

# JOURNAL OF ENVIRONMENTAL MICROBIOLOGY AND TOXICOLOGY

Website: <http://journal.hibiscuspublisher.com/index.php/JEMAT/index>



JEMAT VOL 14 NO 1 2026  
Artemia salina

## Environmentally Relevant Concentrations of Lead Induce Toxic Stress in Nile Tilapia (*Oreochromis niloticus*)

Musa A. Ibrahim<sup>1,4\*</sup>, Musa Ibrahim<sup>1</sup>, Philip W. Edward<sup>1</sup>, Mayelmi H. Markus<sup>1</sup>, Hauwa I. Dibal<sup>1</sup>, Mathias N. Bwala<sup>2</sup>, Usman A. Ibrahim<sup>3,4</sup>

<sup>1</sup>Department of Biology, Faculty of Life Sciences, University of Maiduguri, P.M.B. 1069, Maiduguri 600230, Borno State, Nigeria.

<sup>2</sup>Borno State Field Office, National Environmental Standards and Regulations Enforcement Agency (NESREA), C/O Borno State Afforestation Office Complex, Angwan Doki, Behind Dujima Hotel, Maiduguri, Borno State, Nigeria.

<sup>3</sup>Department of Civil and Water Resources Engineering, Faculty of Engineering, University of Maiduguri, P.M.B. 1069, Maiduguri 600230, Borno State, Nigeria.

<sup>4</sup>North-East Regional Centre for National Water Resources Capacity Building Network, University of Maiduguri, P.M.B. 1069, Maiduguri 600230, Borno State, Nigeria.

\*Corresponding author:

Musa A. Ibrahim  
Department of Biology,  
Faculty of Life Sciences,  
University of Maiduguri,  
P.M.B. 1069, Maiduguri 600230,  
Borno State,  
Nigeria.

Email: [moosaad8@gmail.com](mailto:moosaad8@gmail.com); [maibrahim@unimaid.edu.ng](mailto:maibrahim@unimaid.edu.ng)

### History

Received: 29<sup>th</sup> May 2026  
Received in revised form: 29<sup>th</sup> June 2026  
Accepted: 30<sup>th</sup> July 2026

### Keywords

Lead  
Biomarkers  
Aquatic ecosystems  
Oxidative stress  
Toxicity

### SDG Keywords

SDG 6 Clean water and sanitation  
SDG 12 Responsible consumption and production  
SDG 9 Industry, innovation and infrastructure

### Abstract

Lead (Pb) is a common toxic metal and can be harmful to fish health even at low concentrations. The toxicity of environmentally relevant nominal concentrations of Pb (0.125-1.000 mg/L) to juvenile tilapia (*Oreochromis niloticus*; initial total length 9.1 +/- 0.5 cm; body weight 10.6 +/- 2.3 g) was studied for 28 days with continuous aeration (dissolved oxygen 5.2-5.4 mg/L). Measured and nominal Pb concentrations were highly correlated ( $R^2 = 0.9995$ ,  $p = 0.0002$ ). Tissue Pb concentration increased with waterborne Pb concentration ( $p = 0.0004$ ) and the average bioconcentration factor was 0.33 L/kg. Viscerosomatic and hepatosomatic indices were lower in Pb-exposed fish than in controls while gonadosomatic index was not significantly different among groups. Water temperature and pH were relatively stable and electrical conductivity and total dissolved solids varied among treatments. Reduced glutathione, malondialdehyde and superoxide dismutase differed significantly among groups, while catalase activity did not. Electrical conductivity was positively correlated with total dissolved solids ( $r = 0.97$ ) and negatively correlated with pH ( $r = -0.90$ ). These results show that environmentally relevant Pb exposure can modify organ condition, tissue metal burden, and oxidative-stress responses in juvenile Nile tilapia, supporting the need for routine monitoring of Pb in freshwater and aquaculture systems.

### INTRODUCTION

Lead (Pb) is a naturally occurring element and is toxic to aquatic organisms even at low concentrations [1]. It is widely distributed in environmental media such as soil, water and biota [2, 3]. Anthropogenic inputs are from mining, smelting, production of batteries and paints, industrial effluents, sewage, agricultural runoff and improper disposal of wastes [4-8]. These inputs increased Pb contamination of surface waters and the exposure of aquatic organisms. The Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) is an aquaculture species of global importance, contributing to food and nutrition security [9,10]. Because of its wide environmental tolerance, availability and ecological relevance it is also useful in aquatic toxicology and biomonitoring [7,11].

Studies on freshwater monitoring in Nigeria have reported Pb contamination in water, sediment and fish. The concentrations in water were reported to be in the range of 0.020-1.540 mg/L in Alau Dam, 0.002-0.132 mg/L in Uke Stream and 0.026-0.087 mg/L in Escravos River [11-13]. In Nigeria fish from contaminated waters have been reported to have measurable tissue Pb confirming the transfer from the aquatic environment to biota [11,13-15].

Lead reacts with sulfur and oxygen groups of proteins and can be accumulated in fish tissues [1,16]. Waterborne Pb interferes with branchial ion regulation in freshwater fish by affecting  $\text{Ca}^{2+}$ -ATPase,  $\text{Na}^{+}/\text{Ca}^{2+}$  exchange and  $\text{Na}^{+}/\text{K}^{+}$ -ATPase, disturbing the calcium homeostasis and electrochemical

gradients [17]. Thus, chronic exposure to relatively low Pb concentrations can still have biologically important effects [1]. Long-term exposure to Pb can adversely affect fish growth, respiration, ion regulation, hematology and enzyme activity [18-24]. Reported behavioral effects include reduced feeding and swimming, loss of balance and erratic movement, while tissue injury may occur with increased exposure [1,18,20]. High levels can be lethal [25].

Pb also modifies the pro-oxidant/antioxidant balance, promotes the generation of reactive oxygen species and alters enzymatic and non-enzymatic antioxidant defenses [1,26,27]. The present study therefore evaluated Pb bioaccumulation and oxidative-stress biomarkers in juvenile Nile tilapia exposed to environmentally relevant Pb concentrations.

## MATERIALS AND METHODS

### Source of chemical

Lead(II) acetate trihydrate [ $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$ ; purity 99.9%] and other analytical-grade reagents were obtained from a chemical supplier in Jos, Nigeria.

### Preparation of stock and test solutions of lead

Environmentally relevant nominal Pb concentrations of 0.125, 0.250, 0.500 and 1.000 mg/L were prepared from a 1,000 mg Pb/L stock solution. The stock was prepared by dissolving 1.830 g of lead(II) acetate trihydrate [ $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$ ; molar mass 379.33 g/mol] in distilled water and making the final volume up to 1 L. This quantity supplies approximately 1.000 g of elemental Pb. The required stock volume was calculated as  $V_1 = (C_2 \times V_2)/C_1$ , where  $V_1$  is the stock volume,  $C_1$  is the stock concentration (1,000 mg Pb/L),  $C_2$  is the target Pb concentration and  $V_2$  is the final aquarium volume. The highest nominal concentration was selected to span the range of Pb concentrations reported in contaminated Nigerian freshwaters [11-15].

### Source of fish

The experimental fish, juvenile Nile tilapia (*O. niloticus*), were brought from the Department of Fisheries, University of Maiduguri, Borno State.

### Fish Acclimatisation and Maintenance

Experimental fish were acclimated for 12 days in glass aquaria containing dechlorinated borehole water at  $25 \pm 1^\circ\text{C}$ . Fish were fed daily with commercial feed (Blue Crown; 2.0-mm pellet) obtained in Maiduguri. Water was continuously aerated, and dissolved oxygen was maintained at approximately  $5 \pm 2$  mg/L. Water was renewed every other day to limit the accumulation of metabolic wastes.

### Experimental Design/Setup

Fish were randomly allocated to glass aquaria (60 x 60 x 90 cm; 10 fish per aquarium) in triplicate. The experiment comprised a control and four nominal Pb concentrations (0.125, 0.250, 0.500 and 1.000 mg/L) arranged in a completely randomized design. Temperature, dissolved oxygen, total dissolved solids, electrical conductivity and pH were measured daily with a calibrated handheld water-quality meter.

### Exposure to lead

Feeding was withheld for 24 h before the 28-day exposure. Mortality and behavioural changes were recorded throughout the experiment. Fish were considered dead when gill movement ceased and they failed to respond to gentle stimulation; dead fish were removed promptly.

### Sample preparation

Fish were sacrificed under clove oil anaesthesia. An incision was made through the lateral side to ensure that the internal organs were removed intact. A small median lobe of the liver was cut, and a portion was fixed in Davidson's solution for 24 hours before being transferred to 10% buffered formalin for further analysis.

### Heavy metal analysis (Pb) in water, gills and muscles

Water and fish-tissue samples ( $n = 3$  per aquarium) were prepared using acid-digestion procedures adapted from previously reported methods [28]. Water samples were digested with concentrated nitric and hydrochloric acids (6 mL of 65%  $\text{HNO}_3$  and 3 mL of 37%  $\text{HCl}$ ), followed by 0.25 mL  $\text{H}_2\text{O}_2$ , in a microwave digestion system at  $120^\circ\text{C}$  for 30 min. After cooling, the digest was diluted to 100 mL with deionized water, filtered and retained for analysis using atomic absorption spectrometer (AAS) by injecting 20  $\mu\text{L}$  into the tube.

Fish samples were oven-dried, ground and digested with concentrated  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  (2:1, v/v) using adapted published procedures [28,29]. Approximately 2 g of homogenized dry tissue was placed in a digestion tube, treated with the acid mixture and digested for 3 h until reduced to 10 mL. After homogenizing and digesting the dried materials with a 10 mL tri-acid combination ( $\text{HNO}_3$ :  $\text{HClO}_4$ : $\text{H}_2\text{SO}_4$ ), the digested samples were diluted to a final volume of 100 mL using deionized water. After cooling, each digest was filtered and made up to a defined final volume with deionized water before analysis with AAS (Unicam 919 Model).

### Determination of Pb in water and tissues

Water and muscle digests were analysed for Pb by atomic absorption spectrometry. Concentrations should be reported as mg/L for water and mg/kg dry weight for tissue. Instrumental quality-control information should be added as specified above.

### Biochemical analyses

Fish tissues were excised, rinsed in ice-cold physiological saline and homogenized (10%, w/v) in chilled 0.1 M phosphate buffer (pH 7.4). Homogenates were centrifuged at  $10,000 \times g$  for 15 min at  $4^\circ\text{C}$ , and the supernatants were used for analysis. Superoxide dismutase activity was determined from inhibition of epinephrine autoxidation at 480 nm [30], catalase activity from  $\text{H}_2\text{O}_2$  decomposition at 240 nm [31], reduced glutathione with 5,5'-dithiobis-(2-nitrobenzoic acid) at 412 nm [32], and lipid peroxidation as thiobarbituric-acid-reactive substances at 532 nm [33]. Enzyme activities were normalized to protein concentration measured by the Bradford assay [34].

### Ethical clearance

All experimental procedures involving the *O. niloticus* were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Head of Biology Department, Faculty of Life Sciences of the University of Maiduguri with the recommendation of the Departmental Health Research and Animal Ethics Committee. Additionally, handling, sampling, and euthanasia were performed using humane methods to minimize stress and suffering, and all efforts were made to reduce the number of animals used while ensuring scientific validity.

### Statistical analysis

Data were analysed in GraphPad Prism version 5.03. One-way analysis of variance was used to compare treatment means, followed by the Student-Newman-Keuls multiple-comparison procedure. Results are presented as mean  $\pm$  SD unless otherwise

stated. Statistical significance was set at  $p < 0.05$ . Fisher's LSD was used to compare the means.

### Bioconcentration factor

The bioconcentration factor (BCF) was calculated as the Pb concentration in fish tissue  $C_{fish}$  (mg/kg) divided by the measured Pb concentration in water  $C_{water}$  (mg/L), giving units of L/kg [35].  $BCF (L/kg) = C_{fish} (mg/kg) / C_{water} (mg/L)$ .

## RESULTS

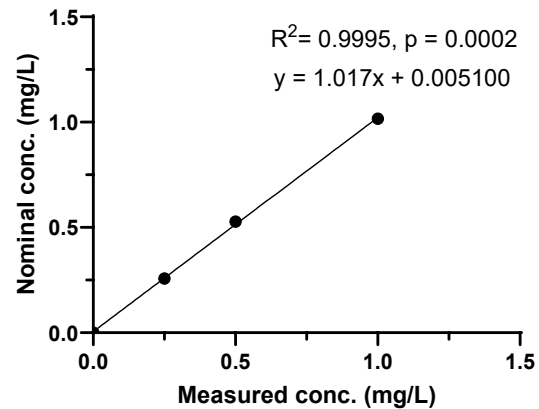
**Table 1** shows the morphometric parameters of *Oreochromis niloticus* in Pb polluted water after 28 days of exposure (28 dpe). The results indicate that body weight (BW) varied significantly across treatments, with higher values recorded in fish exposed to 0.125 and 1.000 mg/L Pb compared to the control ( $P < 0.05$ ). Total length (TL), liver weight (LW), gonad weight (GW), and gonadosomatic index (GSI) did not differ significantly among treatments ( $P > 0.05$ ). In contrast, viscerosomatic index (VSI) and hepatosomatic index (HSI) showed significant reductions in all Pb-exposed groups relative to the control, indicating Pb-induced alterations in visceral and hepatic conditions after 28 days of exposure.

**Table 2** reveals the physicochemical parameters of the different experimental tanks. Water temperature showed minimal variation throughout the experimental period, ranging from 28.5 to 29.4 °C, with no clear concentration-dependent trend observed. Similarly, dissolved oxygen (DO) values remained relatively stable across all treatments, varying narrowly between 5.2 and 5.4 mg/L. Total dissolved solids (TDS) exhibited a gradual decline with increasing concentration. The control and lower concentrations (0.125 and 0.25 mg/L) recorded comparable TDS values (409.3–415.0 mg/L) and were not significantly different ( $p > 0.05$ ).

However, a significant reduction in TDS was observed at 0.5 mg/L (400.3 mg/L), while the lowest value was recorded at 1 mg/L (393.7 mg/L), which differed significantly from the control ( $p < 0.05$ ). Electrical conductivity (EC) increased progressively with increasing concentration. The control and 0.125 mg/L treatments recorded lower EC values (792.0–801.3 µS/cm) and differed significantly from the higher concentrations ( $p < 0.05$ ). In contrast, the 0.25, 0.5, and 1 mg/L treatments showed

significantly higher Evalues, ranging from 818.0 to 827.3 µS/cm. The pH of the media was slightly alkaline across all treatments, with values ranging from 7.5 to 7.9. A marginal decrease in pH was observed with increasing concentration, although the changes remained within a narrow range and indicated relatively stable acid–base conditions during the experiment.

**Fig. 1** presents the relationships among selected physicochemical variables in Pb-exposure water after 28 days. The reported  $R^2 = 0.9995$  refers to the regression between nominal and measured Pb concentrations and should not be described as a general relationship between all physicochemical and morphometric variables.



**Fig. 1.** Linear regression between nominal and measured Pb concentrations in exposure water after 28 days.

**Fig. 2** presents oxidative-stress biomarkers in *O. niloticus* after 28 days of Pb exposure. MDA increased from 4.63 ± 0.86 mM/mL in controls to 14.74 ± 1.50 mM/mL at 0.125 mg/L, then declined at higher concentrations. GSH and SOD also varied among treatments, whereas CAT did not differ significantly. These non-monotonic responses indicate concentration-dependent changes in antioxidant defence and lipid peroxidation.

**Table 1.** Morphometric parameters of *Oreochromis niloticus* in Pb polluted water after 28 days of exposure (28 dpe).

| Treatment | Morphometric           |         |          |          |                        |          |                       |
|-----------|------------------------|---------|----------|----------|------------------------|----------|-----------------------|
|           | BW                     | TL      | LW       | GW       | VSI                    | GSI      | HSI                   |
| Control   | 7.6±2.2 <sup>b</sup>   | 8.5±0.6 | 0.3±0.05 | 0.2±0.05 | 14.8±3.42 <sup>a</sup> | 2.3±0.81 | 3.6±1.12 <sup>a</sup> |
| 0.125     | 13.1±1.6 <sup>a</sup>  | 9.6±0.6 | 0.2±0.09 | 0.3±0.26 | 7.3±3.00 <sup>b</sup>  | 2.1±2.33 | 1.7±0.83 <sup>b</sup> |
| 0.250     | 9.1±0.9 <sup>b</sup>   | 8.8±0.7 | 0.1±0.03 | 0.2±0.14 | 6.5±2.32 <sup>b</sup>  | 2.5±1.47 | 1.0±0.27 <sup>b</sup> |
| 0.500     | 10.7±2.4 <sup>ab</sup> | 9.2±0.5 | 0.2±0.11 | 0.4±0.20 | 8.0±1.94 <sup>b</sup>  | 3.3±1.30 | 1.7±0.63 <sup>b</sup> |
| 1.000     | 12.5±1.4 <sup>a</sup>  | 9.6±0.7 | 0.2±0.06 | 0.5±0.11 | 8.2±1.05 <sup>b</sup>  | 3.8±1.16 | 1.3±0.48 <sup>b</sup> |

Note: Values are expressed as mean ± SD. Different superscript letters within a column indicate significant differences ( $p < 0.05$ ) using Fisher's LSD. BW, body weight; TL, total length; LW, liver weight; GW, gonad weight; VSI, viscerosomatic index; GSI, gonadosomatic index; HSI, hepatosomatic index.

**Table 2.** Physicochemical parameters of Pb conc. in a semi-static system for 28 days of exposure.

| Physicochemical Parameters |            |           |            |           |     |
|----------------------------|------------|-----------|------------|-----------|-----|
| Conc. (mg/L)               | Temp. (°C) | DO (mg/L) | TDS (mg/L) | EC(µS/cm) | pH  |
| Control                    | 28.8       | 5.2       | 415.0a     | 792.0b    | 7.9 |
| 0.125                      | 28.5       | 5.2       | 414.3a     | 801.3b    | 7.7 |
| 0.25                       | 29.4       | 5.4       | 409.3a     | 818.0a    | 7.7 |
| 0.5                        | 29.1       | 5.2       | 400.3b     | 818.0a    | 7.6 |
| 1                          | 28.5       | 5.2       | 393.7c     | 827.3a    | 7.5 |

Note: Values are expressed as mean ± SD. Different superscript letters within a column indicate significant differences ( $p < 0.05$ ) using Fisher's LSD. Temp., temperature; DO, dissolved oxygen; TDS, total dissolved solids; EC, electrical conductivity.

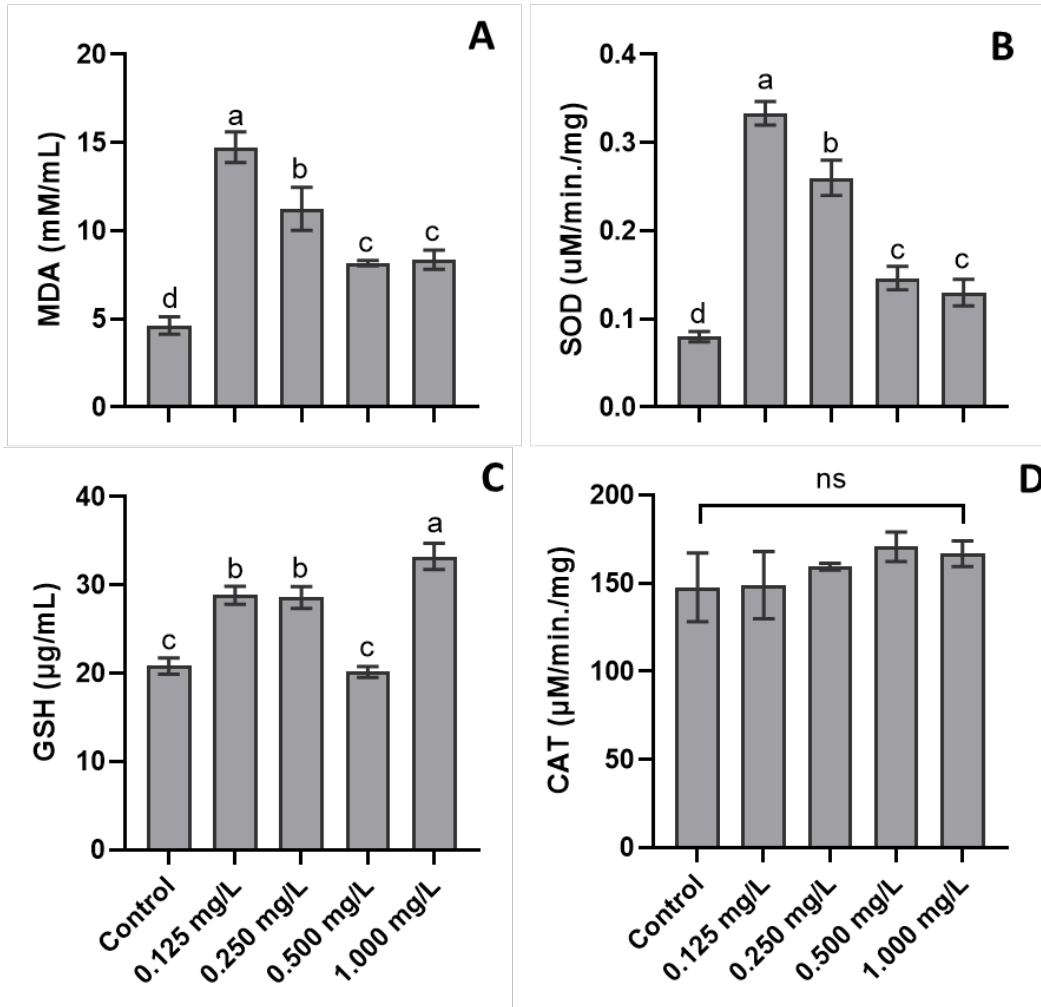


Fig. 2. Oxidative-stress biomarkers in *O. niloticus* after 28 days of Pb exposure. Different letters indicate significant differences ( $p < 0.05$ ); ns, not significant. Error bars represent standard deviations.

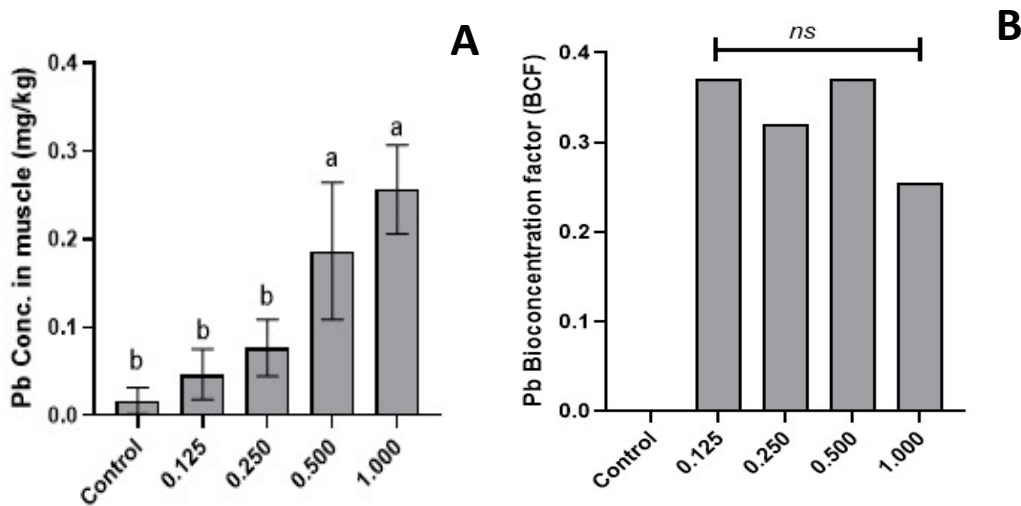


Fig. 3. Muscle Pb concentration (A) and bioconcentration factor (B) in *O. niloticus* after 28 days of exposure. Different letters indicate significant differences ( $p < 0.05$ ); ns, not significant. Error bars represent standard deviations.

The bioaccumulation and the corresponding bioaccumulation factor (BCF) for the different Pb spiked water concentrations of lead in fish increased with increasing Pb concentration in the exposure water (Fig. 3). The minimum Pb in fish (0.010 mg/kg) was found in the control group (0 mg/L) while fish exposed to the highest water concentration (1.0 mg/L) had the highest tissue Pb concentration (0.256 mg/kg) with progressively lower tissue concentrations at 0.5 mg/L (0.185 mg/kg), 0.25 mg/L (0.080 mg/kg) and 0.125 mg/L (0.046 mg/kg). The bioconcentration factor (BCF) differed between treatments and was not strictly linear in relation to water concentration with the highest BCF values (0.37) were observed at 0.5 and 0.125 mg/L. This indicated a relatively higher efficiency of Pb accumulation at these exposure levels.

The BCF value was slightly lower (0.32) at 0.25 mg/L, and the lowest BCF (0.26) was observed at the highest exposure concentration (1 mg/L) while the BCF value for the control group was not determined as no Pb was present in the exposure water. Fig. 4. presents correlations among Pb concentration and water-quality variables after 28 days. Pb concentration correlated positively with EC ( $r = 0.87$ ) and TDS ( $r = 0.75$ ) and negatively with pH ( $r = -0.84$ ). Temperature showed a weak negative association with Pb concentration ( $r = -0.24$ ).

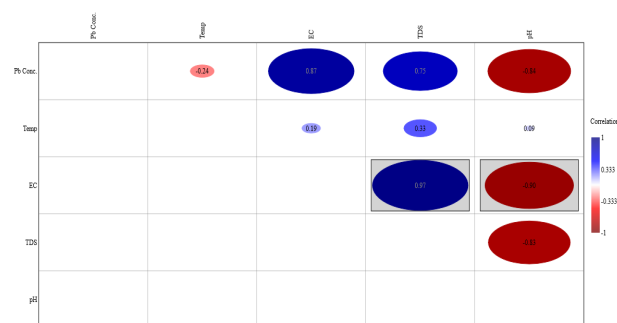


Fig. 4. Correlations among Pb concentration and physicochemical variables after 28 days. Grey cells indicate significant correlations ( $p < 0.05$ ).

Fig. 5. presents correlations among oxidative-stress biomarkers after 28 days. MDA correlated strongly with SOD ( $r = 0.88$ ), while GSH showed moderate positive correlations with MDA ( $r = 0.48$ ) and SOD ( $r = 0.37$ ). CAT was weakly negatively correlated with SOD ( $r = -0.10$ ).

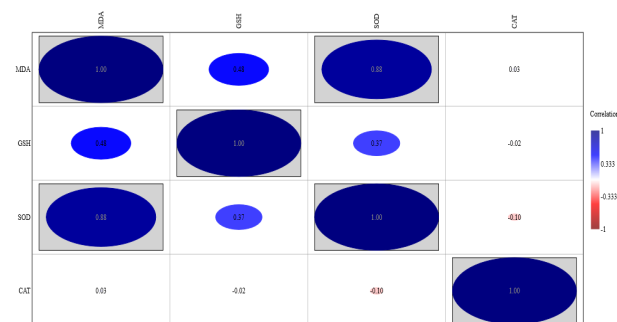


Fig. 5. Correlation matrix of oxidative-stress biomarkers in *O. niloticus* after 28 days of Pb exposure. Grey cells indicate significant correlations ( $p < 0.05$ ).

## DISCUSSION

Based on the results of the present study showed that sub-lethal exposure of Pb caused significant changes in morphometric indices, water quality relationships and oxidative stress responses in *O. niloticus* after a 28-day exposure. The increased BW at 0.125 and 1.00 mg/L Pb relative to control may be an indication of an initial compensatory or stress-related physiological response rather than an increased growth performance. This compensatory effect is similarly seen non-linear growth responses to Pb exposure reported in *O. niloticus* and other freshwater fishes where Pb bioaccumulation disturbs metabolic homeostasis and does not necessarily affect the total length [18,21]. No significant differences of TL, LW, GW and HSI suggest that the internal organ condition was mainly affected by short-term Pb exposure rather than somatic growth as previously reported in tilapia and catfish species exposed to Pb [20,23].

The result from the experiment shows a clear concentration dependent accumulation of Pb in fish tissues with tissue Pb levels increasing from 0.010 mg/L in the control to 0.256 mg/L in the highest exposure (1 mg/L) demonstrating the strong bioaccumulative potential of Pb in aquatic organisms as widely reported for freshwater fishes including *Clarias gariepinus* and *Oreochromis niloticus* [1,5,24]. Higher BCF values at intermediate concentrations (0.5 and 0.125 mg/L) than the highest exposure indicate a possible saturation or regulatory limitation of metal uptake mechanisms at higher level of Pb concentrations, a phenomenon previously reported in fish exposed to chronic heavy metal stress [21,25].

The comparable temperature (28.5–29.4 °C) and DO (5.2–5.4 mg/L) between treatments suggest that Pb accumulation was not due to thermal or oxygen stress, which is consistent with previous studies that demonstrated Pb bioaccumulation even under favorable physicochemical conditions [18,20]. However, the slow decrease in TDS with increasing EC and minor reduction in pH shows changed ionic composition of the water which may increase Pb bioavailability and uptake through ionoregulatory disruption at the gill surface [2,17]. Similar correlations of higher EC, metal mobility and increased accumulation in tissues have been reported in Nigerian and other freshwater systems impacted by anthropogenic activities [11,14,15]. The parallel trends between physicochemical parameters and Pb bioaccumulation show the sensitivity of *O. niloticus* to even moderate Pb exposure and further confirms the ecological and food safety implications of metal contamination in freshwater environments [6,13].

Lower VSI and HSI in all Pb treatments indicate impaired visceral and hepatic conditions and are in agreement with Pb bioaccumulation in metabolically active organs. It has been demonstrated that accumulation of lead in the liver interferes with detoxification pathways, enzyme synthesis and lipid metabolism [22,24]. Chronic exposure to heavy metals has been linked to hepatic stress, cellular degeneration and lack of energy reserves resulting in a decreased HSI [1,19]. The findings confirm the reports of liver dysfunction and changes in organ indices in *O. niloticus* and *Clarias gariepinus* due to Pb [5,18]. The strong positive correlation ( $R^2 = 0.9995$ ) between the physicochemical parameters and morphometric indices suggest that the biological responses of the fish are sensitive to changes in water quality under Pb contamination. The positive correlation of Pb concentration with EC and TDS and negative correlation with pH indicates the progressive deterioration of water quality with increasing Pb levels. Generally, an increase in EC and TDS in metal-contaminated waters is associated with increased ionic

load and dissolved contaminants which might enhance metal bioavailability and toxicity [13,15]. Reducing the pH increases the physiological stress in exposed fish, which further improves the solubility and uptake of Pb [2,6].

The oxidative stress biomarkers responses in *O. niloticus* following Pb exposure confirm the induction of oxidative imbalance. The alterations in antioxidant enzymes and markers of lipid peroxidation indicate that Pb disrupts the redox homeostasis and causes overproduction of reactive oxygen species (ROS). Increased MDA levels reflect the enhancement of lipid peroxidation, a well-known consequence of Pb-induced oxidative damage in fish tissues [1,7]. The strong positive correlation between MDA and SOD shows a compensatory up regulation of antioxidant defenses in response to the increased oxidative pressure as previously observed in Pb exposed tilapia and catfish [4,24].

Further, moderate positive correlations between reduced GSH, MDA and SOD further indicate activation of non-enzymatic antioxidant pathways to ameliorate oxidative stress induced by Pb. However, the low negative correlation between CAT and SOD suggest differential regulation of antioxidant enzymes during chronic Pb exposure, which could be attributed to enzyme inhibition or depletion under increased oxidative loads [16,22]. These imbalances in antioxidant systems have been widely associated with compromised immune function, metabolic disruption and increased susceptibility to secondary stressors in contaminated aquatic environments [1,6].

The present findings of combined morphometric alterations, water quality deterioration and oxidative stress responses further suggest the ecological and physiological hazards of Pb contamination to freshwater fishes. These results are particularly relevant for fisheries sustainability and food security in areas with increasing heavy-metal pollution, where polluted fish may endanger both ecosystem health and human consumers [9,10,11,14].

## CONCLUSION

The exposure of juvenile *O. niloticus* to environmentally relevant concentrations of Pb for 28 days caused changes in tissue Pb burden, organ-condition indices and oxidative-stress biomarkers. The decreased values of VSI and HSI denote an impaired condition of the viscera and the liver with little change in total length and reproductive indices. Changes in antioxidant responses and lipid peroxidation reflect a disruption of redox homeostasis. These results support the use of *O. niloticus* as a sentinel species for Pb contamination in freshwater. Regular monitoring of Pb levels and other important physicochemical parameters in freshwater ecosystems is recommended for prevention of ecological degradation and protection of fisheries and other bio-resources. There should be prioritization of effective regulation and control of anthropogenic Pb inputs to reduce bioavailability and subsequent bioaccumulation in aquatic organisms. In aquaculture, managing water quality is important to minimize exposure to heavy metals and the associated health hazards. The National Environmental Standards and Regulations Enforcement Agency (NESREA) is advised to step up routine compliance monitoring and enforcement of national environmental regulations to conserve and protect the aquatic ecosystems from anthropogenic induced Pb pollution and to protect public health. Long-term and multigenerational exposure scenarios should be investigated in future studies to understand chronic and reproductive effects of Pb in *O. niloticus*. Combining histopathological, molecular and immunological biomarkers

could provide a broader understanding of Pb toxicity mechanisms. Furthermore, the combined effects of Pb with other co-occurring pollutants such as micro and nanoplastics (MNPs), polycyclic aromatic hydrocarbons, pesticides and other aquatic contaminants need to be investigated to better reflect environmentally realistic exposure conditions and inform risk-based management strategies.

## ACKNOWLEDGEMENTS

The researchers acknowledge the Tertiary Education Fund (TETFund) Nigeria for funding this research through Institutional-Based Research (IBR-VC/CRI/ADM.012/VOL.III. 2025), University of Maiduguri, Nigeria.

## AUTHOR CONTRIBUTIONS

Conceptualization; M.A.I., M.I., H.I.D., M.N.B.U.A.I., Data analysis; M.A.I., Data curation; M.I., P.W.E, M.H.M., Writing-original draft; M.A.I., and editing were equally shared by M.I., P.W.E, M.H.M, H.I.D., M.N.B., and U.A.I.

## CONFLICTS OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The raw data supporting the findings of this study are available from the corresponding author upon reasonable request.

## ETHICAL APPROVAL AND CONSENT

Not applicable

## REFERENCES

1. Lee JW, Choi H, Hwang UK, Kang JC, Kang YJ, Kim KI, et al. Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: a review. *Environ Toxicol Pharmacol*. 2019;68:101-108. <https://doi.org/10.1016/j.etap.2019.03.010>
2. Cheng H, Hu Y. Lead (Pb) isotopic fingerprinting and its applications in lead pollution studies in China: a review. *Environ Pollut*. 2010;158:1134-1146. <https://doi.org/10.1016/j.envpol.2009.12.028>
3. Wang Z, Bao J, Wang T, Moryani HT, Kang W, Zheng J, et al. Hazardous heavy metals accumulation and health risk assessment of different vegetable species in contaminated soils from a typical mining city, Central China. *Int J Environ Res Public Health*. 2021;18:2617. <https://doi.org/10.3390/ijerph18052617>
4. Kim JH, Kang JC. The immune responses in juvenile rockfish, *Sebastes schlegelii*, exposed to dietary lead(II). *Environ Toxicol Pharmacol*. 2016;46:211-216.
5. Olaifa FE, Olaifa AK, Lewis OO. Toxic stress of lead on *Clarias gariepinus* (African catfish) fingerlings. *Afr J Biomed Res*. 2003;6:101-104.
6. Emenike EC, Iwuozor KO, Anidiobi SU. Heavy metal pollution in aquaculture: sources, impacts and mitigation techniques. *Biol Trace Elem Res*. 2022;200:4476-4492. <https://doi.org/10.1007/s12011-021-03037-x>
7. Reda RM, Zaki EM, Aioub AAA, Metwally MMM, Yassin AM, Mahsoub F. Behavioral, biochemical, immune, and histological responses of Nile tilapia (*Oreochromis niloticus*) to lead, mercury, and pendimethalin exposure: individual and combined effects. *Environ Sci Eur*. 2025;37:11. <https://doi.org/10.1186/s12302-024-01047-9>
8. Dixit R, Wasiullah, Malaviya D, Pandiyan K, Singh UB, Sahu A, et al. Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental

- processes. Sustainability. 2015;7:2189-2212. <https://doi.org/10.3390/su7022189>
9. Alsaleh M. The role of the fishery industry in the shift towards sustainable food security: a critical study of blue food. Environ Sci Pollut Res Int. 2023;30:105575-105594. <https://doi.org/10.1007/s11356-023-29747-4>
10. Wang J, Zhang R, He T, Belgrano A. Editorial: blue foods security and sustainability. Front Mar Sci. 2024;11:1487645. <https://doi.org/10.3389/fmars.2024.1487645>
11. Bwala MN. Assessment of heavy metals in sediments, water and *Clarias gariepinus* from Alau Dam, Borno State, Nigeria. Sahel J Life Sci FUDMA. 2023;1:155-162.
12. Opaluwa OD, Aremu MO, Ogbo LO, Magaji JI, Odiba IE, Ekpo ER. Assessment of heavy metals in water, fish and sediments from Uke Stream, Nasarawa State, Nigeria. Curr World Environ. 2012;7:213-230.
13. Edward M, Muhib A. Heavy metals concentration in water, sediment, and fish around Escravos River, Nigeria. World J Res Rev. 2020;10:28-37.
14. Akubuo FC, Osuji LC, Hart AI. Temporal variations of heavy metal concentrations in selected fishes, sediments and surface water along an artisanal refinery site, Rivers State, Nigeria. J Environ Sci Health B. 2023;58:235-245.
15. Davies IC, Ekperusi AO. Evaluation of heavy metal concentrations in water, sediment and fishes of New Calabar River in southern Nigeria. J Aquat Sci Mar Biol. 2021;4:12-20.
16. Verstraeten SV, Aimo L, Oteiza PI. Aluminium and lead: molecular mechanisms of brain toxicity. Arch Toxicol. 2008;82:789-802. <https://doi.org/10.1007/s00204-008-0345-3>
17. Rogers JT, Richards JG, Wood CM. Ionoregulatory disruption as the acute toxic mechanism for lead in the rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol. 2003;64:215-234. [https://doi.org/10.1016/S0166-445X\(03\)00053-5](https://doi.org/10.1016/S0166-445X(03)00053-5)
18. Abdelzaher MF, Azab AM, Authman MMN, Shaban WM. Impact of lead and cadmium chronic exposure on some physiological parameters of Nile tilapia (*Oreochromis niloticus*). Egypt J Aquat Biol Fish. 2022;26:421-432.
19. Çiftçi N, Karayakar F, Ay Ö, Cici B, Erdem C. Effects of copper and lead on some haematological parameters of *Oreochromis niloticus*. Fresenius Environ Bull. 2015;24:2771-2775.
20. El-Sayed EE. Accumulation of some heavy metals and its effect on hematological indices of freshwater fish, *Oreochromis niloticus*. Egypt J Aquat Biol Fish. 2015;19:89-100.
21. Dos Santos CR, Cavalcante ALM, Hauser-Davis RA, Lopes RM, Da Costa Mattos RCO. Effects of sub-lethal and chronic lead concentrations on blood and liver ALA-D activity and hematological parameters in Nile tilapia. Ecotoxicol Environ Saf. 2016;129:250-256.
22. Khalesi MK, Abedi Z, Behrouzi S, Kohestan Eskandari S. Haematological, blood biochemical and histopathological effects of sublethal cadmium and lead concentrations in common carp. Bulg J Vet Med. 2017;20:141-150.
23. Kumar M, Kumar D, Kumar R. Effect of heavy metals cadmium, lead and copper on the blood characteristics of freshwater catfish *Clarias batrachus*. Int J Adv Res Biol Sci. 2017;4:129-134.
24. Abdel-Warith AA, Younis EMI, Al-Asgah NA, Rady AM, Allam HY. Bioaccumulation of lead nitrate in tissues and its effects on haematological and biochemical parameters of *Clarias gariepinus*. Saudi J Biol Sci. 2020;27:840-845.
25. Azua ET, Akaahan TJ. Toxic stress exhibited by juveniles of *Clarias gariepinus* exposed to different concentrations of lead. J Res Environ Sci Toxicol. 2017;6:8-11.
26. Gurer H, Ercal N. Can antioxidants be beneficial in the treatment of lead poisoning? Free Radic Biol Med. 2000;29:927-945. [https://doi.org/10.1016/S0891-5849\(00\)00413-5](https://doi.org/10.1016/S0891-5849(00)00413-5)
27. Maiti AK, Saha NC, Paul G. Effect of lead on oxidative stress, Na<sup>+</sup>/K<sup>+</sup>-ATPase activity and mitochondrial electron transport chain activity of the brain of *Clarias batrachus* L. Bull Environ Contam Toxicol. 2010;84:672-676.
28. Musa AM, Oyediji DE, Mada SB. Bioaccumulation of heavy metals in some tissues of croaker fish from oil-spilled rivers of Niger Delta region, Nigeria. Asian Pac J Trop Biomed. 2017;7:563-568. <https://doi.org/10.1016/j.apjtb.2017.05.008>
29. Olabemiwo OM, Alade AO, Tella AC, Adediran GO. Assessment of polycyclic aromatic hydrocarbons content in smoked *Clarias gariepinus* and *Tilapia guineensis* fish species available in western Nigeria. Int J Basic Appl Sci. 2011;11:135-150.
30. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247:3170-3175. [https://doi.org/10.1016/S0021-9258\(19\)45228-9](https://doi.org/10.1016/S0021-9258(19)45228-9)
31. Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-126. [https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3)
32. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70-77. [https://doi.org/10.1016/0003-9861\(59\)90090-6](https://doi.org/10.1016/0003-9861(59)90090-6)
33. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351-358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
34. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
35. Arnot JA, Gobas FAPC. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ Rev. 2006;14:257-297. <https://doi.org/10.1139/a06-005>