induced toxicity. Similar biochemical disturbances associated with sublethal cadmium exposure have been reported in *Clarias gariepinus*, where heavy metal exposure altered metabolic enzyme activities and induced cellular stress responses (Elarabany & Bahnasawy, 2019).

Table 2: Stress Enzyme Activities in *Clarias gariepinus* Exposed to Different Concentrations of Cadmium.

Cadmium Concentration	Acetylcholinesterase (AChE) Activity (U/mL) Mean ± SD	Glutathione S-Transferase (GST) Activity (U/mg Protein) Mean ± SD	Lactate Dehydrogenase (LDH) Activity (U/L) Mean ± SD
Control	10.83 ± 0.35	21.13 ± 0.35	45.00 ± 0.30
0.1 mg/L	9.03 ± 0.21	18.10 ± 0.20	47.87 ± 0.32
0.5 mg/L	7.77 ± 0.25	15.20 ± 0.30	52.33 ± 0.76
1.0 mg/L	5.50 ± 0.20	10.07 ± 0.25	57.43 ± 0.65

Values are expressed as mean ± standard deviation (n = 3).

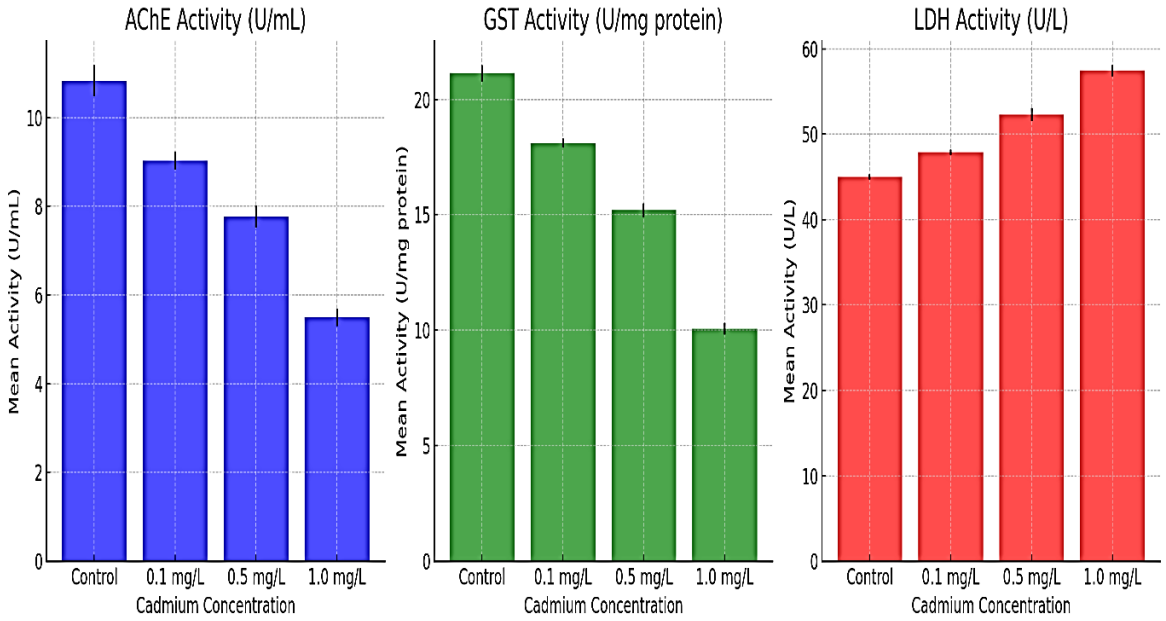

Figure 2: Effects of Different Cadmium Concentrations on Stress Enzyme Activities in *Clarias gariepinus* After 28 Days of Exposure.

Figure 2 illustrates the changes in stress enzyme activities of *Clarias gariepinus* following 28 days of exposure to different concentrations of cadmium. The figure shows a concentration-dependent response pattern, characterized by a progressive reduction in acetylcholinesterase (AChE) and glutathione S-transferase (GST) activities with increasing cadmium concentrations, while lactate dehydrogenase (LDH) activity increased progressively. The observed inhibition of AChE and GST indicates cadmium-induced neurotoxicity and disruption of antioxidant defence mechanisms, whereas elevated LDH activity suggests increased cellular membrane damage and tissue injury.

Values are presented as mean ± standard deviation (n = 3). Significant differences among treatment groups were determined using one-way ANOVA followed by Tukey's post-hoc test at $p < 0.05$.

DISCUSSION

Physicochemical Parameters

Physicochemical conditions of the exposure medium are important determinants of toxicity responses in aquatic organisms because they influence fish metabolism, physiological stability, and contaminant availability. In the present study, temperature, pH, and dissolved oxygen were maintained within acceptable ranges throughout the exposure period, indicating that the observed biochemical alterations were primarily associated with cadmium exposure rather than environmental stress.

Temperature plays a significant role in regulating metabolic activity, enzymatic processes, oxygen consumption, and the toxicity of dissolved contaminants. Changes in temperature can influence metal uptake by affecting physiological processes and altering the chemical behaviour of toxicants in aquatic systems. The maintenance of temperature at **25 ± 2°C** ensured that thermal conditions remained suitable for *Clarias gariepinus* and minimized temperature-related variations in cadmium toxicity responses (Lee et al., 2023; Yu et al., 2024).

Water pH is another important factor controlling the chemical speciation, solubility, and bioavailability of heavy metals. Acidic conditions generally increase the availability of free metal ions, thereby enhancing their uptake and toxic effects in aquatic organisms. In this study, the pH remained stable at **7.0 ± 0.2**, within the recommended range for freshwater fish culture, suggesting that cadmium toxicity was not influenced by abnormal pH conditions. Stable pH conditions therefore allowed the observed biochemical responses to be attributed mainly to cadmium exposure (Lee et al., 2023).

Dissolved oxygen concentration is essential for maintaining fish respiration, energy metabolism, and physiological performance. Low oxygen conditions can increase the susceptibility of fish to pollutants by inducing additional physiological stress and reducing detoxification capacity. The dissolved oxygen level maintained in this study (**6.0 ± 1.0 mg/L**) was adequate for fish health, indicating that hypoxic stress did not contribute significantly to the observed toxicological responses (Yu et al., 2024).

Biochemical Responses of *Clarias gariepinus* Exposed to Different Cadmium Concentrations

The present study demonstrated concentration-dependent alterations in biochemical biomarkers of *Clarias gariepinus* following 28 days of cadmium exposure. The activities of acetylcholinesterase (AChE), glutathione S-transferase (GST), and lactate dehydrogenase (LDH) showed significant responses to increasing cadmium concentrations, indicating progressive neurotoxicity, oxidative stress, and cellular damage.

Acetylcholinesterase (AChE) Activity

The significant reduction in AChE activity observed in cadmium-exposed fish indicates disruption of normal neurotransmission. AChE is responsible for the hydrolysis of acetylcholine at synaptic junctions, and inhibition of this enzyme can result in impaired nerve signalling and neurological dysfunction.

In this study, AChE activity decreased progressively from **10.83 U/mL in the control group** to **5.50 U/mL at 1.0 mg/L cadmium exposure**, demonstrating a clear concentration-dependent inhibitory effect. The reduction suggests that increasing cadmium accumulation interfered with neuronal function, possibly through oxidative damage, enzyme inhibition, and disruption of cellular signalling pathways.

Previous studies have demonstrated that cadmium can induce neurological disturbances in fish by promoting oxidative stress and altering enzyme activities associated with nervous system function (Lee et al., 2023). Similar reductions in biochemical biomarkers following cadmium exposure have also been reported, confirming the sensitivity of AChE as an indicator of metal-induced neurotoxicity.

Glutathione S-Transferase (GST) Activity

GST is an important phase II detoxification enzyme involved in the conjugation of reactive metabolites with glutathione, thereby protecting cells against oxidative damage. In the present study, GST activity decreased significantly with increasing cadmium concentrations, declining from **21.13 U/mg protein in the control group** to **10.07 U/mg protein at 1.0 mg/L cadmium exposure**.

The observed reduction indicates impairment of antioxidant defence mechanisms and reduced detoxification capacity following cadmium exposure. Cadmium is known to generate reactive oxygen species (ROS) by disrupting antioxidant systems and interfering with cellular redox balance. Continuous exposure may overwhelm antioxidant enzymes, resulting in oxidative damage to cellular components.

Espinoza et al. (2011) reported that cadmium exposure altered GST and metallothionein responses in coho salmon tissues, demonstrating the involvement of detoxification pathways during metal stress. The decline in GST activity observed in the present study agrees with previous findings that prolonged cadmium exposure can compromise antioxidant protection and increase oxidative stress in fish.

Lactate Dehydrogenase (LDH) Activity

Unlike AChE and GST, LDH activity increased progressively with increasing cadmium concentration. The LDH level increased from **45.00 U/L in the control group** to **57.43 U/L at 1.0 mg/L cadmium exposure**, indicating enhanced cellular stress and tissue injury.

LDH is an intracellular enzyme involved in anaerobic metabolism, and increased activity is commonly associated with membrane disruption and leakage of intracellular enzymes into circulation. The elevated LDH activity observed in this study suggests that cadmium exposure induced cellular damage, metabolic disturbance, and possible tissue degeneration.

Elarabany and Bahnasawy (2019) reported significant biochemical alterations in *Clarias gariepinus* exposed to sublethal concentrations of cadmium and lead, demonstrating that heavy metal exposure can disrupt metabolic enzymes and induce cellular injury. The present findings support previous observations that increased cadmium accumulation results in oxidative damage and compromised cellular integrity.

Overall Toxicological Implication

The combined response patterns of AChE, GST, and LDH demonstrate that cadmium exposure produced multiple physiological disturbances in *Clarias gariepinus*. The inhibition of AChE indicates neurological impairment, reduced GST activity reflects weakened antioxidant defence, while increased LDH activity confirms cellular injury. The concentration-dependent responses observed across all biomarkers indicate that increasing cadmium exposure severity resulted in progressive physiological damage.

These findings support the application of biochemical biomarkers as sensitive tools for evaluating sublethal heavy metal toxicity in aquatic organisms and highlight the potential ecological risks associated with cadmium contamination in freshwater environments.

CONCLUSION

The physicochemical conditions of the experimental water, including temperature, pH, and dissolved oxygen, were maintained within acceptable ranges recommended by WHO,

USEPA, and DPR throughout the exposure period. The stability of these environmental parameters confirms that the observed biochemical alterations in *Clarias gariepinus* were primarily associated with cadmium exposure rather than secondary stress arising from unfavorable water conditions.

The findings of this study demonstrate that cadmium exposure significantly affected the biochemical health of juvenile *Clarias gariepinus*. Increasing cadmium concentrations resulted in a concentration-dependent inhibition of acetylcholinesterase (AChE) and glutathione S-transferase (GST) activities, accompanied by increased lactate dehydrogenase (LDH) activity. These responses indicate that cadmium induced neurotoxicity, oxidative stress, and cellular damage in exposed fish.

The observed alterations in biochemical biomarkers highlight the potential ecological risks associated with cadmium contamination in aquatic environments. Since *Clarias gariepinus* is an economically important freshwater species widely consumed by humans, continuous exposure to cadmium-contaminated environments may pose risks to aquatic biodiversity, fisheries productivity, and food safety. Therefore, effective monitoring, regulatory control, and pollution prevention strategies are essential for reducing cadmium contamination and protecting aquatic ecosystems.

Recommendations

Based on the findings of this study, the following recommendations are proposed:

a. Establishment of Heavy Metal Monitoring Programmes

Regular monitoring of aquatic environments for cadmium and other potentially toxic metals should be implemented, particularly in areas influenced by industrial activities, mining operations, agricultural runoff, and urban wastewater discharge. Continuous assessment of water quality and metal concentrations will support early detection of contamination and reduce long-term ecological impacts on aquatic organisms.

b. Strengthening of Cadmium Regulation and Environmental Policies

The demonstrated toxic effects of cadmium, even at relatively low exposure concentrations, highlight the need for strict enforcement of environmental regulations governing heavy metal discharge. Regulatory agencies should periodically review permissible cadmium limits in aquatic environments and ensure compliance by industries and other potential sources of contamination.

c. Implementation of Pollution Control and Waste Management Strategies

Effective pollution prevention measures should be adopted to reduce cadmium entry into aquatic systems. Industries involved in metal processing, mining, manufacturing, and related activities should implement appropriate wastewater treatment technologies before effluent discharge. Sustainable agricultural practices and proper waste disposal methods should also be encouraged to minimize heavy metal contamination.

d. Improved Cadmium Management in Aquaculture Systems

Aquaculture facilities should incorporate routine monitoring of water quality and heavy metal concentrations as part of standard management practices. Maintaining suitable water conditions, using uncontaminated feed sources, and applying appropriate water treatment methods are recommended to reduce the risk of cadmium accumulation in cultured fish species.

e. Further Research on Chronic Cadmium Exposure and Bioaccumulation

Although this study evaluated acute and sublethal cadmium toxicity, further investigations are required to examine long-term effects of chronic low-level exposure. Future studies should focus on cadmium bioaccumulation, tissue-specific distribution, reproductive effects, molecular responses, and potential transfer through aquatic food chains to improve ecological risk assessment.

f. Public Awareness and Community Engagement

Environmental awareness programmes should be developed for communities involved in fishing and aquaculture activities. Training workshops and educational campaigns should emphasize the sources, effects, and prevention of heavy metal contamination, thereby promoting safer aquatic resource management and reducing human exposure risks.

g. Development of Sustainable Bioremediation Approaches

Further research should explore environmentally friendly approaches for cadmium removal from contaminated aquatic systems. The application of biological remediation methods involving plants, algae, microorganisms, and other biosorbent materials represents a promising strategy for reducing heavy metal concentrations and restoring polluted aquatic environments.

REFERENCES

1. Ali, H., Khan, E., & Ilahi, I. (2013). Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry, 2013*, Article 673030.

https://doi.org/10.1155/2013/673030

2. American Public Health Association (APHA), American Water Works Association (AWWA), & Water Environment Federation (WEF). (2023). *Standard methods for the examination of water and wastewater* (24th ed.). American Public Health Association.
3. Atif, F., Parvez, S., Pandey, S., Ali, M., Kaur, M., Rehman, H., Khan, H. A., & Raisuddin, S. (2005). Modulatory effect of cadmium exposure on antioxidant enzymes in freshwater fish. *Aquatic Toxicology, 72*(3), 281–291. https://doi.org/10.1016/j.aquatox.2005.01.006
4. Authman, M. M. N., Zaki, M. S., Khallaf, E. A., & Abbas, H. H. (2015). Use of fish as bio-indicator of the effects of heavy metals pollution. *Journal of Aquaculture Research & Development, 6*, Article 328. https://doi.org/10.4172/2155-9546.1000328
5. Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon, 6*(9), e04691. https://doi.org/10.1016/j.heliyon.2020.e04691
6. Elarabany, N., & Bahnasawy, M. (2019). Comparative and interactive biochemical effects of sub-lethal concentrations of cadmium and lead on some tissues of the African catfish (*Clarias gariepinus*). *Toxicological Research, 35*(3), 249–255. https://doi.org/10.5487/TR.2019.35.3.249
7. Engwa, G. A., Ferdinand, P. U., Nwalo, F. N., & Unachukwu, M. N. (2019). Mechanism and health effects of heavy metal toxicity in humans. In B. Karcioglu & A. Arslan (Eds.), *Poisoning in the modern world—New tricks for an old dog?* IntechOpen. https://doi.org/10.5772/intechopen.82511
8. Espinoza, H. M., Williams, C. R., & Gallagher, E. P. (2011). Effect of cadmium on glutathione S-transferase and metallothionein gene expression in coho salmon liver, gill and olfactory tissues. *Aquatic Toxicology, 110–111*, 37–44. https://doi.org/10.1016/j.aquatox.2011.12.012
9. Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture, 500*, 237–242. https://doi.org/10.1016/j.aquaculture.2018.10.030
10. Finney, D. J. (1971). *Probit analysis* (3rd ed.). Cambridge University Press.
11. Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The effects of cadmium toxicity. *International Journal of Environmental Research and Public Health, 17*(11), Article 3782. https://doi.org/10.3390/ijerph17113782

12. Hodson, P. V. (1988). The effect of metal metabolism on uptake, disposition and toxicity in fish. *Aquatic Toxicology, 11*(1), 3–18. https://doi.org/10.1016/0166-445X(88)90003-4
13. Järup, L., & Åkesson, A. (2009). Current status of cadmium as an environmental health problem. *Toxicology and Applied Pharmacology, 238*(3), 201–208. https://doi.org/10.1016/j.taap.2009.04.020
14. Livingstone, D. R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine Pollution Bulletin, 42*(8), 656–666. https://doi.org/10.1016/S0025-326X(01)00060-1
15. Lushchak, V. I. (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology, 101*(1), 13–30. https://doi.org/10.1016/j.aquatox.2010.10.006
16. Mansour, S. A., & Sidky, M. M. (2002). Ecotoxicological studies: Heavy metals contaminating water and fish from Egypt. *Food Chemistry, 78*(1), 15–22. https://doi.org/10.1016/S0308-8146(01)00197-8
17. McKim, J. M. (1977). Evaluation of tests with early life stages of fish for predicting long-term toxicity. *Journal of the Fisheries Research Board of Canada, 34*(8), 1148–1154. https://doi.org/10.1139/f77-172
18. Ochu, A., Ejime, C. F., Timipere, T. D., Tebekaemi, O. D., & Friday, A. O. (2025). Analyzing toxic heavy metals in industrial discharge: Implications for public health. *Iconic Research and Engineering Journals, 8*(7), 746–750.
19. Organisation for Economic Co-operation and Development (OECD). (2019). *OECD Guidelines for the testing of chemicals, Section 2: Test No. 203—Fish, acute toxicity test*. OECD Publishing. https://doi.org/10.1787/9789264069961-en
20. Rand, G. M. (1995). *Fundamentals of aquatic toxicology: Effects, environmental fate, and risk assessment* (2nd ed.). Taylor & Francis.
21. Rainbow, P. S. (2007). Trace metal bioaccumulation: Models, metabolic availability and toxicity. *Environment International, 33*(4), 576–582. https://doi.org/10.1016/j.envint.2006.05.007
22. Sprague, J. B. (1969). Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. *Water Research, 3*(11), 793–821. https://doi.org/10.1016/0043-1354(69)90050-5

23. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. In A. Luch (Ed.), *Molecular, clinical and environmental toxicology: Volume 3: Environmental toxicology* (pp. 133–164). Springer. https://doi.org/10.1007/978-3-7643-8340-4_6
24. van der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology, 13*(2), 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6
25. Wang, J., & Chen, C. (2006). Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review. *Biotechnology Advances, 24*(5), 427–451. https://doi.org/10.1016/j.biotechadv.2006.03.001
26. World Health Organization (WHO). (2021). *Guidelines for drinking-water quality* (4th ed., incorporating the first and second addenda). World Health Organization.
27. Witeska, M., Kondera, E., & Bojarski, B. (2023). Hematological and hematopoietic analysis in fish toxicology—A review. *Animals, 13*(16), Article 2625. https://doi.org/10.3390/ani13162625
28. Yu, Y. B., Lee, J. W., Jo, A. H., Choi, Y. J., Choi, C. Y., Kang, J. C., & Kim, J. H. (2024). Toxic effects of cadmium exposure on hematological and plasma biochemical parameters in fish. *Toxics, 12*(10), Article 699. https://doi.org/10.3390/toxics12100699