Journal of Himalayan Earth Sciences Volume 59, No. 1, 2026 pp. 70-87

Toxicity Potential of Lead in Nile Tilapia (Oreochromis niloticus) Using Bioaccumulation and Oxidative Stress Indicators

Arooj Atiq[1], Asma Jamil[1*], Rija Khalid[1], and Sajida Rasheed[2]

[1] Department of Earth and Environmental Sciences, Bahria School of Engineering & Applied Sciences, Bahria University, H-11 Islamabad;

[2] Department of Biotechnology, University of Kotli, AJK

**Correspondence Author: asmajamil.buic@bahria.edu.pk*

Submitted Date:21/09/2025 Acceptance Date: 12/12/2025 Publication Date: 31/03/2026

Abstract

This contemporary study was carried out to determine the toxicity potential of lead (Pb) using the fish *Oreochromis niloticus* as a bioindicator. Further, the study was also undertaken to determine the oxidative stress, bioaccumulation factor, metal pollution index, and antioxidant enzymatic and non-enzymatic activities of CAT, GST, and GSH in different fish organs due to absorption of Pb. A total of 50 fish were exposed for 30 days to graded Pb concentrations (0.08, 0.016, 0.0016, and 0.000016 mg/L) under controlled laboratory conditions. Pb accumulation was organ-specific, with the highest concentrations in muscles (0.409 mg/kg) and gills (0.247 mg/kg), both exceeding the WHO permissible limits for fish tissues. The bioaccumulation factor (BAF) followed the order muscle > brain > heart > liver > gills, indicating preferential storage in metabolically active tissues, while the metal pollution index (MPI) reflected an overall low contamination degree. Oxidative stress biomarkers revealed marked alterations in enzymatic activities: catalase (CAT) activity decreased by 41.3 % in liver and 2.0 % in gills, glutathione S-transferase (GST) by 13 % in liver and 27.7 % in gills, and reduced glutathione (GSH) by 12.7 % in liver and 14.7 % in gills, demonstrating a clear Pb-induced oxidative imbalance. The enzyme suppression corresponded with elevated Pb residues in tissues, reflecting compromised antioxidant defense mechanisms. These findings confirm that even low Pb exposure disrupts biochemical homeostasis and promotes oxidative stress in *O. niloticus*. The results emphasize the utility of oxidative biomarkers as sensitive indicators of heavy-metal pollution and highlight the urgent need for stricter monitoring of aquaculture and freshwater systems to mitigate potential ecological and public-health risks.

***Keywords*:** Antioxidant; Bioindicator; Bioaccumulation factor; Catalase; Glutathione S-transferase; Lead

1. Introduction

Lead (Pb) is a pervasive environmental contaminant of great concern in freshwater systems. As a_non-essential heavy metal, Pb is persistent, non-biodegradable, highly toxic even at low (0.025–0.100 mg/L) and exposure significantly disrupted enzymatic activities (acid amylase, protease, lipase, chymotrypsin) and impaired growth and metabolism in fish (Álvarez-González et al., 2020) It has been widely used in industry (e.g. batteries, paints, solder, electroplating, ammunition, and gasoline additives) (Velusamy et al., 2021), resulting in extensive anthropogenic release into water bodies. Pb ranks 82nd in atomic number and is naturally present in the earth's crust (though at low abundance) (Saha et al., 2024). Natural mobilization (e.g., volcanic emissions, soil and rock weathering, and atmospheric deposition) can also introduce Pb into freshwater (Jin et al., 2025). Notably, Pb's stability and tendency to bind to particulates cause it to accumulate in sediments and aquatic biota over time (Taslima et al., 2022). Studies from Dir, Shangla, and the Kurram River regions of Pakistan reported elevated heavy metal levels in water and sediments, indicating potential health and ecological risks through contamination and bioaccumulation pathways (Ali et al., 2018; Ilyas et al., 2017; Nawab et al., 2015)

Freshwater Pb contamination arises from multiple sources. Anthropogenic inputs – especially industrial and urban activities are dominant. Mining, smelting of ores, and metal processing release Pb into rivers, while Pb-acid battery production, waste recycling, and legacy leaded gasoline and paints contribute additional loads (Tang et al., 2023; World Health Organization, 2024). Agricultural runoff and untreated effluents from factories and landfills can introduce Pb into streams and lakes. On the other side, naturally occurring sources such as weathering of lead-bearing rocks and sediments, soil erosion, and volcanic ash also contribute to background Pb levels (Saha et al., 2024). Seasonal variations in runoff (e.g., high rainfall or flooding) could help to increase Pb flux from soils and the remobilization of contaminated sediments as well, leading to more metal moving up into the water column (Jin et al., 2025). Thus, both legacy pollution and ongoing human activities continually feed Pb into freshwater ecosystems.

Once in the water, Pb enters the aquatic food web through well-known mechanisms. Dissolved Pb and particulate-bound Pb can be taken up directly by plankton and aquatic plants, which are then consumed by higher trophic levels (Habib et al., 2023; Oros, 2025; Parolini et al., 2022). Pb readily adsorbs to fine sediments, which act as long-term reservoirs. Disturbance of these sediments (by floods, bioturbation, or dredging) can release Pb back into the water, where it becomes bioavailable to fish and invertebrates (Habib et al., 2023). Fish absorb waterborne Pb across gill membranes and ingest Pb via contaminated food or sediment particulates. Because Pb is not readily excreted, it bioaccumulates in fish tissues (e.g., liver, kidney, muscle) over time (Authman et al., 2015; Łuszczek-Trojnar et al., 2013; Spokas et al., 2006). This food chain biomagnification process is compromised by the fact that predators at higher trophic levels can be burdened with very high Pb doses. In fact, Pb levels in the organs of freshwater fish from polluted sites are frequently higher and may be indicative of a history or extent of environmental contamination.

Fish exposure to Pb delves into various physiological systems as it interferes with survival, growth, and reproduction, which in turn causes neuro-, endo-, and immunotoxicity leading to neurological, behavioral, and immunological disorders (Saha et al., 2024). The major pathway of Pb poisoning is oxidative stress, as through this mechanism Pb causes excessive production of ROS; reduces antioxidant defenses, and disrupts cell membranes and DNA (Wang et al., 2024). Fish exposed to Pb via chronic exposure show increased oxidative biomarkers (e.g., malondialdehyde) and reduced activities of antioxidant enzymes (superoxide dismutase, catalase) in gill, liver, and kidney tissues as well. Pb causes cells to spend energy on detoxification and makes less energy for growth and normal metabolism (Lee et al., 2019). In severe cases, Pb poisoning causes organ failure and death. Importantly, these toxic effects are not limited to fish – aquatic invertebrates and amphibians can similarly suffer genetic damage, impaired development, and mortality under high Pb loads (Botté et al., 2022; Mouchet et al., 2007). Because Pb is persistent and bioaccumulative, its entry into aquatic food webs produces cascading ecological and public-health effects: contaminated fish and shellfish act as dietary vectors of Pb, contributing to neurological, renal, and cardiovascular harms in humans (Habib et al., 2023). While chronic contamination degrades ecosystems, it reduces biodiversity and alters food-web structure. Field studies from Bangladesh, China, and elsewhere report elevated Pb in water and sediments often alongside other metals that exceed safety thresholds and raise carcinogenic and other health risks from fish consumption (Lipy et al., 2025). Pb is a probable human carcinogen linked to kidney damage, neurodevelopmental deficits, and reproductive harm, so no safe level is accepted in drinking water or seafood (World Health Organization, 2024).

Previous studies on lead in freshwater fish have focused on field surveys of tissue concentrations, controlled exposure experiments, biomarker assessments of oxidative damage, and trials of mitigation measures (El-Sappah et al., 2022; Kaya and Akbulut, 2015; Lee et al., 2019). This study differs by combining organ-specific Pb measurements (gill, liver, muscle, kidney) with antioxidant biomarkers in the same cohort, allowing direct comparison of tissue burdens and biochemical responses. By doing so, we test the hypothesis that Pb partitions differentially among organs and that edible muscle may indicate a greater consumer risk than liver or whole-body indices. The resulting organ-level baselines and biomarker profiles will inform more effective monitoring and aquaculture management strategies. This approach aims to examine how Pb is distributed in the fish body and its impact on antioxidant defenses. Tissue burdens of Pb and stress responses are compared to assess the value of these fish organs as bioindicators of Pb pollution. Eventually, such data will advance our knowledge of Pb dynamics in freshwater food webs and enhance monitoring efforts for the protection of aquatic life and human health.

2. Materials and Methods

2.1. Study Area and Fish Sampling

The present study was carried out at the Government Fisheries Farm, Islamabad, Pakistan. A total of 50 Nile tilapia (*Oreochromis niloticus*) specimens were obtained from the farm using standard fishing nets with the help of professional fishermen. Collected fish had an average body weight of 660–670 g and total body length of 36–39 cm (Fig. 1). Immediately after collection, fish were transported to the laboratory in ice boxes with sealed zip-lock plastic bags to ensure hygienic handling, minimize metabolic activity, and reduce chances of external contamination.

Upon arrival at the laboratory, fish were initially stunned in a 5% formalin solution to immobilize them, after which they were thoroughly rinsed with deionized/distilled water to remove adhering debris or contaminants. Morphometric data, including total length and body weight, were recorded prior to experimentation.

2.2. Experimental Design and Exposure Setup

To evaluate the toxicity potential of Pb, fish were allocated into five experimental groups, including four treatment groups exposed to increasing Pb concentrations and one control group without exposure. Each group was maintained in a separate glass aquarium containing water from the farm to avoid sudden osmotic shock. Juvenile *Oreochromis niloticus* were acclimated for 14 days and exposed for 28 days to five treatments in a semi-static system (50% renewal and re-spiking every 48 h). Each treatment had three replicate aquaria with 10 fish per aquarium (n = 30 per treatment).

Fig. 1. *Oreochromis niloticus* (Tilapia/Nile Tilapia).

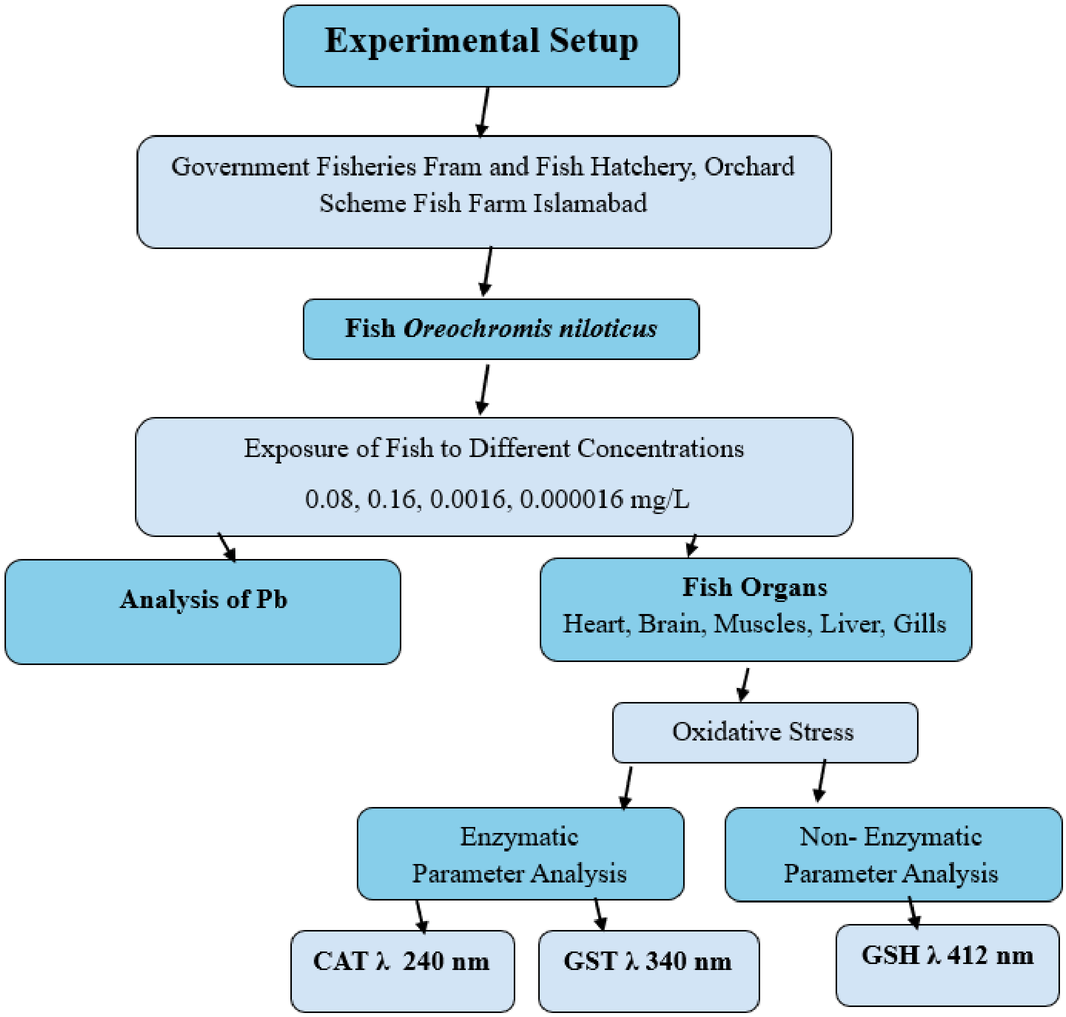

Fig. 2. Experimental Design for Pb Accumulation and Oxidative Stress Biomarker Analysis in Fish Organs

Pb was introduced into the experimental water using analytical-grade salts, and the fish were exposed to different nominal concentrations of the metal. The experimental setup (Fig. 2) consisted of four treatment groups and one control group. Group 1 was exposed to 0.08 mg/L Pb, Group 2 to 0.016 mg/L Pb, Group 3 to 0.0016 mg/L Pb, and Group 4 to 0.000016 mg/L Pb, while the control group was maintained without any Pb exposure (0.0 mg/L Pb). Concentrations (0.000016–0.08 mg/L) were chosen to span near-environmental to supra-environmental levels, bracketing the WHO provisional drinking-water guideline for Pb (0.01 mg/L) (World Health Organization, 2022).

Each treatment was performed in triplicate, and fish were acclimatized prior to, to reduce stress. Throughout the experimental duration, fish were maintained under standard laboratory conditions and monitored regularly. Temperature (25 °C, i.e., room temperature), pH (6.5) were measured daily, photoperiod was 12L:12D, fish were fed twice daily, and mortalities/abnormal behavior were recorded.

At the end of the exposure period, fish were humanely euthanized in accordance with OECD guidelines and the institutional ethical committee for animal experimentation, and dissection was performed using sterilized instruments (Fig. 3) to carefully excise the gills, liver, brain, muscle, and heart. Each tissue was collected in triplicate, rinsed thoroughly with deionized water, blotted with sterile filter paper to remove excess surface moisture, and immediately weighed to record fresh weight, which is shown in Table 1. The samples were then dried at a controlled temperature, homogenized into fine powder, and stored at −20 °C in sterile containers until further processing. For Pb quantification, 1.0 g of each dried tissue sample was transferred to a digestion vessel and treated with 10 mL of concentrated analytical-grade nitric acid (HNO_3). The digestion procedure was conducted in two phases, beginning with initial digestion by heating the samples at 90 °C for 45 minutes on a hot plate, followed by final digestion at 150 °C for 8 hours until a clear solution was obtained, indicating complete decomposition of organic matter. After cooling, the digested samples were diluted to a final volume of 25 mL with 2.5% HNO_3, filtered through Whatman No. 42 filter paper to remove any particulate matter, and subsequently stored at 4 °C in amber bottles until instrumental analysis. Blank samples and duplicates were processed alongside the tissues to ensure quality control of the analytical procedure.

Table 1: Mean Fresh Weight and Dry Weight of Fish Organs in grams (g)

Fish Organs	Mean Fresh Weight (g)	Mean Dry Weight (g)
Brain	1.68	1.092
Liver	1.403	0.833
Heart	1.196	1.037
Gills	1.966	1.05

Fig. 3. Dissection of Tilapia

2.3. Pb Quantification by Atomic Absorption Spectrophotometry

Pb concentrations in the digested tissue samples were measured with a Flame Atomic Absorption Spectrophotometer (Shimadzu AA-7000, Japan) at the Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi. The instrument was initially calibrated for Pb by analyzing a set of standard solutions prepared using certified reference material to confirm the accuracy and reliability of the measurements. A calibration curve was taken in the concentration range of expected Pb levels in the samples for each analysis run, and linearity ($R^2 \geq 0.999$) was checked before we analyzed the samples. Strict quality control measures were applied during the whole process and included analysis of procedural blanks to exclude contamination, replicate samplings to test reliability, and spike recovery experiments to validate procedures (accuracy/efficiency) in Pb recoveries from the biological matrix. The detection and quantification limits were established based on the standard deviation of the blanks and instrument sensitivity. Final concentrations of Pb in the tissues were calculated and expressed on a dry weight basis as milligrams of Pb per kilogram of tissue (mg/kg dry weight).

2.4. Metal Pollution Assessment

The gradation of heavy metal Pb accretion was assessed by defining the bioaccumulation factor/transfer factor and metal pollution index of the tissues of fish to get a brief idea of the effects of Pb concentrations on fish tissues.

The estimation of the bioaccumulation factor (BAF) was approved by utilizing the proportion of the absorption of Pb metal found in different fish tissues (expressed in mg/Kg dry weight) (Dwivedi et al., 2015; Monikh et al., 2015). The equation (1) is the formulation to compute BAF:

$$BAF = \frac{\textit{Metal Conc.in dry Fish Tissue } (mg/Kg)}{\textit{Conc.of Same Metal in Site Water } (mg/L)} \quad \text{………….. (1).}$$

The tissues of the collected fish specimens were assessed through the collective procedures of the metal pollution index (MPI). The concentration of Pb was calculated according to the protocols of (Usero et al., 1997). MPI is well-defined as the nth root of the reproductions of contamination factor of heavy metals and is calculated by the consequent equation (2):

$$\boldsymbol{MPI = (Cf_1 * Cf_2 * Cf_n)\ 1/n \ldots.. (2)}$$

Cf_1, Cf_2, up to Cf_n are the absorptions of metallic elements n in the sample (Abdel-Satar et al., 2017; Hassaan et al., 2016). The ratio of the calculated concentrations of the natural profusion of an assumed metal can be projected as the contamination factor (CF), which is categorized into four levels for measuring the enhancement of a solitary metal contaminant in fish for a certain period (Voigt et al., 2015).

2.5. Oxidative Stress Biomarker Analysis

To investigate oxidative stress induced by Pb exposure, both enzymatic and non-enzymatic antioxidant markers were quantified in the dissected tissues of exposed and control fish.

For sample preparation, freshly excised tissues were immediately homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4) using a pre-chilled homogenizer to preserve enzymatic activity and minimize oxidative degradation during processing. The homogenates were centrifuged at 1000 rpm for 10 min at 4°C to remove any cellular debris, and the post-mitochondrial supernatant (PMS) was collected. This PMS fraction was employed for further biochemical analyses, since it is the soluble portion of the tissue that comprises antioxidant enzymes and low molecular weight antioxidants.

Catalase (CAT) activity was assayed by spectrophotometric measurement at 240 nm based on the decomposition of hydrogen peroxide (H_2O_2) with a UV–Visible spectrometer. Read absorbance at 30-sec intervals, poised over 3 min. Enzymatic activity was measured by using an extinction coefficient of 3.94 mM/cm and expressed as units/mg protein (U/mg protein), where one unit corresponds to the conversion of 1 μ/mol of H_2O_2 per minute under assay conditions.

Glutathione S-transferase (GST) activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The PMS was incubated in phosphate buffer containing 30 mM 1-chloro-2,4-dinitrobenzene (CDNB; 30 mmol/L), and the conjugation reaction was followed spectrophotometrically at 340 nm. Absorbance readings were taken every 30 seconds for 3 minutes. GST activity was expressed as U/mg protein, using the extinction coefficient of 9.6 mM/cm, where one unit corresponds to the formation of 1 μmol of CDNB-GSH conjugate per minute.

Reduced glutathione (GSH) levels were quantified. Aliquots of tissue homogenates were treated with 4% sulfosalicylic acid to precipitate proteins, followed by centrifugation to obtain the supernatant. This supernatant was reacted with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) reagent, which forms a yellow-colored chromophore (TNB) upon reaction with GSH. Absorbance was measured at 412 nm, and GSH concentration was calculated using an extinction coefficient of 14.15 mM/cm, with results expressed as μ/mol GSH per gram of tissue.

The protein content in fish tissues was determined based on the absorbance at 260 nm and 280 nm. This operation consisted in three steps: the activity of the enzyme was expressed in units per milliliter by applying the corresponding assay formula (3); then, spectrophotometric measurement and determination of protein concentration (Eq. 4) (mg/mL) were performed according to the 280-absorbance method; the enzymatic activity was finally standardized as units/mg protein.

$$\boldsymbol{Unit/ml\,enzyme\ (nanomoles\ of\ enzyme\ present\ per\ gram\ of\ sample\ tissue} =$$

$$\frac{(\Delta\, OD/min\ Sample - \Delta\, OD/min\ Blank)*1}{Coefficient*\frac{total\ reaction\ volumn}{volumn\ of\ enzyme\ taken}} * \frac{Total\ volumn\ of\ enzyme\ extract}{Fresh\ weight\ of\ tissues\ (g)} * Total\ Protein * 100 \ldots\ldots\ldots\ldots \quad \mathbf{(3)}$$

$$\boldsymbol{Unit/mg\ \ Protein} = \frac{Unit/ml\ enzyme}{mg\ Protein/ml\ enzyme} \ldots\ldots\ldots\ldots \quad \mathbf{(4)}$$

This method relies on the ultraviolet absorbance of aromatic amino acids present in proteins, allowing rapid and non-destructive estimation without the need for additional reagents. The protein concentration was calculated using the standard Christian and Warburg equation (5), where the constants 1.55 and 0.76 represent correction factors specific to protein absorbance at the respective wavelengths.

$$\boldsymbol{Protein\ Concentration\ mg/ml} = 1.55 * A280 - 0.76 * A260 \ldots\ldots\ldots\ldots \quad \mathbf{(5)}$$

After the above analysis measures, the specimens of the collected fish samples were equipped in the research laboratory of Bahria University, Islamabad. The samples of the fish specimens were prepared by following the standard operating procedures (SOPs) in the research laboratory. The spectrophotometry for determining the oxidative stress examination of GSH, CAT, and GST activities in the fish specimen was performed by Ultraviolet UV) Spectrophotometer. The readings from the UV spectrophotometry were noted for a time duration of 3 minutes with an intermission of every 30 seconds at the optical densities of 240nm (for CAT Activity, 340nm (for GST), and 412nm (For GSH), correspondingly.

2.6. Comparison of the Results with the WHO permissible limits of Pb

WHO (2011) has recommended the allowable limits of all heavy metals, specifically Pb, to be accumulated by fish species in the aquatic environment. In this context, after analyzing these activities and attaining the readings, the readings were then compared with the WHO (2011) guidelines for the accumulation of Pb by the fish to analyze the amount of Pb in the body structures of *Oreochromis niloticus.*

3. Results

The analysis and concentration of heavy metals such as Pb were recognized in the brain, muscles, liver, kidneys, and gills of the fish *Oreochromis niloticus* (Tilapia/Nile Tilapia) that was gathered from the Government Fisheries Farm, i.e., Fish Hatchery, Orchard Scheme Fish Farm, Islamabad. The analysis was conducted by exposing the organs of fish to different concentrations of Pb, as discussed later. After the analysis of Pb in fish organs, the results were then compared with the permissible limits as the WHO guidelines (2011) of the acceptable absorption of Pb in the edible organs of the fish.

Table 2: Taxonomical Classification of *Oreochromis niloticus*

Kingdom	**Animalia**
Phylum	Chordata
Class	Actinopterygii
Order	Cichliformes
Genus	*Oreochromis*
Species	*Niloticus*
Binomial name	*Oreochromis niloticus*
Common name	Tilapia/Nile Tilapia

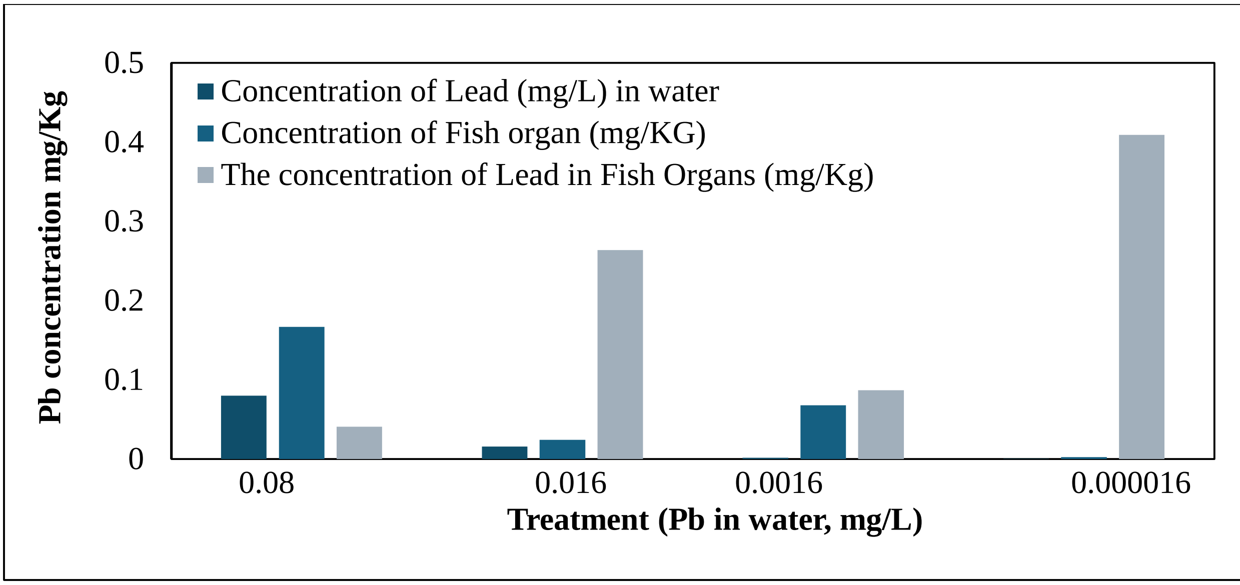

Fig. 4. Lead concentration in the heart tissue of *Oreochromis niloticus*. Pb (mg/kg dry weight) (n = 6). Statistical differences were tested by one-way ANOVA with Tukey's post hoc test ($p < 0.05$).

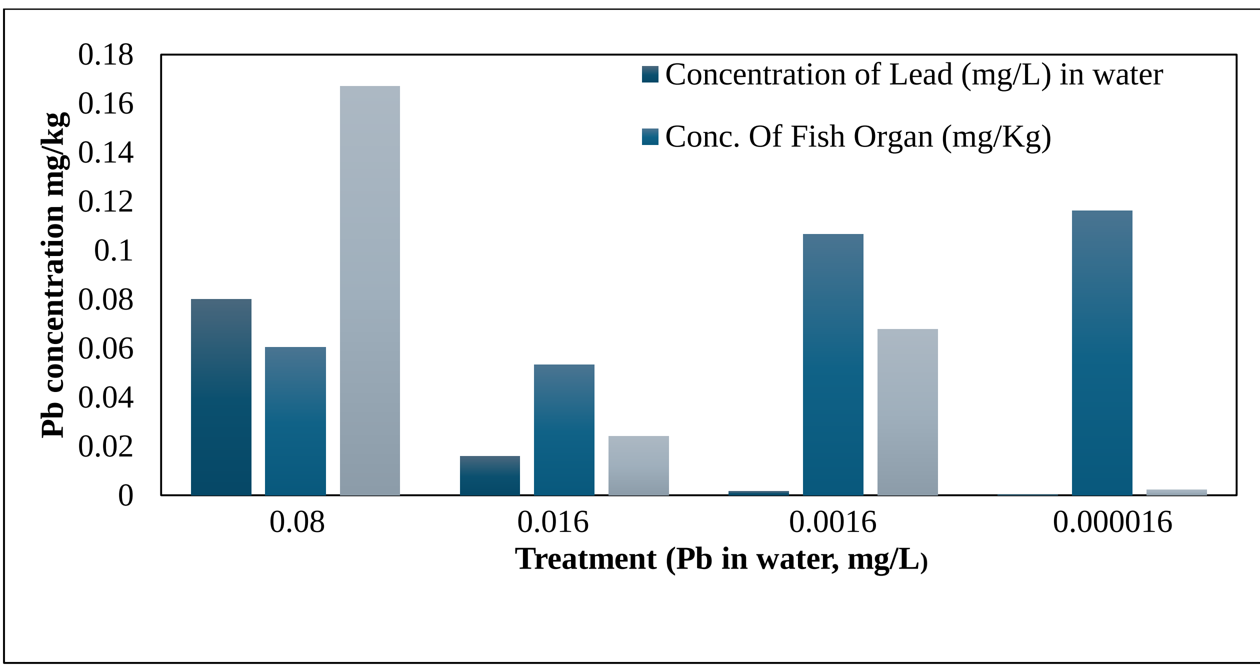

Fig. 5. Lead concentration in the brain tissue of *Oreochromis niloticus*. Pb (mg/kg dry weight) (n = 6). Statistical differences were tested by one-way ANOVA with Tukey's post hoc test ($p < 0.05$).

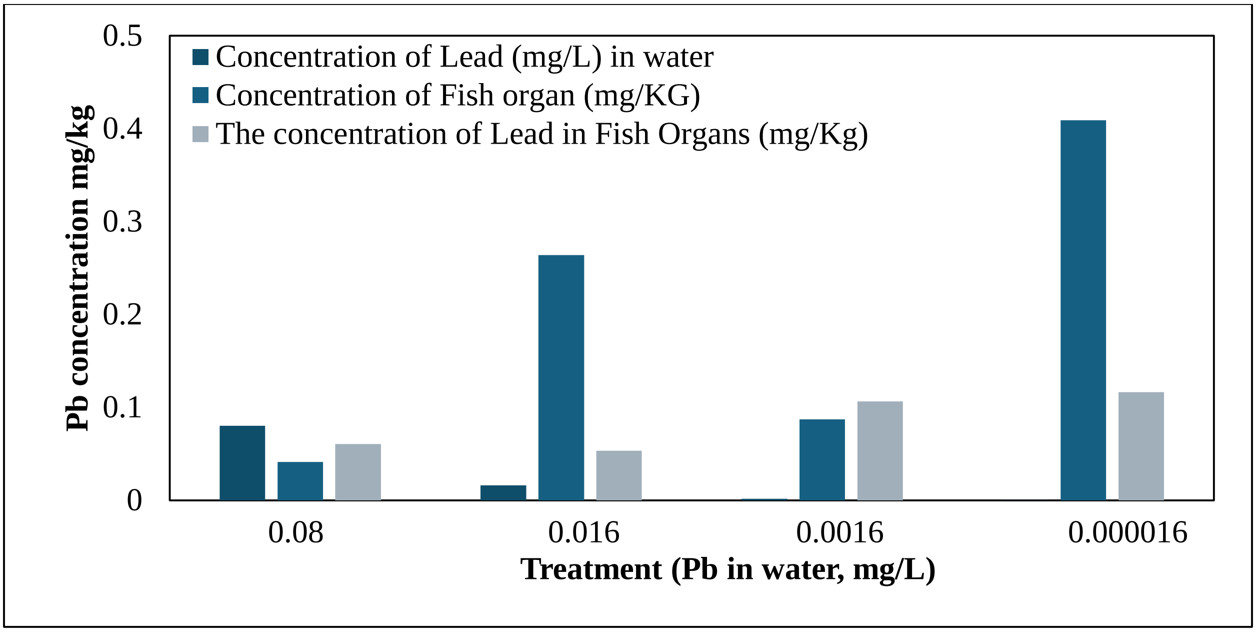

Fig. 6. Lead concentration in the muscle tissue of *Oreochromis niloticus*. Pb (mg/kg dry weight), (n = 6). Statistical differences were tested by one-way ANOVA with Tukey's post hoc test ($p < 0.05$).

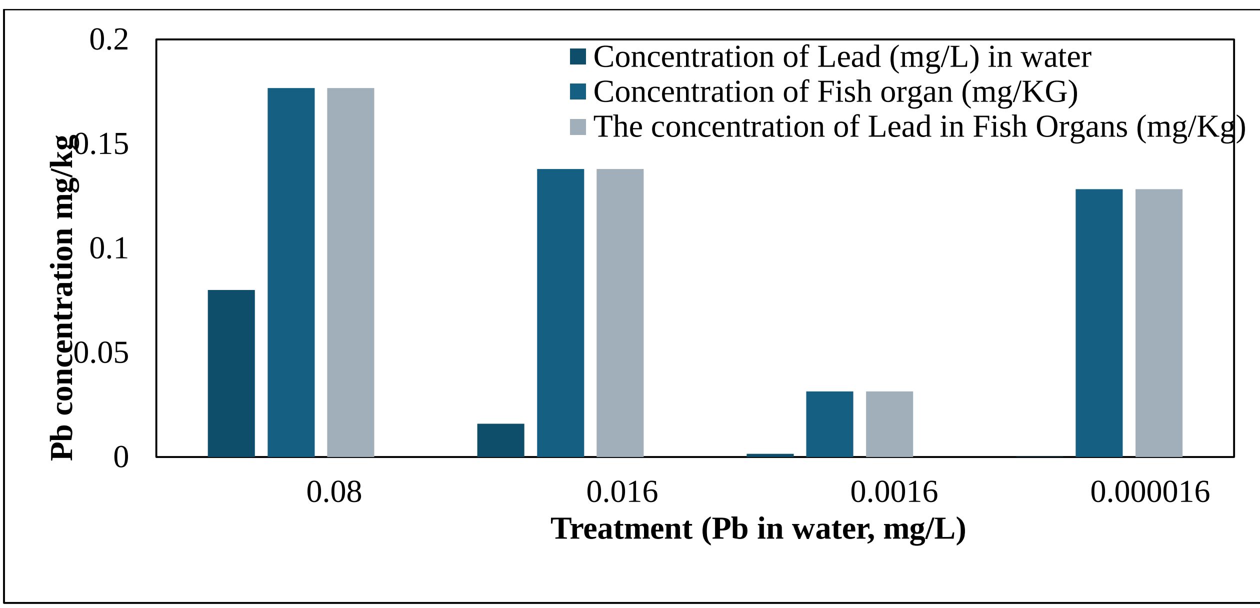

Fig. 7. Lead concentration in the liver tissue of *Oreochromis niloticus*. Pb (mg/kg dry weight), (n = 6). Statistical differences were tested by one-way ANOVA with Tukey's post hoc test ($p < 0.05$).

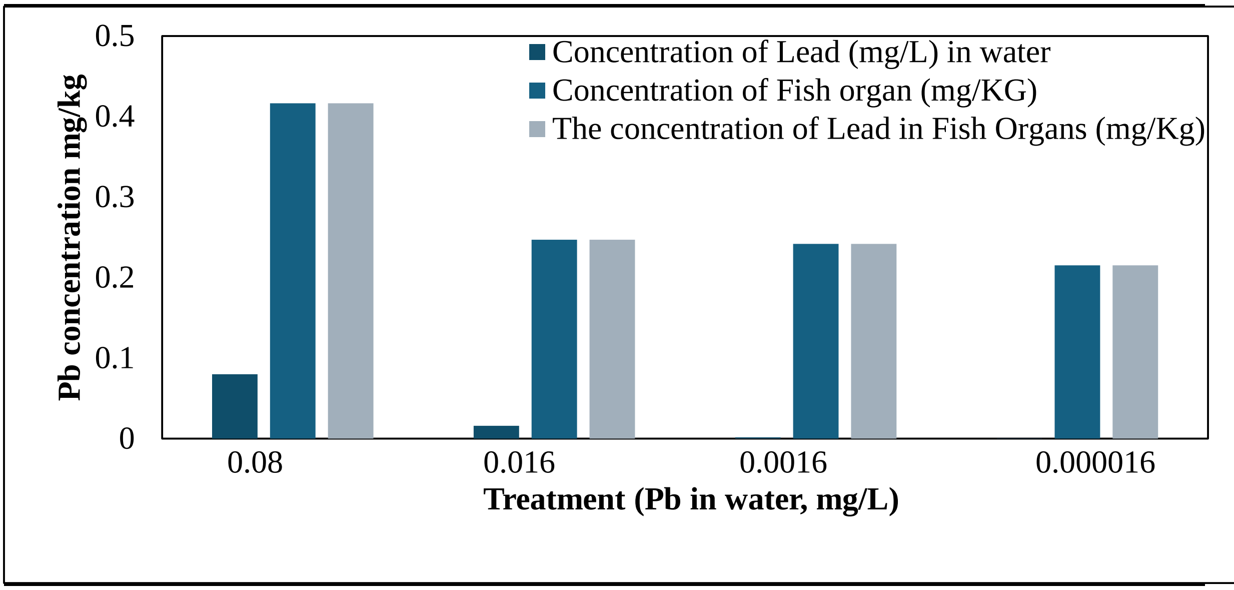

Fig. 8. Lead concentration in the gill tissue of *Oreochromis niloticus*. Pb (mg/kg dry weight). (n = 6). Statistical differences were tested by one-way ANOVA with Tukey's post hoc test ($p < 0.05$).

3.1. Distribution of Pb Concentrations in Fish Organs (mg/Kg)

Pb was detected in all examined tissues (heart, brain, muscle, liver, and gills; Figs. 4–8), but its accumulation was organ-specific and did not follow a simple exposure–response pattern. The maximum concentrations of Pb measured in each tissue were: muscle: 0.4091 mg/kg, gills: 0.2469 mg/kg, liver: 0.1767 mg/kg, heart: 0.1670 mg/kg, and brain: 0.1162 mg/kg.

3.2. Bioaccumulation Factor

Analysis of the bioaccumulation factor (BAF) in different organs of *Oreochromis niloticus* (Table 3) revealed the highest concentration of Pb in the muscles (1.946 mg/kg at 0.08 mg/L), followed by the brain (1.322 mg/kg at 0.08 mg/L), heart (0.661 and 0.479 mg/kg at 0.016 mg/L), liver (0.452 mg/kg at 0.08 mg/L), and gills (0.192 mg/kg at 0.08 mg/L). These findings indicate significant Pb accumulation in vital tissues, which may disrupt metabolic functions in fish and pose health risks to humans through fish consumption. Overall, the order of Pb bioaccumulation was: muscles > brain > heart > liver > gills, and results showed a direct relationship between Pb concentration and BAF.

3.3. Metal Pollution Index

The metal load supports the analysis of oxidative stress regarding metal pollution. From the results mentioned above in the present study (Table 4), it could be determined that the absorption of the metal pollution index is low in the organs of *Oreochromis niloticus,* as per the classification of the Contamination factor by (Ali et al., 2016), because the concentration of Pb is lower than 1, which indicates the presence of Pb in low amounts in the organs of fish. Further, the presence of Pb in the organs of fish could be elucidated in the Gills, Muscles, Liver, Brain, and Heart. However, from the results of BAF in the organs of *Oreochromis niloticus*, the absorption of metals was highest in the muscles and lowest in the gills. But the results of MPI indicate that the presence of Pb is most increased in the gills, i.e., 0.001339456 mg/Kg, and lowest in the heart, i.e., 0.000000164 mg/Kg.

Table 3: Bioaccumulation Factor in Different Organs of *Oreochromis niloticus.*

Name of Fish Organs	Concentration of Pb (mg/L) in water	Conc. Of Fish Organ (mg/Kg)	WHO permissible Limit of Pb in Fish Organs (mg/Kg)	BAF (mg/Kg)
Brain	0.08	0.060	0.3	1.32231
	0.016	0.053	0.3	0.30018
	0.0016	0.106	0.3	0.01502
	0.000016	0.116	0.3	0.00013
Liver	0.08	0.176	0.3	0.45274
	0.016	0.138	0.3	0.1159
	0.0016	0.031	0.3	0.05079
	0.000016	0.128	0.3	0.00012
Heart	0.08	0.167	0.3	0.47904
	0.016	0.024	0.3	0.66115
	0.0016	0.067	0.3	0.02359
	0.000016	0.002	0.3	0.00666
Muscles	0.08	0.041	0.3	1.94647
	0.016	0.263	0.3	0.06065
	0.0016	0.087	0.3	0.01836
	0.000016	0.409	0.3	0.00003
Gills	0.08	0.416	0.3	0.19216
	0.016	0.246	0.3	0.06480
	0.0016	0.242	0.3	0.00661
	0.000016	0.215	0.3	0.00007

Table 4: Metal Pollution Index (MPI) in Different Organs of *Oreochromis niloticus*

Organs	Metal Pollution Index (MPI)	Degree of Concentration
Brain	0.000009976	Low
Liver	0.000024637	Low
Gills	0.001339456	Low
Muscles	0.000096583	Low
Heart	0.000000164	Low

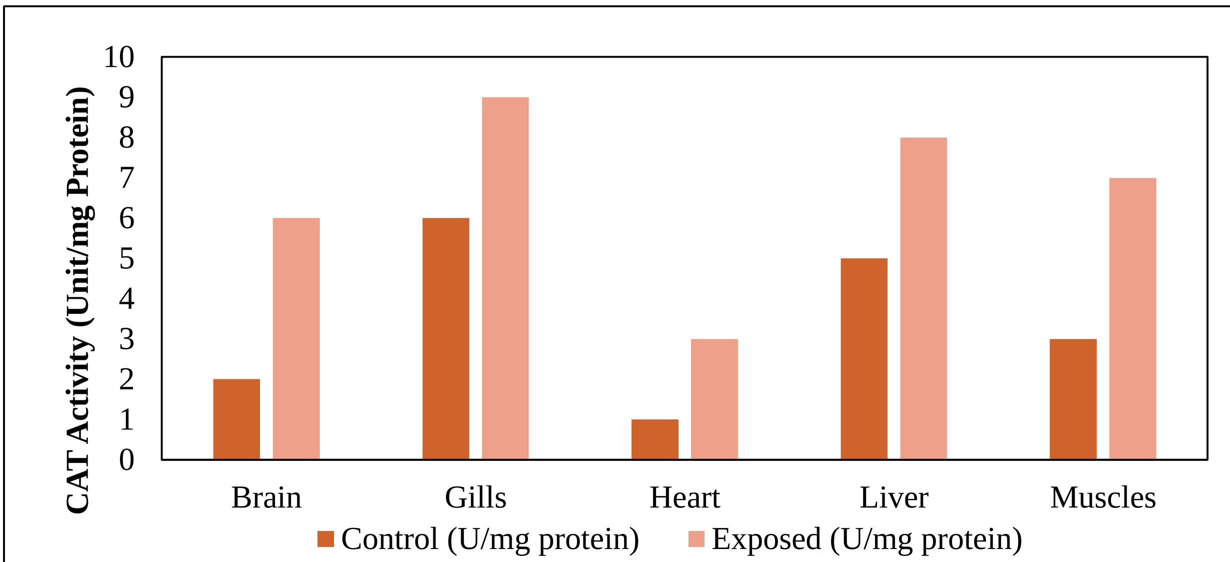

Fig. 9. Catalase (CAT) activity in different organs of *Oreochromis niloticus* under control and Pb-exposed conditions. (CAT catalyzes decomposition of H_2O_2 to H_2O and O_2, protecting cells from peroxide-induced oxidative damage.) (Activity: U/mg protein; mean ± SD, n = 6).

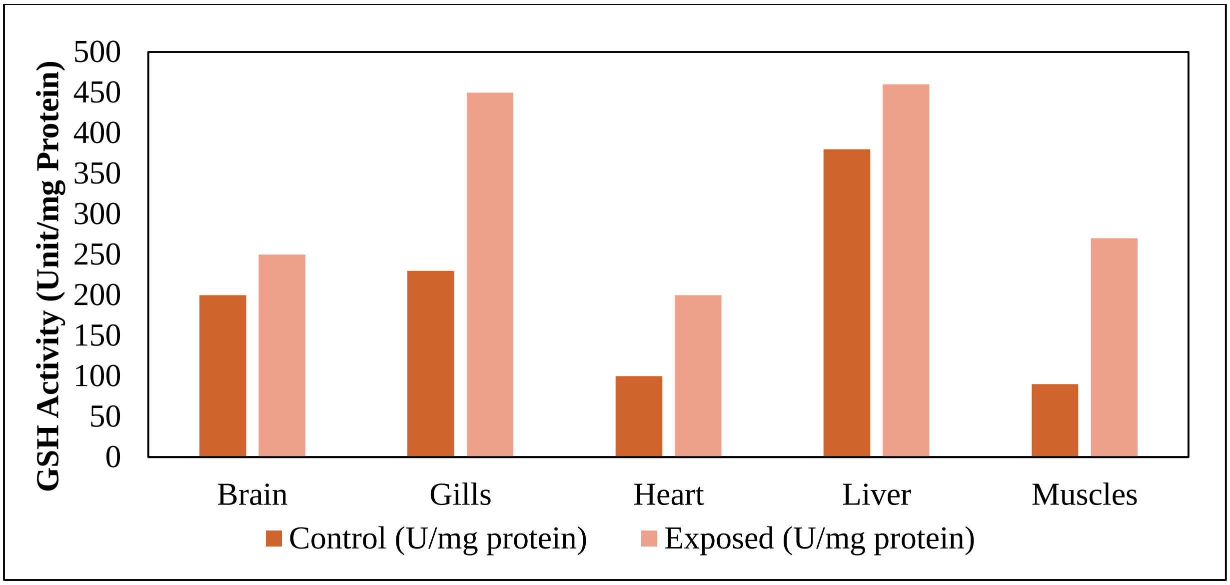

Fig. 10. Glutathione (GSH) activity in different organs of *Oreochromis niloticus* under control and Pb-exposed conditions (GSH is the primary intracellular thiol antioxidant and substrate for detoxifying enzymes, buffering cellular redox state.) (Level: U/mg protein; mean ± SD, n = 6).

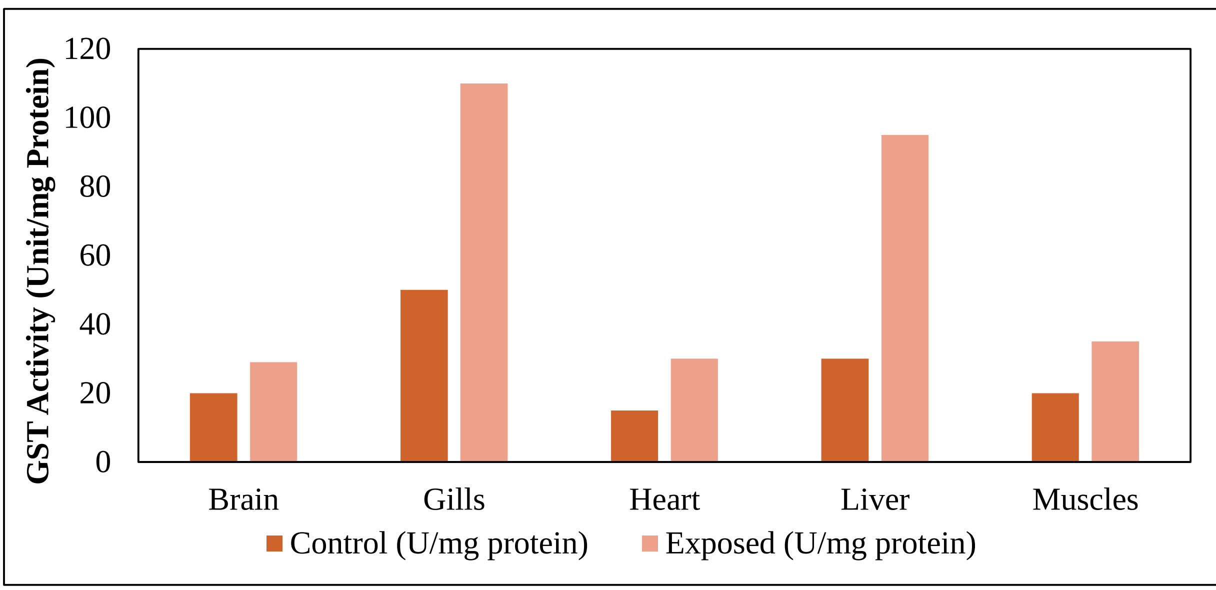

Fig. 11. Glutathione S-transferase (GST) activity in different organs of *Oreochromis niloticus* under control and Pb-exposed conditions (GST catalyzes conjugation of reduced glutathione to xenobiotics and lipid-peroxidation products, facilitating detoxification). (Activity: U/mg protein; mean ± SD, n = 6).

4. Discussion

Although the gills and liver are typically considered primary accumulation sites due to their direct exposure to the aquatic environment and roles in uptake and detoxification, the highest concentration in this study occurred in muscle tissue. Different studies show variation in Pb accumulation sites in *Oreochromis niloticus*. Kaoud and El-Dahshan (2010) reported the highest levels in the liver, while Ishak et al. (2020) found maximum accumulation in the gills. In contrast, Abdel-Mohsien and Mahmoud (2015) observed the highest Pb levels in the muscles of fish from Egyptian freshwater, differing from the present study's findings

The absence of a monotonic increase in tissue Pb with nominal exposure (for example, the largest muscle value occurring at a low nominal concentration) likely reflects a combination of biological and methodological factors rather than a simple analytical artefact. Possible explanations include nonlinear uptake and elimination kinetics, temporal differences in accumulation and depuration among organs, inter-individual variability (age, size, condition, or feeding behavior), and sampling or labeling inconsistencies in the dataset. These processes can cause peak residues to appear at intermediate or low nominal exposures, depending on exposure duration and timing relative to sampling.

Figures 4-8 show that Pb accumulation was highest in the muscles and gills of fish from the Islamabad Fish Hatchery, with the order of accumulation being muscle > gills > liver > heart > brain. In natural environments, fish absorb more Pb than under controlled conditions, posing serious health risks. Similarly, a researcher found that Pb exposure increased its concentration in multiple organs and tissues of *Oreochromis niloticus,* impairing enzyme activity and nerve conductivity. According to Álvarez-González that low concentrations of Pb, such as "0.025 and 0.100 mg/L, were sufficient to disturb the actions of acid amylases, proteases, lipases, and chymotrypsin. Further, when the enzyme activities are disturbed, the growth pattern of the fish is also disturbed (Álvarez-González et al., 2020).

Variable accumulation in a situation-dependent manner has been described in previous studies. For example, Pb accumulation in the liver and gills was reported to be higher by Lee and colleagues (Lee et al., 2019), while Ishak et al. (2020) found elevated levels in the kidney and liver. On the other hand, Oyeleke obtained similar findings to what we observed with respect to Pb being concentrated in muscles and liver. They observed higher accumulation of Pb in the liver, which was associated with an increase in glucose levels, oxidative stress, and decreased liver lipid-protein-moisture profile, resulting in hyperglycemia, intimating that metabolism is disrupted in fish (Oyeleke et al., 2018).

These findings highlight that Pb and other heavy metals can disturb aquatic ecological balance in rivers, lakes, and streams due to their persistence and tendency to biomagnify in the food chain (Paul and Sengupta, 2013). Pb accumulation in fish organs not only induces oxidative stress and metabolic disorders but also renders fish consumption unsafe for humans. To comprehensively evaluate the toxicological burden, the Metal Pollution Index (MPI) can be applied to summarize overall Pb accumulation across fish tissues.

Although the overall MPI indicates a low degree of contamination, organ-specific BAF reveals meaningful Pb burdens in key tissues, notably muscle and gill. Low MPI, therefore, does not preclude biological effect: even modest whole-body pollution can concentrate in particular organs and elicit oxidative stress. In our study, the elevated BAF in muscle (edible tissue) and gill was accompanied by altered CAT, GST, and GSH, showing that tissue-level accumulation, not MPI alone, best predicts biochemical disturbance and food-safety risk. Hence, MPI should be interpreted alongside organ-specific BAF and biomarker data when assessing environmental and human-health relevance.

Figures 9–11 show coordinated, organ-specific disturbances of antioxidant defenses in *Oreochromis niloticus* associated with Pb bioaccumulation. Measured Pb concentrations were highest in the gills and liver, which is consistent with many field and laboratory reports that identify gills (direct water contact, thin epithelium) and liver (detoxification and storage) as primary sink tissues for waterborne metals (El-Moselhy et al., 2014). At the biochemical level, Pb exposure produced a pattern typical of metal-induced oxidative stress: a marked induction of GSH activity in some tissues (Fig. 10) reflecting activation of thiol-based detoxification pathways, but signs of hepatic GSH depletion consistent with rapid utilization under sustained reactive oxygen species (ROS) production. Glutathione (GSH) participates both in direct radical scavenging and as a substrate for conjugation reactions, so an initial increase followed by tissue-specific depletion is a commonly observed response to metal stress (Lushchak, 2012).

Glutathione S-transferase (GST) activity (Fig. 11) was altered in a tissue-dependent way, with reductions in the gills that likely reflect enzyme inactivation and lipid peroxidation at the site of direct metal uptake; GST changes therefore act as sensitive indicators of cellular oxidative damage and impaired phase II detoxification. Concurrently, catalase (CAT) activity (Fig. 9) was depressed in the organs examined, indicating impaired H_2O_2 breakdown and an overwhelmed enzymatic defence against peroxide-derived ROS. The combined pattern elevated GSH turnover, disrupted GST activity, and suppressed CAT is consistent with Pb-driven overproduction of ROS/H_2O_2 and failure of antioxidant systems to fully neutralize oxidative insults, which can promote protein damage, lipid peroxidation, and downstream physiological impairment (Srikanth et al., 2013).

Pb exposure promotes ROS generation through Fenton-like reactions and disruption of redox homeostasis, leading to lipid peroxidation and enzyme inhibition. In *O. niloticus*, CAT activity increased markedly in the gills (from 6.0 ± 0.4 to 9.0 ± 0.6 U/mg protein) and muscles (from 3.0 ± 0.3 to 7.0 ± 0.5 U/mg protein), while GSH levels rose sharply in the gills (230 ± 20 to 450 ± 25 U mg^{-1} protein) and liver (380 ± 22 to 460 ± 28 U/mg protein). GST activity also showed notable elevation, especially in gills (50 ± 4 to 110 ± 6 U/mg protein) and liver (30 ± 3 to 95 ± 5 U/mg protein). These changes indicate an initial compensatory antioxidant response followed by possible enzyme inhibition at higher Pb exposure. Similar ROS-mediated disruptions have been reported under Cd and Cu toxicity in fish, confirming that Pb triggers comparable oxidative-damage pathways and impairs membrane stability and metabolic efficiency.

Our results demonstrate organ-specific Pb accumulation and related oxidative stress, underscoring the novelty and practical relevance of this study. The highest Pb level was found in muscle (0.4091 $mg \cdot kg^{-1}$), followed by gill, liver, heart, and brain, contrasting with reports that identify liver and gill as primary metal sinks (Abdel-Moneim et al., 2016). This muscle-dominant accumulation highlights a greater food-safety risk, as edible tissue contained the highest Pb burden. Although our biomarker patterns (altered CAT, GST, and GSH) align with Abdel-Moneim et al. (2016) in indicating Pb-induced oxidative imbalance, the tissue distribution differs, suggesting that Pb may persist in muscle even when hepatic detoxification occurs. Zhai and colleagues showed that probiotics can mitigate Pb uptake, supporting two complementary management paths: reducing environmental Pb inputs and applying biological interventions (Zhai et al., 2017). Together, our findings provide baseline organ-specific Pb levels and antioxidant responses useful for aquaculture risk assessment and for developing mitigation strategies.

5. Conclusions

1. This study demonstrated the toxic potential of Pb in *Oreochromis niloticus*, highlighting significant bioaccumulation and oxidative stress responses.
2. Pb accumulation in fish organs followed the order muscle > gills > liver > heart > brain, with the highest concentration detected in muscles (1.946 mg/kg at 0.08 mg/L).
3. The metal pollution index indicated that, despite low absorption levels, Pb presence was sufficient to disrupt metabolic and physiological processes.
4. Concentrations in fish organs exceeded the WHO permissible limits, confirming advanced accumulation.
5. Notably, exposed fish showed higher Pb concentrations in gills and muscles compared to controls.

6. Antioxidant responses revealed inhibition of CAT (41.3% in liver, 2.0% in gills), GST (13% in liver, 27.7% in gills), and GSH (12.7% in liver, 14.7% in gills), suggesting oxidative stress as a key toxicological effect.
7. Overall, these findings indicate that the Orchard Scheme Fish Farm, Islamabad, is contaminated with Pb, likely from sewage discharge and industrial activities, posing risks to fish health and reflecting broader environmental concerns for aquatic ecosystems.
8. Pb exposure in *O. niloticus* caused organ-specific accumulation and oxidative stress, with the highest level in muscle, indicating food-safety concerns. These findings stress the need to monitor edible tissues and antioxidant biomarkers for accurate risk assessment and to improve aquaculture management and environmental monitoring practices.

Acknowledgements

The authors are thankful to the Environmental Science lab, Bahria University, Islamabad, for laboratory support.

Author's Contribution

AA conducted experiments, AJ conceptualized and supervised the research, RK performed data analysis and wrote up, SR edited and finalized the manuscript

Funding

No specific funding was available from any source.

Conflict of Interest

The authors declare no conflict of interest

References

Abdel-Mohsien, H. S., & Mahmoud, M. A. (2015). Accumulation of some heavy metals in Oreochromis niloticus from the Nile in Egypt: potential hazards to fish and consumers. *Journal of Environmental Protection*, *6*(09), 1003.

Abdel-Moneim, A. M., Essawy, A. E., El-Din, N. K. B., & El-Naggar, N. M. (2016). Biochemical and histopathological changes in liver of the Nile tilapia from Egyptian polluted lakes. *Toxicology and industrial health*, *32*(3), 457-467.

Abdel-Satar, A. M., Ali, M. H., & Goher, M. E. (2017). Indices of water quality and metal pollution of Nile River, Egypt. *Egyptian Journal of Aquatic Research*, *43*(1), 21-29. https://doi.org/https://doi.org/10.1016/j.ejar.2016.12.006

Ali, J., Khan, M. A., Nazneen, S., Muhammad, J., Nasir, M. J., Shah, M. T., & Khan, S. (2018). Assessment of heavy metals and physico-chemical characteristics of water and sediments, Kurram River (Pakistan). *Journal of Himalayan Earth Sciences*, *51*(1), 113-126.

Ali, M. M., Ali, M. L., Islam, M. S., & Rahman, M. Z. (2016). Preliminary assessment of heavy metals in water and sediment of Karnaphuli River, Bangladesh. *Environmental nanotechnology, monitoring & management*, *5*, 27-35. https://doi.org/https://doi.org/10.1016/j.enmm.2016.01.002

Álvarez-González, C., Martínez-Sánchez, L., Peña-Marín, E., Guerrero-Zárate, R., Jesús-Ramírez, F., Morales-García, V., Uribe-López, M., & Núñez-Nogueira, G. (2020). Effects on the growth and digestive enzyme activity in Nile tilapia fry (Oreochromis niloticus) by lead exposure. *Water, Air, & Soil Pollution*, *231*(9), 477.

Authman, M., 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*(4), 1-13. https://doi.org/http://dx.doi.org/10.4172/2155-9546.1000328

Botté, A., Seguin, C., Nahrgang, J., Zaidi, M., Guery, J., & Leignel, V. (2022). Lead in the marine environment: concentrations and effects on invertebrates. *Ecotoxicology*, *31*(2), 194-207.

Dwivedi, A. C., Tiwari, A., & Mayank, P. (2015). Seasonal determination of heavy metals in muscle, gill, and liver tissues of Nile tilapia, Oreochromis niloticus (Linnaeus, 1758) from the tributary of the

Ganga River, India. *Zoology and Ecology*, *25*(2), 166-171.

El-Moselhy, K. M., Othman, A., Abd El-Azem, H., & El-Metwally, M. (2014). Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. *Egyptian journal of basic and applied sciences*, *1*(2), 97-105.

El-Sappah, A. H., Seif, M. M., Abdel-Kader, H. H., Soaud, S. A., Elhamid, M. A. A., Abdelghaffar, A. M., El-Sappah, H. H., Sarwar, H., Yadav, V., & Maitra, P. (2022). Genotoxicity and trace elements contents analysis in Nile Tilapia (Oreochromis niloticus) indicated the levels of aquatic contamination at three Egyptian areas. *Frontiers in Veterinary Science*, *9*, 818866.

Habib, S. S., Batool, A. I., Rehman, M. F. U., & Naz, S. (2023). Assessment and bioaccumulation of heavy metals in fish feeds, water, and some tissues of Cyprinus carpio cultured in different environments (Biofloc Technology and Earthen Pond System). *Biological Trace Element Research*, *201*(7), 3474-3486. https://doi.org/https://doi.org/10.1007/s12011-022-03415-z

Hassaan, M. A., El Nemr, A., & Madkour, F. F. (2016). Environmental assessment of heavy metal pollution and human health risk. *American Journal of Water Science and Engineering*, *2*(3), 14-19.

Ilyas, M., Khan, S., Khan, A., Amin, R., Khan, A., & Aamir, M. (2017). Analysis of drinking water quality and health risk assessment-A case study of Dir, Pakistan. *Journal of Himalayan Earth Sciences*, *50*(1A), 100-110.

Ishak, A. R., Zuhdi, M. S. M., & Aziz, M. Y. (2020). Determination of lead and cadmium in tilapia fish (Oreochromis niloticus) from selected areas in Kuala Lumpur. *Egyptian Journal of Aquatic Research*, *46*(3), 221-225.

Jin, X., Wu, Q., Peñuelas, J., Sardan, J., Peng, Y., Li, Z., Peng, X., Heděnec, P., Yang, Q., & Yuan, C. (2025). Climate and anthropogenic activities control the concentrations of copper, zinc, cadmium, and chromium in global inland waters. *Communications Earth & Environment*, *6*(1), 520. https://doi.org/https://doi.org/10.1038/s43247-025-02508-6

Kaoud, H., & El-Dahshan, A. (2010). Bioaccumulation and histopathological alterations of the heavy metals in Oreochromis niloticus fish. *Nature and science*, *8*(4), 147-156.

Kaya, H., & Akbulut, M. (2015). Effects of waterborne lead exposure in Mozambique tilapia: oxidative stress, osmoregulatory responses, and tissue accumulation. *Journal of Aquatic Animal Health*, *27*(2), 77-87.

Lee, J.-W., Choi, H., Hwang, U.-K., Kang, J.-C., Kang, Y. J., Kim, K. I., & Kim, J.-H. (2019). Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. *Environmental toxicology and pharmacology*, *68*, 101-108.

Lipy, E. P., Rahman, M. T., Islam, S., Raknuzzaman, M., Islam, M. M., Akhter, S., Islam, D., Naim, M. R., Moniruzzaman, M., & Biswas, A. (2025). Occurrence of heavy metals in fish species, water, and sediments of the Pasur River in Bangladesh, along with assessments of ecological and human health risks. *Discover Environment*, *3*(1), 49. https://doi.org/https://doi.org/10.1007/s44274-025-00238-2

Lushchak, V. I. (2012). Glutathione homeostasis and functions: potential targets for medical interventions. *Journal of Amino Acids*, *2012*(1), 736837.

Łuszczek-Trojnar, E., Drąg-Kozak, E., & Popek, W. (2013). Lead accumulation and elimination in tissues of Prussian carp, Carassius gibelio (Bloch, 1782), after long-term dietary exposure, and depuration periods. *Environmental Science and Pollution Research*, *20*(5), 3122-3132.

Monikh, F. A., Maryamabadi, A., Savari, A., & Ghanemi, K. (2015). Heavy metals' concentration in sediment, shrimp, and two fish species from the northwest Persian Gulf. *Toxicology and industrial health*, *31*(6), 554-565. https://doi.org/https://doi.org/10.1177/0748233713475498

Mouchet, F., Cren, S., Cunienq, C., Deydier, E., Guilet, R., & Gauthier, L. (2007). Assessment of lead ecotoxicity in water using the amphibian larvae (Xenopus laevis) and preliminary study of its immobilization in meat and bone meal combustion residues. *Biometals*, *20*(2), 113-127.

Nawab, J., Ali, S., Rauf, A., Rehman, U. U., Khan, A. A., Sajjad, M., & Khan, W. (2015). Health risk associated with heavy metals via consumption of surface and groundwater in the district Shangla, Pakistan. *Journal of Himalayan Earth Sciences*, *48*(2), 62-73.

Oros, A. (2025). Bioaccumulation and trophic transfer of heavy metals in marine fish: Ecological and ecosystem-level impacts. J. Xenobiotics, 15 (2), 59. In.

Oyeleke, P. O., Okparaocha, F. J., & Abiodun, O. A. (2018). Human health risk assessment of heavy metals (Lead, Cadmium, and Copper) in fresh water tilapia fish (Oreochromis niloticus) from Eleyele River, Ibadan, Southwestern Nigeria. *Chemistry Research Journal*, *3*(4), 134-142.

Parolini, M., Panseri, S., Håland Gaeta, F., Rossi, L., Dell'Anno, M., Ceriani, F., De Felice, B., Rafoss, T., Arioli, F., & Pilu, S. (2022). Trends and potential human health risk of trace elements accumulated in transplanted blue mussels during restoration activities of Flekkefjord fjord (Southern Norway). *Environmental Monitoring and Assessment*, *194*(3), 208.

Paul, N., & Sengupta, M. (2013). Lead-induced overactivation of phagocytes and variation in enzymatic and non-enzymatic antioxidant defenses in intestinal macrophages of Channa punctatus. *Modern Research in Inflammation*, *2*(2), 28-35. https://doi.org/10.4236/mri.2013.22004

Saha, N. C., Chatterjee, A., Banerjee, P., Bhattacharya, R., Sadhu, A., Pastorino, P., & Saha, S. (2024). Toxic Effects of Lead Exposure on Freshwater Climbing Perch, Anabas testudineus, and Bioremediation Using Ocimum sanctum Leaf Powder. *Toxics*, *12*(12), 927. https://doi.org/10.3390/toxics12120927

Spokas, E. G., Spur, B. W., Smith, H., Kemp, F. W., & Bogden, J. D. (2006). Tissue lead concentration during chronic exposure of Pimephales promelas (fathead minnow) to lead nitrate in aquarium water. *Environmental science & technology*, *40*(21), 6852-6858.

Srikanth, K., Pereira, E., Duarte, A., & Ahmad, I. (2013). Glutathione and its dependent enzymes' modulatory responses to toxic metals and metalloids in fish—a review. *Environmental Science and Pollution Research*, *20*(4), 2133-2149.

Tang, Z., Liu, X., Niu, X., Yin, H., Liu, M., Zhang, D., & Guo, H. (2023). Ecological risk assessment of aquatic organisms induced by heavy metals in the estuarine waters of the Pearl River. *Scientific Reports*, *13*(1), 9145. https://doi.org/https://doi.org/10.1038/s41598-023-35798-x

Taslima, K., Al-Emran, M., Rahman, M. S., Hasan, J., Ferdous, Z., Rohani, M. F., & Shahjahan, M. (2022). Impacts of heavy metals on early development, growth, and reproduction of fish–A review. *Toxicology reports*, *9*, 858-868. https://doi.org/https://doi.org/10.1016/j.toxrep.2022.04.013

Usero, J., Gonzalez-Regalado, E., & Gracia, I. (1997). Trace metals in the bivalve molluscs Ruditapes decussatus and Ruditapes philippinarum from the Atlantic Coast of Southern Spain. *Environment International*, *23*(3), 291-298. https://doi.org/https://doi.org/10.1016/S0160-4120(97)00030-5

Velusamy, S., Roy, A., Sundaram, S., & Kumar Mallick, T. (2021). A review on heavy metal ions and containing dyes removal through graphene oxide-based adsorption strategies for textile wastewater treatment. *The Chemical Record*, *21*(7), 1570-1610. https://doi.org/https://doi.org/10.1002/tcr.202000153

Voigt, C. L., Da Silva, C. P., Doria, H. B., Randi, M. A. F., de Oliveira Ribeiro, C. A., & de Campos, S. X. (2015). Bioconcentration and bioaccumulation of metal in freshwater Neotropical fish

Geophagus brasiliensis. *Environmental Science and Pollution Research*, *22*(11), 8242-8252. https://doi.org/https://doi.org/10.1007/s11356-014-3967-4

Wang, Y., Noman, A., Zhang, C., Al-Bukhaiti, W. Q., & Abed, S. M. (2024). Effect of fish-heavy metals contamination on the generation of reactive oxygen species and its implications on human health: a review. *Frontiers in Marine Science*, *11*, 1500870. https://doi.org/https://doi.org/10.3389/fmars.2024.1500870

World Health Organisation (2011). *Guidelines for World Health Statistics:* W. H. Organization. https://www.who.int/publications/i/item/9789241564199

World Health Organisation (2022). *Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda*. W. H. Organization. https://www.ncbi.nlm.nih.gov/books/NBK579460/table/ch12.tab88/

World Health Organization (2024). *Lead poisoning*. https://www.who.int/health-topics/lead-poisoning#tab=tab_1

Zhai, Q., Wang, H., Tian, F., Zhao, J., Zhang, H., & Chen, W. (2017). Dietary Lactobacillus plantarum supplementation decreases tissue lead accumulation and alleviates lead toxicity in Nile tilapia (Oreochromis niloticus). *Aquaculture Research*, *48*(9), 5094-5103.