CIBTech Journal of Zoology ISSN: 2319–3883
An Online International Journal, Available at http://www.cibtech.org/cjz.htm
2025 Vol.14, pp.344-348/Chavan and Jaiswal
Research Article

IMPACT OF LEAD ACETATE GLYCOGEN CONTENT IN MUSCLES OF *OREOCHROMIS MOSSAMBICUS*

***Chavan Rohini Sudhakar and Jaiswal Neetarani Ramesh**
Department of Zoology, Yeshwant Mahavidyalaya, Nanded, India - 431605
**Author for Correspondence: jneeta474@gmail.com*

ABSTRACT

Inadequate disposal or handling of materials containing lead, including electronic waste, batteries and industrial waste, has the potential to pollute soil and groundwater. Lead acetate may seep into the surroundings through aging plumbing resulting in the pollution of water bodies and soil. Certain consumer goods, such as toys, jewellery and cosmetics may contain lead, particularly if imported from areas with less rigorous regulations. Bioaccumulation of lead acetate in body of aquatic organism. Fish *Oreochromis mossambicus* is adaptable to diverse environmental conditions and it is consumed all over the world. In this study impact of increase concentration of lead acetate on glycogen contain of muscles of fish *Oreochromis mossambicus*. Lead acetate shows toxic effect and ultimately affects food chain. In the present study the *Oreochromis mossambicus* of length 15 ± 0.21 cm and weight 123 ± 3.0 gm underwent treatment of lead acetate (0.5 mg/L) for duration 1 day, 5 days, 10 days, 15 days. The Anthrone reagent test method is used for determination of glycogen content of fish muscles. Glycogen content muscles of *Oreochromis mossambicus* is decrease in lead acetate exposed fish. As duration of lead acetate 0.5 mg/L (5% of 96 hours LC_{50}) exposure increases decrease in glycogen contain of *Oreochromis mossambicus* was observed. This study current research emphasizes metabolic abnormalities induced by excessive lead acetate exposure in *Oreochromis mossambicus* fish. Lead acetate changes the functional state of muscles in *Oreochromis mossambicus*, the organism under exposure.

***Keywords**: Glycogen, Lead Acetate, Oreochromis mossambicus, Fish*

INTRODUCTION

Industrial activities contribute significantly to the release of lead acetate into the atmosphere, soil, and aquatic systems. The combustion of lead acetate–containing fuels in vehicles and the deterioration of lead-based paints, especially those used before regulatory restrictions, further add to environmental contamination. Over time, degraded paint and fuel residues release lead acetate particles that persist in the environment, increasing the risk of ecological damage. Elevated concentrations of lead acetate in aquatic ecosystems are particularly harmful. They not only impair water quality but also promote bioaccumulation of the metal in aquatic organisms. *Oreochromis mossambicus*, a fish species widely consumed worldwide due to its adaptability and ease of culture, serves as a key bioindicator in studies of heavy metal pollution. Exposure to high levels of lead acetate disrupts vital physiological and metabolic processes in fish. It adversely affects gill function, thereby impairing respiration, nutrient absorption, and growth. Prolonged exposure can also damage the reproductive system, elevate stress responses, and ultimately increase mortality. Invertebrates and other aquatic organisms are similarly affected, indicating the far-reaching ecological consequences of lead pollution. Heavy metal contamination, largely driven by industrialization and chemical discharges, has therefore emerged as a global environmental concern. Lead acetate, among other metals, poses severe threats to aquatic biodiversity, food web stability, and overall ecosystem health. Given its resilience to varying water conditions, *Oreochromis mossambicus* is frequently employed in ecotoxicological studies to assess the toxicological impacts of such pollutants (Gupta & Singh, 2011; Karami *et al.,* 2016; Naigaga *et al.,* 2011; Tchounwou *et al.,* 2012). However, even this robust species can suffer from physiological and metabolic disturbances when exposed to high levels of lead acetate, as this compound disrupts glycogen synthesis, enzyme activity, and nutrient absorption, along with other critical biological functions (Mahi *et al.,* 2022; Kim *et al.,* 2020). Lead acetate toxicity in aquatic ecosystems primarily manifests through

bioaccumulation, where metal ions are retained within tissues of fish and other organisms over time, ultimately leading to oxidative stress and organ damage (Zahran *et al.,* 2025). The interaction of lead with biological systems alters metabolic processes, causing respiratory dysfunction, growth retardation, and tissue damage (Gupta & Singh, 2011; U.S. EPA, 1992). Notably, several studies have demonstrated the accumulation of lead in gill tissues, impairing oxygen exchange and reducing overall respiratory efficiency in fish (Kim *et al.,* 2020; Naigaga *et al.,* 2011). Chronic exposure to elevated lead acetate levels also affects reproductive performance, inducing endocrine disruption and reducing survival rates, which ultimately threatens population dynamics and ecosystem stability (Ferreira *et al.,* 2023; Tchounwou *et al.,* 2012).

MATERIALS AND METHODS

The *Oreochromis mossambicus* is used in research study. Fish *Oreochromis mossambicus* of length 15 ± 0.21 cm and weight 123 ± 3.0 gm. In minimum stress condition fish are transported using clean aerated water. *Oreochromis mossambicus* acclimatise in laboratory condition for seven days. Acclimatised fishes are used for experiment. Temperature is maintained at 26º C ± 2º C and measure by using digital thermometer. Dissolved oxygen is measure at 6.2-6.7 mg/L by using are aerator and adequate oxygen supply is maintained. *Oreochromis mossambicus* were exposed to darkness for 12 hours and to light for 12 hours. During the acclimatization period, fish were provided with a commercial diet, and all individuals were fasted for 24 hours before the start of the experiment to standardize metabolic conditions. The experimental design included one control group and one experimental group. The Anthrone method is a widely applied biochemical assay for estimating glycogen and other carbohydrate levels in biological materials. This colorimetric technique is based on the reaction between carbohydrates and anthrone reagent under strongly acidic conditions. In the presence of concentrated sulfuric acid, glycogen is hydrolysed into glucose monomers, which are subsequently dehydrated to form furfural derivatives. These derivatives react with anthrone to produce a characteristic blue-green complex, the intensity of which is directly proportional to the carbohydrate concentration (Hassid & Abraham, 1957; Van Handel, 1965). For analysis, a 0.2% anthrone solution is freshly prepared in concentrated sulfuric acid and chilled before use. Standard glycogen solutions, typically ranging from 10–100 µg/mL, are prepared to generate a calibration curve. Muscle tissue samples are first digested with potassium hydroxide (KOH), followed by neutralization, to release glycogen. One milliliter of either a glycogen standard or tissue extract is transferred into test tubes, and 4 mL of chilled anthrone reagent is added. After thorough mixing, the tubes are heated in a boiling water bath for 10 minutes and then rapidly cooled in an ice bath. Absorbance is measured at 620 nm using a spectrophotometer. A standard curve is constructed by plotting absorbance (Y-axis) against known glycogen concentrations (X-axis), and the glycogen concentration in the samples is determined accordingly. The Anthrone assay remains one of the most widely used carbohydrate determination methods because it is inexpensive, simple, sensitive, and applicable to a wide variety of biological materials, including tissues and cells (Hodge & Hofreiter, 1962; Albalasmeh *et al.,* 2013).

Experimental Setup

The experimental design included two groups: one control group (Set I) and experimental groups exposed to concentrations 0.5 mg/L of lead acetate (Set II).

1. Control Group (Set I): Fish in the control group were maintained in clean, untreated freshwater for the entire study duration to serve as a baseline for comparison.
2. Experimental Group (Set II): Fish *Oreochromis mossambicus* were exposed to 0.5 mg/L lead acetate concentration, equivalent to 5% of 96 hours the LC_{50}.

The 96 hours LC_{50} (lethal concentration for 50% of the population) values of lead acetate was calculated for *Oreochromis mossambicus,* ensuring the selected concentrations are sub-lethal and appropriate for assessing metabolic and glycogen responses over time. Each group consisted of at least 10 fish to ensure sufficient statistical power for detecting significant differences in glycogen content across conditions. Fish were sampled at intervals of 1, 5, 10, and 15 days to assess the time-dependent effects of lead acetate exposure.

RESULTS AND DISCUSSION

Table 1: Effect of lead acetate on glycogen content in muscles of *Oreochromis mossambicus* (Mean ± SD, glycogen in mg/g weight of the muscles)

Duration (days)	Control set (glycogen content in muscles)	0.5 mg/L lead acetate (5% of 96 hours LC_{50})
1 day	11.87± 2.64	10.67± 1.73
5 days	11.93± 1.89	10.81± 2.45
10 days	12.82±2.38	09.76± 1.37
15 days	12.95± 1.73	09.43± 1.56

Values are mean ± standard deviation of 6 replicants

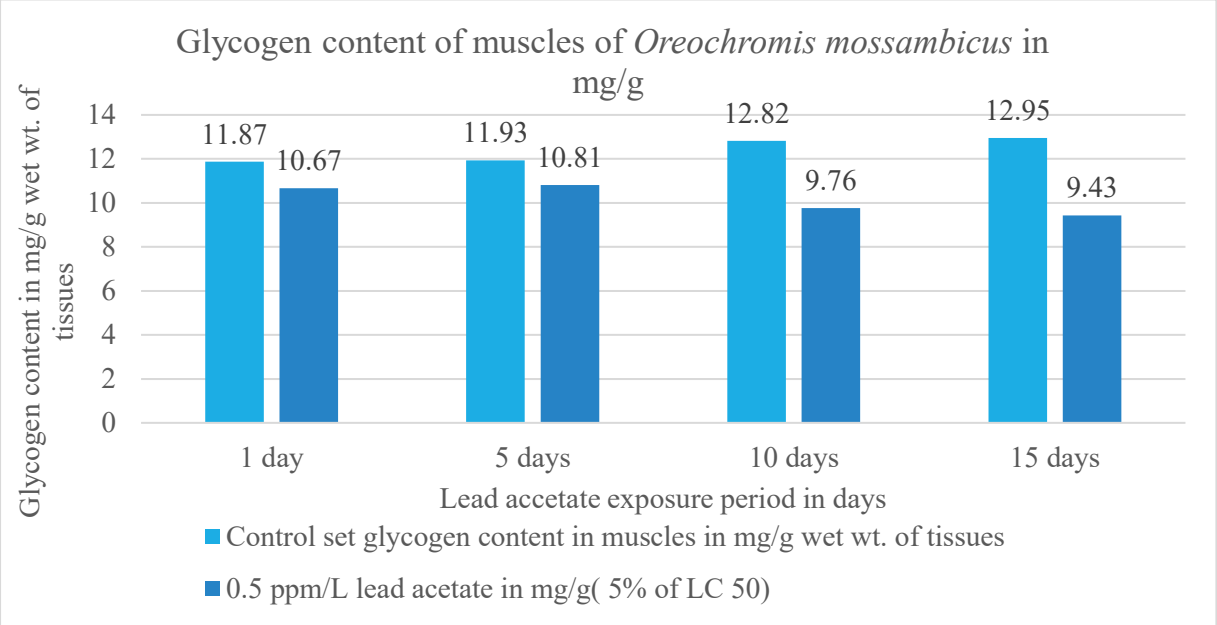

Figure 1: Effect of lead acetate on glycogen content of muscles tissues of fish *Oreochromis mossambicus*

ANOVA Results

A Two-way ANOVA was conducted to assess the significance of differences in glycogen content across time points and lead acetate concentrations for muscles. The ANOVA results indicate:

Muscle: Glycogen content showed a similar decreasing trend. Significant differences ($p < 0.05$) were also observed between the control and experimental group, with notable decreases in glycogen levels as exposure duration increased. These results confirm that lead acetate exposure leads to time and dose-dependent inhibition of glycogen synthesis in muscles. Figure shows the graph depicts in the glycogen content in muscles over time for *Oreochromis mossambicus* exposed to lead acetate (0.5 mg/L) compared to the control. Each point represents the mean value, with error bars indicating the standard deviation. Data points indicate mean values, with standard deviations as error bars. The data reveal a clear, exposure dependent reduction in glycogen content in muscle. The glycogen depletion pattern suggests that lead acetate disrupts glycogen metabolism, likely through oxidative stress and inhibition of glycogen synthesis pathways. The consistent decline in glycogen content over time further indicates cumulative toxicity. This trend suggests a possibly exponential effect of lead acetate on glycogen

inhibition, where longer exposures significantly amplify the toxic impact. It is noticed that the changes in glycogen content changes during different exposure period. The glycogen content of set II (lead acetate of concentration 0.5 mg/L) content very less glycogen as compare to control (Set I). Muscle content very less glycogen of set II which expose to lead acetate (0.5 mg/L) for 15 days as compare to control, 1 Day, 5 Days, 10 Days. It is noticed that glycogen contain in muscle was decreases as exposure period of lead acetate was increased. In lead acetate treated fish *Oreochromis mossambicus* shows decrease in glycogen content of muscle as compare to control fish.

The present study demonstrated that glycogen content in the muscle of *Oreochromis mossambicus* was significantly reduced following exposure to lead acetate (Figure 1). A progressive decline in muscle glycogen levels was observed with increasing exposure duration when compared to the control group (Table 1). Specifically, as the exposure period extended from 1 day to 5, 10, and 15 days, glycogen content declined consistently in muscle. Fish maintained in the experimental group (Set II; exposed to 0.5 mg/L lead acetate, equivalent to 5% of 96 hours LC_{50}) showed a greater reduction in glycogen compared to the control group (Set I). These findings indicate that lead acetate induces metabolic disturbances by inhibiting glycogen storage in muscle. The results support earlier reports that heavy metals, including lead, interfere with carbohydrate metabolism, enzyme activity, and energy balance in fish (Mahi *et al.,* 2022; Kim *et al.,* 2020; Van Handel, 1965). This study thus provides evidence for the acute toxic effects of lead acetate on glycogen metabolism in *Oreochromis mossambicus*.

CONCLUSION

Overall, the depletion of glycogen reserves in *Oreochromis mossambicus* under lead acetate exposure reflects a disruption of energy metabolism that may compromise physiological resilience. Since glycogen serves as a critical energy source during stress, its reduction could diminish the fish's ability to cope with additional environmental challenges. In aquaculture systems and natural ecosystems alike, such metabolic disturbances may lead to decreased growth, reduced reproductive output, and increased mortality, underscoring the ecological risks associated with heavy metal contamination.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to the Mahatma Jyotiba Phule Research Fellowship, Nagpur, Maharashtra, India, for providing the financial assistance that made this research possible. Their support has been invaluable in advancing this study, and I am deeply appreciative of the opportunity to contribute to the field with their generous backing.

REFERENCES

Albalasmeh, A. A., Berhe, A. A., & Ghezzehei, T. A. (2013). A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydrate Polymers*, **97**(2), 253–261. *https://doi.org/10.1016/j.carbpol.2013.04.072*

Ferreira, C. S., *et al.* (2023). Reproductive toxicity induced by lead exposure: Effects on fish gametogenesis and endocrine disruption. Chemosphere, **339**, 139496. *https://doi.org/10.1016/j.chemosphere.2023.139496*

Gupta, P., & Singh, S. (2011). Lead toxicity and its impact on aquatic life. Environmental Monitoring and Assessment, **177**(1–4), 99–107. *https://doi.org/10.1007/s10661-010-1613-5*

Hassid, W. Z., & Abraham, S. (1957). Chemical procedures for analysis of polysaccharides. In Methods in Enzymology **3**, pp. 34–50. Academic Press. *https://doi.org/10.1016/S0076-6879(57)03403-0*

Hodge, J. E., & Hofreiter, B. T. (1962). Determination of reducing sugars and carbohydrates. In R. L. Whistler & M. L. Wolfrom (Eds.), Methods in Carbohydrate Chemistry. *Academic Press.***1**, pp. 380–394.

Karami, A., Romano, N., Galloway, T. S., & Hamzah, H. (2016). Heavy metals in aquaculture: Sources, toxicity, and prevention. Aquaculture International, **24**(4), 1181–1193. *https://doi.org/10.1007/s10499-016-9995-0*

Kim, H. J., Choi, S.-O., & Lee, Y. J. (2020). Determination of toxic effects of lead acetate on different tissues of zebrafish (Danio rerio). *Ecotoxicology and Environmental Safety*, **187**, 109831. *https://doi.org/10.1016/j.ecoenv.2019.109831*

Mahi, T. F., Islam, S., Mamun, M. A. A., & Ahmed, M. K. (2022). Assessment of lead (Pb) toxicity in juvenile Nile tilapia (Oreochromis niloticus): Physiological, histological, and biochemical changes. Scientific Reports, **12**, 14209. *https://doi.org/10.1038/s41598-022-17459-2*

Naigaga, I., Kaiser, H., & Muller, W. (2011). The role of tilapia (Oreochromis spp.) as bioindicators of aquatic pollution. *African Journal of Aquatic Science*, **36**(1), 1–7. *https://doi.org/10.2989/16085914.2010.536682*

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. EXS, 101, 133–164. *https://doi.org/10.1007/978-3-7643-8340-4_6*

U.S. Environmental Protection Agency. (1992). Lead hazards to fish, wildlife, and invertebrates: A synoptic review (Biological Report No. **85**(1.11)). U.S. Fish and Wildlife Service. *https://semspub.epa.gov/work/03/137426.pdf*

Van Handel, E. (1965). Estimation of glycogen in small amounts of tissue. Analytical Biochemistry, **11**(2), 256–265. https://doi.org/10.1016/0003-2697(65)90016-X

Zahran, E., Mamdouh, A.-Z., Elbahnaswy, S., El-Sayed, A., & El Sebaei, M. G. (2025). The impact of heavy metal pollution: Bioaccumulation, oxidative stress, and histopathological alterations in fish across diverse habitats. *Aquaculture International*. *https://doi.org/10.1007/s10499-025-02045-1*