GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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# Temporal Toxicodynamics of Lead Nitrate in African Catfish (*Clarias gariepinus*) Fingerlings: Behavioural responses, probit-derived toxicity thresholds and environmental risk characterization

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GSC Biological and Pharmaceutical Sciences, 2026, 36(01), 159–171

Publication history: Received on 31 May 2026; revised on 14 July 2026; accepted on 16 July 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.36.1.0259

## Abstract

Lead contamination of freshwater ecosystems remains a major environmental challenge with serious implications for fish health, aquaculture production, and ecological sustainability. The present study evaluated the acute toxicodynamics of lead nitrate in African catfish (*Clarias gariepinus*) fingerlings through a 96-hour laboratory bioassay. Fish were exposed to graded concentrations of lead nitrate (0.00, 39.96, 79.93, 159.85 and 319.70 mg $L^{-1}$), and cumulative mortality, behavioural alterations, and Probit-derived toxicity thresholds were determined. Mortality increased progressively with both concentration and exposure duration, reaching 66.7% at the highest concentration after 96 hours. Correspondingly, the median lethal concentration ($LC_{50}$) declined from 9.987 mg $L^{-1}$ at 24 hours to 4.174 mg $L^{-1}$ at 96 hours, representing approximately a 58% reduction in toxicity threshold over time. Behavioural responses, including increased air gulping, erratic swimming, abnormal opercular movement, impaired equilibrium, abnormal tail beating, skin peeling, body discolouration, and loss of reflex, intensified progressively with increasing exposure. A Behavioural Toxicity Index (BTI) developed in this study demonstrated a clear concentration-dependent increase in behavioural impairment. Estimated safe exposure concentrations derived using the Sprague and National Academy of Sciences/National Academy of Engineering (NAS/NAE) application factors also declined with increasing exposure duration, indicating progressively reduced environmental safety margins. Collectively, the findings demonstrate that lead nitrate toxicity is strongly influenced by exposure duration and highlight the value of integrating behavioural biomarkers with Probit-derived toxicity thresholds for environmental risk assessment. The study provides baseline toxicological information that will support freshwater environmental monitoring, ecological risk assessment, and sustainable aquaculture management in regions affected by heavy metal contamination.

**Keywords:** Acute toxicity; Behavioural biomarkers; Environmental risk assessment; Heavy metals; Lead nitrate; *Clarias gariepinus*; Probit analysis; Toxicodynamics.

## 1. Introduction

Freshwater ecosystems are increasingly threatened by anthropogenic pollutants resulting from rapid industrialization, urbanization, mining activities, agricultural intensification, and indiscriminate waste disposal. Among these pollutants, heavy metals are of particular concern because they are non-biodegradable, environmentally persistent, and capable of bioaccumulating in aquatic organisms, thereby posing significant ecological and public health risks [1–4]. Unlike many organic contaminants that undergo degradation, heavy metals remain in aquatic environments for extended periods and are continuously recycled between water, sediments, and aquatic biota, leading to long-term ecosystem contamination and disruption of aquatic food webs.

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Lead (Pb) is one of the most widespread and hazardous heavy metals contaminating freshwater ecosystems. Despite global efforts to reduce its environmental release, lead continues to enter rivers, lakes, reservoirs, and aquaculture systems through mining activities, battery manufacturing, metal processing industries, electronic waste recycling, agricultural runoff, municipal wastewater discharge, and atmospheric deposition [3, 5, 6]. In developing countries, including Nigeria, inadequate environmental monitoring and poor waste management practices have exacerbated lead contamination of aquatic ecosystems, raising concerns about fish health, aquaculture productivity, and food safety [6]. As a non-essential metal with no known biological function, lead readily accumulates in fish tissues following uptake through the gills, digestive tract, and skin, where it disrupts physiological and biochemical processes through oxidative stress, mitochondrial dysfunction, ion regulatory imbalance, enzyme inhibition, and tissue degeneration [5, 7, 8].

The toxicity of lead is influenced by both concentration and exposure duration, making toxicity a dynamic rather than static process. Consequently, acute toxicity bioassays remain fundamental tools for evaluating the hazard potential of aquatic contaminants and establishing scientifically defensible environmental safety thresholds. Probit regression analysis is widely regarded as the standard statistical approach for estimating lethal concentrations ($LC_{10}$, $LC_{50}$ and $LC_{90}$), providing quantitative measures of toxicant potency and supporting ecological risk assessment, water quality guideline development, and experimental design for chronic toxicity studies [9, 10]. Furthermore, temporal changes in $LC_{50}$ values provide valuable insights into toxicodynamic processes by illustrating how increasing exposure duration progressively reduces organismal tolerance to toxicants.

The African sharp tooth catfish, *Clarias gariepinus* (Burchell, 1822), is one of the most economically important freshwater fish species cultured throughout Africa because of its rapid growth, high market value, broad environmental tolerance, efficient feed conversion, and resistance to handling stress [11, 12]. Beyond its commercial importance, *C. gariepinus* has become one of the most widely accepted model organisms in aquatic toxicology due to its hardiness, adaptability to laboratory conditions, sensitivity to environmental contaminants, and well-documented physiological responses to chemical stressors [11]. Consequently, toxicity studies using *C. gariepinus* provide valuable information not only for aquaculture management but also for freshwater environmental monitoring and ecological risk assessment.

Behavioural responses are increasingly recognized as some of the earliest and most sensitive indicators of toxicant-induced stress in fish. Alterations such as erratic swimming, increased opercular movement, air gulping, loss of equilibrium, abnormal tail movements, reduced escape responses, and loss of reflex often occur before measurable changes in physiological, biochemical, or histopathological parameters, making behavioural assessment an effective early warning tool in aquatic ecotoxicology [13, 14]. Because behaviour integrates neurological, respiratory, muscular, and metabolic functions, behavioural biomarkers provide rapid, non-invasive evidence of toxicant-induced physiological impairment and have become increasingly incorporated into contemporary environmental risk assessment frameworks.

Although several studies have investigated the toxicity of lead in freshwater fishes, most have focused primarily on reporting $LC_{50}$ values or evaluating isolated physiological endpoints, with comparatively limited emphasis on integrating behavioural responses, temporal toxicodynamic patterns, Probit-derived toxicity thresholds, and environmentally relevant safety benchmarks within a single experimental framework. Furthermore, information on the acute toxicity profile of lead nitrate in *Clarias gariepinus* fingerlings remains limited despite the species' ecological and economic importance in African aquaculture. Generating comprehensive toxicity thresholds together with behavioural evidence is essential for improving ecological risk assessment and supporting evidence-based management of lead-contaminated aquatic environments.

Therefore, the present study investigated the acute toxic effects of lead nitrate on *Clarias gariepinus* fingerlings through a 96-hour laboratory bioassay. Specifically, the study evaluated cumulative mortality and behavioural alterations, estimated $LC_{10}$, $LC_{50}$ and $LC_{90}$ values using Probit regression analysis, and derived environmentally relevant safe exposure concentrations for ecological risk characterization. It was hypothesized that increasing lead nitrate concentration and exposure duration would produce concentration- and time-dependent increases in mortality and behavioural impairment, resulting in progressively lower lethal concentration estimates and reduced environmental safety thresholds.

## 2. Materials and Methods

### 2.1. Study Area

The acute toxicity experiment was conducted at the Wet Laboratory of the Department of Fisheries and Aquaculture, University of Cross River State (UNICROSS), Okuku Campus, Cross River State, Nigeria. The laboratory was equipped

with continuous aeration facilities and maintained under ambient tropical conditions throughout the experimental period. All experimental procedures were conducted in accordance with standard guidelines for aquatic toxicity testing to ensure consistency, reproducibility, and animal welfare.

### 2.2. Experimental Fish and Acclimation

Healthy African catfish (*Clarias gariepinus*) fingerlings with an average body weight of 51.54 ± 0.37 g and mean total length of 4.61 ± 0.54 cm were obtained from Federal College of Fisheries and Marine Technology, fish farm, Enugu campus, Nigeria. The fish were transported to the laboratory in oxygenated plastic containers to minimize handling stress.

Upon arrival, the fish were acclimated for 14 days in 500-L fiberglass tanks containing dechlorinated borehole water under continuous aeration. During acclimation, fish were fed a commercial floating diet (coppens) containing approximately 40% crude protein at 5% of body weight per day, divided into two equal meals (09:00 and 17:00 h). Approximately 50% of the water was renewed daily to maintain optimum water quality, while uneaten feed and faecal materials were siphoned before feeding. Fish exhibiting signs of injury, abnormal swimming behaviour, disease, or physical deformities were excluded from the experiment.

Feeding was discontinued 24 hours before the commencement of the toxicity test, and no feed was supplied throughout the 96-hour exposure period to prevent deterioration of water quality and minimize interference with toxicant uptake, consistent with standard acute toxicity testing procedures described by the Organization for Economic Co-operation and Development [15].

### 2.3. Test Chemical

Analytical-grade lead nitrate [Pb $(NO_3)_2$] with a purity of ≥ 99% was used as the test toxicant. The chemical was obtained from a certified laboratory chemical supplier and prepared immediately before use using distilled water. Appropriate quantities of the stock solution were diluted with dechlorinated borehole water to obtain the required experimental concentrations.

### 2.4. Experimental Design

The acute toxicity evaluation consisted of two sequential phases: a preliminary range-finding test followed by a definitive 96-hour acute toxicity bioassay. The preliminary range-finding test was conducted to establish the concentration range capable of producing partial mortality and to identify suitable concentrations for the definitive toxicity test. Based on the outcome of the preliminary trial, five experimental treatments consisting of one control and four graded lead nitrate concentrations were selected for the definitive bioassay:

| Treatment | Lead nitrate concentration (mg $L^{-1}$) |
|---|---|
| T1 | 0.00 |
| T2 | 39.96 |
| T3 | 79.93 |
| T4 | 159.85 |
| T5 | 319.70 |

Each treatment consisted of 30 fingerlings, distributed into three replicate tanks containing 10 fish each in a completely randomized design (CRD). The experiment was conducted under static exposure conditions for 96 hours, during which fish were continuously observed for behavioural alterations and mortality.

### 2.5. Acute Toxicity Bioassay

The acute toxicity test followed the general procedures recommended in the OECD Guideline 203 (Fish, Acute Toxicity Test) and the American Public Health Association [16] for static toxicity bioassays.

Individual groups of fish were exposed to their respective lead nitrate concentrations for 96 hours without feeding. Mortality was recorded at 24, 48, 72, and 96 hours. Fish were considered dead when they failed to respond to gentle mechanical stimulation and exhibited complete cessation of opercular movement.

Dead fish were removed immediately after observation to prevent deterioration of water quality. Mortality data recorded at each observation period were cumulative and subsequently used for Probit regression analysis to estimate the median lethal concentration ($LC_{50}$) and other toxicity thresholds.

### 2.6. Behavioural Observations

Behavioural responses were monitored continuously throughout the exposure period, with detailed observations recorded at 12, 48, 72, and 96 hours. The following behavioural endpoints were evaluated: air gulping, erratic swimming, loss of balance, opercular movement, abnormal tail beat, skin peeling, body discolouration, loss of reflex. Behavioural responses were assessed using a semi-quantitative scoring system in which:

- (–) = absence of behavioural alteration
- (+) = mild response
- (++) = moderate response
- (+++) = severe response

Opercular movement was scored separately as:

- Normal
- Increased
- Rapid
- Hyperventilation
- Gasping

To facilitate graphical presentation, qualitative behavioural observations were transformed into numerical scores for the computation of a Behavioural Toxicity Index (BTI). General behavioural responses were assigned scores of 0, 1, 2, and 3 corresponding to –, +, ++, and +++, respectively, whereas opercular movement was scored as 0 (Normal), 1 (Increased), 2 (Rapid), 3 (Hyperventilation), and 4 (Gasping). The BTI for each treatment was calculated as the sum of individual behavioural scores, with higher values indicating greater behavioural impairment.

### 2.7. Water Quality Determination

Physicochemical characteristics of the test water, including temperature (°C), dissolved oxygen (mg $L^{-1}$), and pH, were monitored throughout the experiment using calibrated portable meters [16]. Water quality measurements were recorded before the commencement of the experiment and subsequently at regular intervals to ensure that environmental conditions remained within acceptable limits for *Clarias gariepinus* culture. Water quality variables were maintained as uniformly as possible across treatments to minimize confounding effects on toxicity responses.

### 2.8. Statistical Analysis

Mortality data obtained during the 96-hour exposure period were subjected to Probit regression analysis using IBM SPSS Statistics to estimate the $LC_{10}$, $LC_{50}$, and $LC_{90}$ values together with their 95% fiducial confidence limits. Pearson's chi-square ($\chi^2$) goodness-of-fit test was used to evaluate the adequacy of the Probit model [17].

Descriptive statistics were used to summarize behavioural observations and water quality parameters. Behavioural scores were further integrated into the Behavioural Toxicity Index (BTI) to facilitate quantitative comparison among treatments and exposure periods. Safe exposure concentrations were estimated from the derived $LC_{50}$ values using the Sprague application factor ($0.1 \times LC_{50}$) and the National Academy of Sciences/National Academy of Engineering (NAS/NAE) application factor ($0.01 \times LC_{50}$). All statistical tests were conducted at a 95% confidence level, and statistical significance was accepted at $P < 0.05$.

## 3. Results

### 3.1. Cumulative mortality following acute lead nitrate exposure

No mortality was recorded in the control group throughout the 96-hour exposure period, indicating that the experimental conditions remained suitable for the survival of *Clarias gariepinus* fingerlings (Table 1). Mortality increased progressively with both lead nitrate concentration and exposure duration, demonstrating a clear concentration- and time-dependent toxic effect (Figure 1). At the lowest test concentration (39.96 mg $L^{-1}$), cumulative mortality increased from 6.67% after 24 hours to 20.00% after 96 hours. Similarly, mortality at 79.93 mg $L^{-1}$ increased

from 10.00% to 30.00%, while exposure to 159.85 mg $L^{-1}$ resulted in cumulative mortality increasing from 16.67% to 40.00% over the same period. The highest exposure concentration (319.70 mg $L^{-1}$) produced the greatest toxic effect, with mortality increasing from 26.67% at 24 hours to 66.67% at 96 hours. Overall, cumulative mortality exhibited a progressive increase with increasing toxicant concentration and exposure duration, confirming the acute toxicity of lead nitrate to *C. gariepinus* fingerlings.

**Table 1** Cumulative mortality of *Clarias gariepinus* fingerlings exposed to graded concentrations of lead nitrate during a 96-hour acute toxicity test

| Treatment | Lead nitrate (mg/L) | 24 h | 48 h | 72 h | 96 h | Cumulative Mortality (%) |
|---|---|---|---|---|---|---|
| T1 | 0 | 0 | 0 | 0 | 0 | 0.00% |
| T2 | 39.96 | 2 | 4 | 5 | 6 | 20.0% |
| T3 | 79.93 | 3 | 6 | 7 | 9 | 30.0% |
| T4 | 159.85 | 5 | 7 | 9 | 12 | 40.0% |
| T5 | 319.7 | 8 | 10 | 14 | 20 | 66.7% |

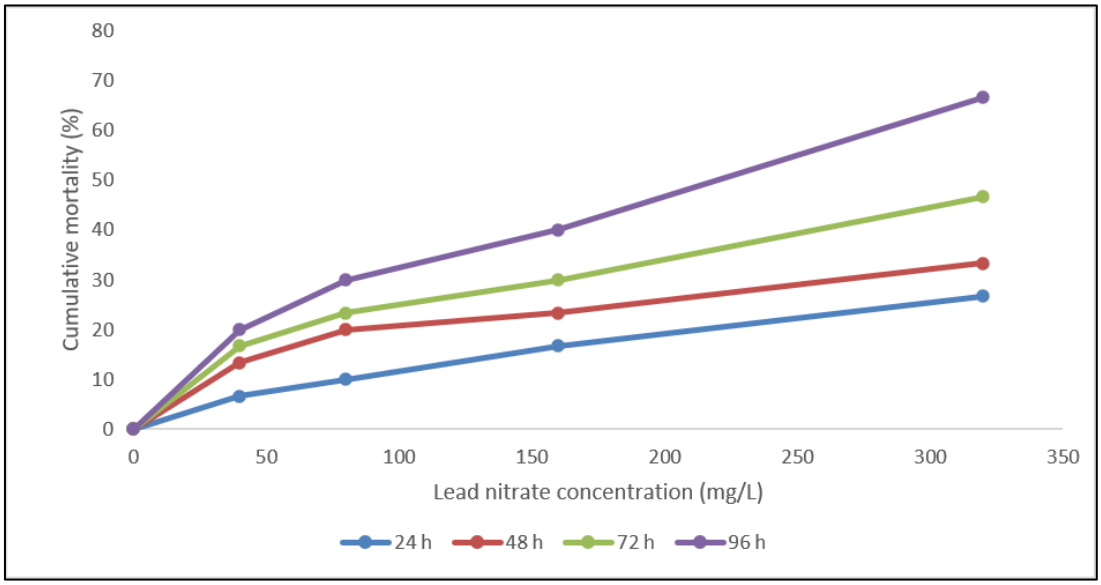


**Figure 1** Concentration- and time-dependent cumulative mortality of *Clarias gariepinus* fingerlings exposed to lead nitrate during a 96-hour acute toxicity test

### 3.2. Probit-derived toxicity thresholds of lead nitrate

Probit regression analysis demonstrated that the toxicity of lead nitrate increased progressively with exposure duration, as reflected by the continuous decline in the estimated lethal concentration values (Table 2). The median lethal concentration ($LC_{50}$) decreased from 9.987 mg $L^{-1}$ after 24 hours to 9.935 mg $L^{-1}$ after 48 hours, before declining further to 6.143 mg $L^{-1}$ and 4.174 mg $L^{-1}$ after 72 and 96 hours, respectively (Figure 2). Similar temporal reductions were observed for $LC_{10}$ and $LC_{90}$ values, indicating increasing toxic potency with prolonged exposure. The Probit regression slopes ranged from 1.629 ± 0.897 to 3.025 ± 0.844, while Pearson's chi-square goodness-of-fit statistics were non-significant ($P > 0.05$) at all exposure periods, indicating satisfactory agreement between the observed mortality data and the fitted Probit models.

**Table 2** Probit regression parameters and estimated lethal concentrations ($LC_{10}$, $LC_{50}$ and $LC_{90}$) of lead nitrate for *Clarias gariepinus* fingerlings following acute exposure

| Exposure Time (h) | Slope ± SE | $LC_{10}$ (mg/L) | $LC_{50}$ (mg/L) | $LC_{90}$ (mg/L) | 95% Lower CL ($LC_{50}$) | 95% Upper CL ($LC_{50}$) | $\chi^2$ | *P*-value |
|---|---|---|---|---|---|---|---|---|
| 24 | 2.284 ± 1.049 | 2.743 | 9.987 | 36.364 | 5.920 | 94,097.273 | 0.267 | 0.875 |
| 48 | 1.629 ± 0.897 | 1.624 | 9.935 | 60.773 | NE | NE | 0.172 | 0.918 |
| 72 | 2.149 ± 0.861 | 1.556 | 6.143 | 24.256 | 4.443 | 53.289 | 0.630 | 0.730 |
| 96 | 3.025 ± 0.844 | 1.574 | 4.174 | 11.069 | 3.487 | 5.905 | 1.764 | 0.414 |

Footnote: Values were estimated using Probit regression analysis in IBM SPSS Statistics. $LC_{10}$, $LC_{50}$ and $LC_{90}$ represent the concentrations expected to cause 10%, 50% and 90% mortality, respectively. Confidence limits (CL) are the 95% fiducial limits of the $LC_{50}$ estimate. Pearson's chi-square ($\chi^2$) goodness-of-fit test was used to evaluate the adequacy of the Probit model. A goodness-of-fit probability ($P > 0.05$) indicates that the Probit model adequately fitted the observed mortality data. NE = Confidence limits not estimated by SPSS due to insufficient information for reliable interval estimation.

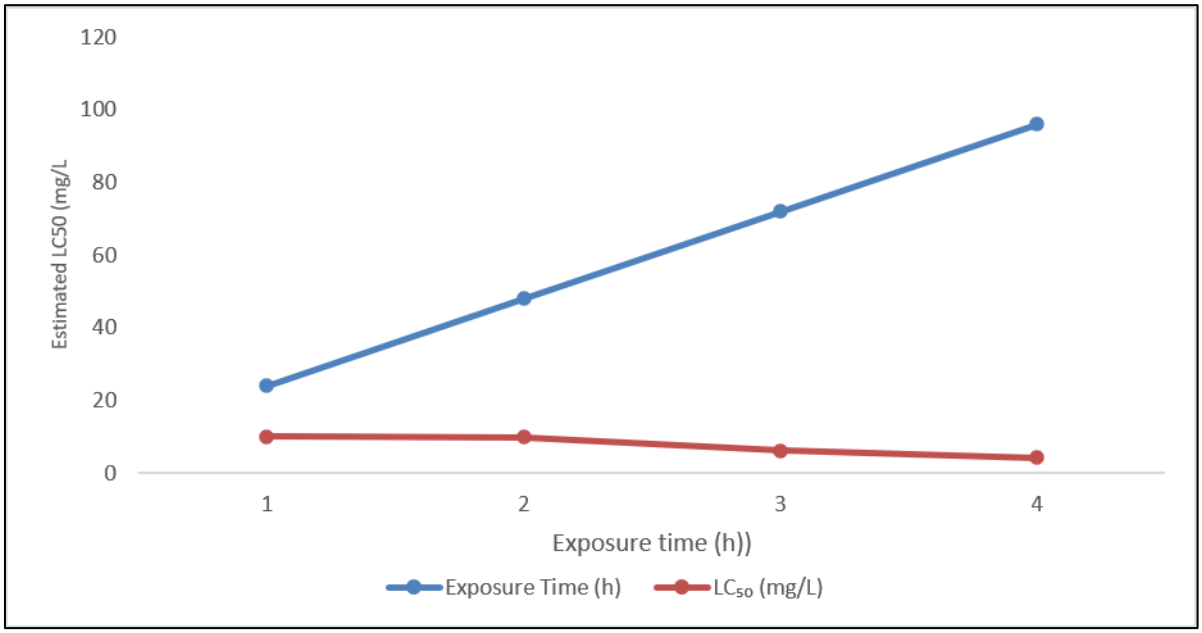


**Figure 2** Temporal decline in the median lethal concentration ($LC_{50}$) of lead nitrate with increasing exposure duration

### 3.3. Estimated safe exposure concentrations

Estimated safe exposure concentrations derived from the acute $LC_{50}$ values decreased progressively with increasing exposure duration (Table 3). Using the Sprague application factor, estimated safe concentrations declined from 0.999 mg $L^{-1}$ at 24 hours to 0.417 mg $L^{-1}$ after 96 hours. Likewise, the National Academy of Sciences/National Academy of Engineering (NAS/NAE) application factor produced corresponding estimates ranging from 0.100 mg $L^{-1}$ to 0.042 mg $L^{-1}$ across the exposure period. These findings indicate that prolonged exposure substantially reduced the concentration of lead nitrate considered environmentally safe for *C. gariepinus* fingerlings.

**Table 3** Estimated safe exposure concentrations of lead nitrate for *Clarias gariepinus* derived from acute $LC_{50}$ values

| Exposure Time (h) | $LC_{50}$ (mg/L) | Sprague Safe Concentration (0.1 × $LC_{50}$) (mg/L) | NAS/NAE Safe Concentration (0.01 × $LC_{50}$) (mg/L) |
|---|---|---|---|
| 24 | 9.987 | 0.999 | 0.100 |
| 48 | 9.935 | 0.994 | 0.099 |
| 72 | 6.143 | 0.614 | 0.061 |
| 96 | 4.174 | 0.417 | 0.042 |

Footnote: $LC_{50}$ = Median lethal concentration estimated by Probit regression analysis. Sprague safe concentration was calculated as 10% of the corresponding $LC_{50}$ (0.1 × $LC_{50}$), while the NAS/NAE safe concentration was calculated as 1% of the corresponding $LC_{50}$ (0.01 × $LC_{50}$), following conventional approaches for deriving estimated safe concentrations from acute toxicity data.

### 3.4. Behavioural alterations during acute exposure

Behavioural abnormalities became increasingly pronounced with increasing lead nitrate concentration and exposure duration (Table 4). Fish in the control treatment maintained normal swimming activity, equilibrium, opercular movement, and external appearance throughout the experiment. In contrast, exposed fish exhibited progressive behavioural disturbances characterized by increased air gulping, erratic swimming, abnormal tail beat, accelerated opercular movement, loss of equilibrium, skin peeling, body discolouration, and diminished reflex responses. These alterations were initially mild at lower concentrations but became progressively more severe as both concentration and exposure time increased. The Behavioural Toxicity Index (BTI) also increased consistently across treatments, indicating an overall escalation in behavioural impairment with increasing toxicant exposure (Figure 3). Similarly, temporal behavioural response profiles showed that respiratory- and locomotion-related behaviours were among the earliest responses to lead exposure and intensified throughout the 96-hour experimental period (Figure 4).

**Table 4** Behavioural alterations exhibited by *Clarias gariepinus* fingerlings during acute exposure to graded concentrations of lead nitrate

| **Exposure Time** | **12 hours** | **48 hours** | **72 hours** | **96 hours** |
|---|---|---|---|---|
| Conc. Mg/L Treatment | T1, T2, T3, T4, T5 | T1, T2, T3, T4, T5 | T1, T2, T3, T4, T5 | T1, T2, T3, T4, T5 |
| Air gulping | -, -, +, +, ++ | -, +, ++, ++, +++ | -, +, ++, +++, +++ | -, ++, +++, +++, +++ |
| Erratic swimming | -, -, +, +, ++ | -, -, +, +, ++ | -, -, +, +, ++ | -, -, +, +, ++ |
| Loss of balance | -, -, -, -, + | -, -, -, -, + | -, -, -, -, + | -, -, -, -, + |
| Operculum movement | Norm, Norm, Inc, Inc, Rap | Norm, Inc, Inc, Rap, Rap | Norm, Inc, Rap, Rap, Hyp | Norm, Rap, Rap, Hyp, Gasp |
| Abnormal tail beat | -, -, -, +, + | -, +, +, +, ++ | -, +, ++, ++, ++ | -, +, ++, +++, +++ |
| Skin peeling | -, -, -, -, - | -, -, -, -, + | -, -, +, +, ++ | -, +, +, ++, ++ |
| Discolouration | -, -, -, -, - | -, -, +, +, + | -, +, +, ++, ++ | -, +, ++, ++, +++ |
| Loss of reflex | -, -, -, -, - | -, -, -, -, - | -, -, +, +, ++ | -, -, +, ++, +++ |

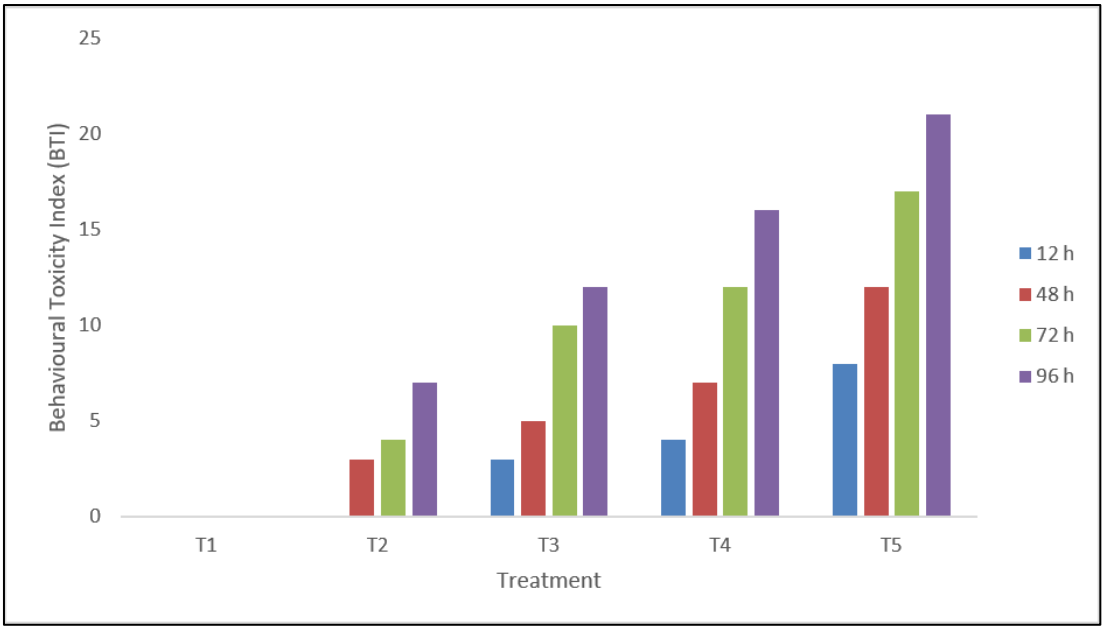


**Figure 3** Behavioural Toxicity Index (BTI) of *Clarias gariepinus* fingerlings during acute exposure to graded concentrations of lead nitrate

The BTI was calculated as the sum of standardized behavioural scores, where − = 0, + = 1, ++ = 2, +++ = 3; operculum movement was scored as Normal = 0, Increased = 1, Rapid = 2, Hyperventilation = 3, and Gasping = 4. Higher BTI values indicate greater behavioural impairment.

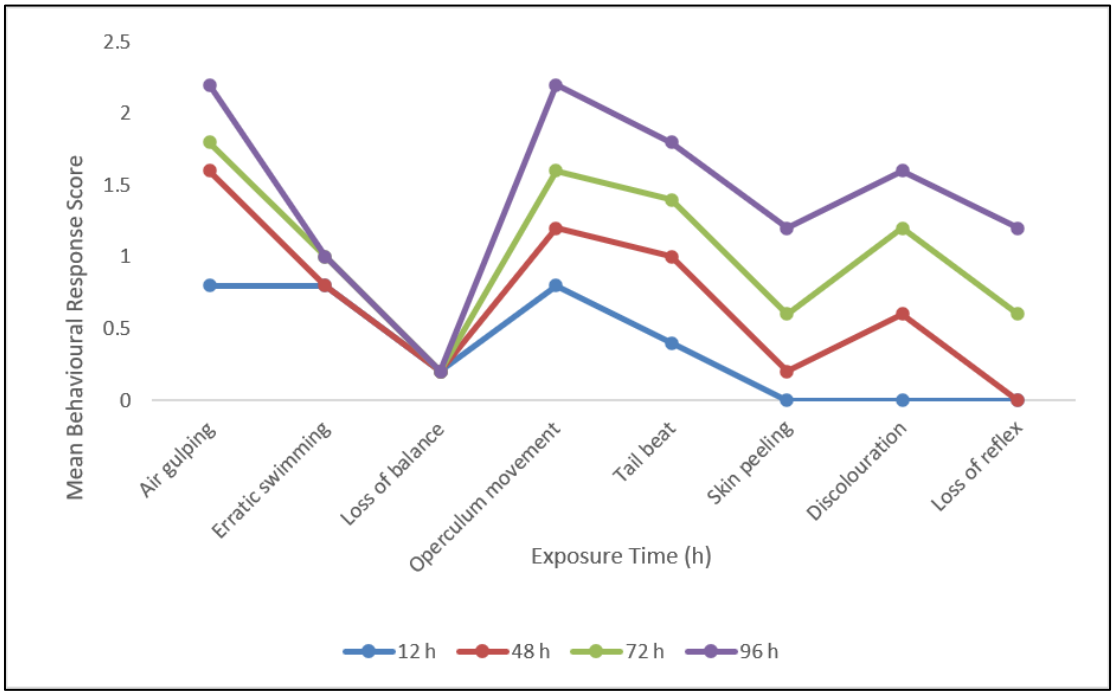


**Figure 4** Temporal progression of behavioural response scores in *Clarias gariepinus* fingerlings during acute exposure to lead nitrate

### 3.5. Temporal progression of toxicity

Lead nitrate exhibited a progressive increase in toxic potency with increasing exposure duration, as evidenced by the declining $LC_{50}$ values and corresponding percentage reduction in toxicity thresholds (Table 5). Relative to the 24-hour $LC_{50}$, only a marginal reduction was observed after 48 hours, whereas substantially greater reductions occurred after

72 and 96 hours. Overall, the $LC_{50}$ decreased by approximately 58% between 24 and 96 hours, indicating that prolonged exposure considerably enhanced the toxic effect of lead nitrate. This progressive reduction demonstrates the cumulative nature of lead toxicity and highlights exposure duration as an important determinant of acute toxicity in *C. gariepinus* fingerlings.

**Table 5** Toxicity progression of lead nitrate with exposure duration

| Exposure Time (h) | $LC_{50}$ (mg/L) | Percentage reduction in $LC_{50}$ (%) |
|---|---|---|
| 24 | 9.987 | – |
| 48 | 9.935 | 0.52 |
| 72 | 6.143 | 38.49 |
| 96 | 4.174 | 58.20 |

Footnote: The estimated $LC_{50}$ of lead nitrate declined progressively from 9.987 mg $L^{-1}$ at 24 h to 4.174 mg $L^{-1}$ at 96 h, representing a 58.2% reduction in the concentration required to induce 50% mortality over the exposure period.

Collectively, the observed concentration-dependent mortality, progressive decline in lethal concentration estimates, increasing behavioural impairment, and reduced safe exposure thresholds demonstrate the temporal evolution of lead nitrate toxicity in *Clarias gariepinus* fingerlings.

## 4. Discussion

The present study demonstrated that lead nitrate exerts pronounced acute toxic effects on *Clarias gariepinus* fingerlings, as evidenced by progressive increases in mortality, behavioural impairment, declining lethal concentration thresholds, and reduced estimated safe exposure concentrations with increasing exposure duration. Collectively, these findings confirm that the toxicity of lead nitrate is both concentration- and time-dependent and underscore the importance of integrating behavioural observations with conventional toxicity endpoints to improve ecological risk assessment.

The concentration-dependent increase in mortality observed during the 96-hour exposure period agrees with the established principle that heavy metal toxicity intensifies with increasing toxicant concentration and duration of exposure [10]. The absence of mortality in the control group confirms that the observed lethal effects resulted solely from lead nitrate exposure rather than experimental stress or poor water quality. Progressive mortality during exposure reflects the continuous uptake and accumulation of dissolved lead ions through the gills and gastrointestinal tract, resulting in increasing physiological dysfunction over time. Lead is recognized as a non-essential toxic metal that interferes with calcium homeostasis, inhibits numerous enzyme systems, disrupts ion transport, induces oxidative stress, and impairs mitochondrial function, ultimately leading to cellular damage and mortality [5, 7].

The mortality pattern observed in the present study is consistent with previous investigations reporting concentration-dependent mortality in freshwater fishes exposed to lead and other heavy metals. [18] reported similar toxic responses in *Oreochromis niloticus*, while recent studies have demonstrated that lead exposure induces progressive physiological deterioration and mortality in common carp (*Cyprinus carpio*) and other freshwater species through oxidative stress, inflammatory responses, and disruption of energy metabolism [3]. These findings reinforce the view that lead remains one of the most ecologically significant heavy metal contaminants threatening freshwater fisheries and aquaculture.

A major contribution of the present study is the characterization of the temporal toxicodynamics of lead nitrate through Probit-derived toxicity thresholds. The progressive decline in $LC_{50}$ values from 24 to 96 hours indicates that the toxic potency of lead nitrate increased substantially as exposure duration increased. This observation supports the concept that toxicity is a dynamic biological process rather than a fixed characteristic of a contaminant. Prolonged exposure allows continuous uptake and accumulation of lead within metabolically active organs, including the gills, liver, kidney, and nervous tissue, progressively reducing the physiological capacity of fish to maintain homeostasis [10]. Consequently, lower concentrations become sufficient to induce mortality after longer exposure periods.

The approximately 58% reduction in $LC_{50}$ between 24 and 96 hours highlights the cumulative nature of lead toxicity and emphasizes the importance of exposure duration in toxicity assessment. Similar temporal declines in $LC_{50}$ values have been reported for lead, cadmium, mercury, and copper in freshwater fishes, demonstrating that increasing exposure duration consistently enhances toxicological responses [2, 4, 18]. The relatively wide confidence interval associated with the 24-hour $LC_{50}$ estimate and the absence of estimated confidence limits at 48 hours are attributable

to the comparatively low mortality recorded during the early stages of exposure. Such observations are common in Probit analysis because reliable confidence interval estimation depends on adequate mortality across the concentration range [9]. As mortality increased by 96 hours, the confidence intervals narrowed considerably, indicating improved precision of the toxicity estimates.

Behavioural alterations constituted one of the earliest and most sensitive responses to lead nitrate exposure in the present study. Exposed fish exhibited progressive increases in air gulping, erratic swimming, abnormal opercular movement, impaired equilibrium, abnormal tail beating, body discolouration, skin peeling, and loss of reflex, all of which became more pronounced with increasing concentration and exposure duration. These findings support previous reports that behavioural responses provide rapid and highly sensitive indicators of toxicant-induced stress in fish because behaviour integrates the functional status of the nervous, respiratory, muscular, and endocrine systems [13, 14].

The behavioural abnormalities observed are likely associated with the neurotoxic and respiratory effects of lead. Lead readily crosses biological membranes and accumulates within nervous tissues, where it disrupts calcium-mediated neurotransmission, inhibits acetylcholinesterase activity, alters synaptic signaling, and promotes oxidative damage within neurons [7, 19]. Respiratory distress, reflected by increased opercular movement and air gulping, may result from impaired branchial ion regulation and reduced oxygen uptake following lead-induced damage to gill tissues. Similar behavioural abnormalities have recently been reported in common carp and African catfish exposed to lead nitrate, where swimming abnormalities, respiratory distress, and reduced escape responses were associated with increased oxidative stress and tissue damage [3]. These observations confirm that behavioural assessment provides valuable early-warning information that may precede measurable biochemical or histopathological alterations.

An important feature of the present study was the development of a Behavioural Toxicity Index (BTI), which integrated multiple qualitative behavioural observations into a single quantitative measure of toxicological stress. Although developed specifically for this investigation, the BTI facilitated objective comparison of behavioural impairment among treatments and exposure periods and demonstrated a clear increase in behavioural toxicity with increasing lead exposure. Such integrated behavioural indices may complement conventional toxicity endpoints by providing sensitive, rapid, and non-invasive tools for monitoring aquatic pollution. Future studies should evaluate the applicability of this index across different contaminants and fish species to determine its broader usefulness in aquatic ecotoxicology.

The estimation of environmentally safe exposure concentrations further enhances the practical significance of this study. Safe concentrations derived using both the Sprague application factor and the National Academy of Sciences/National Academy of Engineering (NAS/NAE) application factor decreased progressively with increasing exposure duration, reflecting the cumulative nature of lead toxicity. These findings indicate that environmental safety thresholds based solely on short-term toxicity data may underestimate ecological risk under natural conditions where aquatic organisms experience prolonged exposure to low concentrations of contaminants. Similar concerns have been raised in recent environmental risk assessments, which emphasize that persistent heavy metal contamination poses significant long-term risks to freshwater ecosystems through bioaccumulation, trophic transfer, and chronic physiological impairment [3, 6].

From an environmental management perspective, the toxicity thresholds generated in this study provide valuable baseline data for freshwater monitoring programmes, aquaculture management, and environmental regulation. Because *Clarias gariepinus* is one of the most economically important cultured fish species in Africa, the estimated safe concentrations may assist environmental agencies in developing discharge standards for lead-containing effluents and evaluating the suitability of freshwater resources for aquaculture production. Furthermore, the generated LC values provide an important reference for designing chronic toxicity experiments involving environmentally relevant sublethal lead exposure.

One of the strengths of the present investigation is the integration of mortality assessment, behavioural biomarkers, Probit-derived toxicity thresholds, and estimated environmental safety limits within a single experimental framework. While many previous studies have focused primarily on $LC_{50}$ determination, the present study demonstrates that combining behavioural observations with conventional toxicity endpoints provides a more comprehensive understanding of acute toxicodynamics. Nevertheless, certain limitations should be acknowledged. The study was conducted under controlled laboratory conditions using a single toxicant, whereas natural aquatic environments are characterized by fluctuating physicochemical conditions and complex mixtures of contaminants that may modify toxic responses. In addition, the present investigation focused on acute toxicity and behavioural responses without examining tissue lead accumulation, oxidative stress biomarkers, antioxidant enzyme activity, or molecular mechanisms of toxicity.

Future research should therefore investigate the chronic effects of environmentally relevant lead concentrations on growth performance, haematology, serum biochemistry, oxidative stress, histopathology, reproductive physiology, and immune function in *C. gariepinus*. Integrating these physiological endpoints with molecular biomarkers would provide a more comprehensive understanding of the mechanisms underlying lead toxicity and strengthen ecological risk assessments for tropical freshwater ecosystems.

Overall, the present study demonstrates that lead nitrate induces progressive mortality, behavioural dysfunction, declining toxicity thresholds, and reduced environmental safety margins in *Clarias gariepinus* fingerlings. The combined use of behavioural biomarkers, Probit-derived toxicity estimates, and safe exposure calculations provides a robust framework for evaluating acute heavy metal toxicity and generates valuable baseline information for environmental monitoring, ecological risk assessment, and sustainable aquaculture management.

## 5. Conclusion

The present study demonstrated that lead nitrate poses a significant acute toxicological risk to *Clarias gariepinus* fingerlings, with toxicity increasing progressively as both exposure concentration and duration increased. The concentration-dependent rise in cumulative mortality, together with the progressive decline in $LC_{10}$, $LC_{50}$, and $LC_{90}$ values over the 96-hour exposure period, confirms that lead toxicity is a dynamic process characterized by increasing toxic potency with prolonged exposure. The derived Probit-based toxicity thresholds provide robust estimates of acute toxicity and establish valuable baseline data for environmental hazard assessment.

Behavioural alterations, including increased air gulping, erratic swimming, abnormal opercular movement, impaired equilibrium, abnormal tail beating, body discolouration, skin peeling, and loss of reflex, were among the earliest observable responses to lead exposure and intensified with increasing concentration and exposure duration. The Behavioural Toxicity Index developed in this study further demonstrated the value of integrating multiple behavioural endpoints into a quantitative indicator of toxicological stress, highlighting the potential of behavioural biomarkers as rapid and sensitive tools for monitoring heavy metal contamination in freshwater ecosystems.

The estimated safe exposure concentrations derived from the acute $LC_{50}$ values declined progressively with increasing exposure duration, emphasizing that prolonged exposure substantially reduces the concentration of lead considered environmentally safe. These findings reinforce the need to incorporate exposure duration into ecological risk assessment and water quality guideline development rather than relying solely on short-term toxicity estimates.

Overall, this study provides a comprehensive evaluation of the acute toxicodynamics of lead nitrate in *Clarias gariepinus* by integrating mortality responses, behavioural biomarkers, Probit-derived toxicity thresholds, and environmentally relevant safety benchmarks within a single experimental framework. The findings contribute important baseline information for freshwater environmental monitoring, ecological risk assessment, and sustainable aquaculture management in regions vulnerable to heavy metal contamination. Future investigations should extend these observations by examining the chronic physiological, biochemical, molecular, and histopathological consequences of environmentally relevant lead exposure under conditions that more closely resemble natural freshwater ecosystems.

## Compliance with ethical standards

### *Acknowledgements*

This research was supported by the Tertiary Education Trust Fund (TETFund), Nigeria, under the Institutional Based Research (IBR) Intervention (Grant Ref. No: UNICROSS/DRD/IBR/009/003), for the project titled: "Turning a weed into wealth: Ameliorative Potential of Water Hyacinth (*Eichhornia crassipes*) Leaf Protein Concentrate against Lead-Induced Toxicity in *Clarias gariepinus* Fingerlings". The authors sincerely thank the Department of Fisheries and Aquaculture, University of Cross River State, as well as the laboratory personnel (Ernest Dan Sunday, Anthony Onah Ogine, and Tordo Elias Anyiman) for their invaluable technical assistance and support.

### *Disclosure of conflict of interest*

The authors declare that they have no competing or conflicting interests.

*Statement of ethical approval*

All experimental procedures involving fish were conducted in strict accordance with institutional guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Cross River State, Nigeria (Approval No: UNICROSS/FAEC/2006-F001).

*Statement of informed consent*

This manuscript does not contain any studies involving human participants performed by any of the authors; therefore, informed consent was not required.

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