



Comparison of Lethal Concentration of Lead Acetate in Rosy Barb (*Pethia sp*) and Buenos Aires Tetra (*Hyphessobrycon sp*)

Sk. Kabita¹, Sehnaz Parvin¹, Md. Tamim Firdous¹

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ABSTRACT

Background: Most industrial toxic effluents and human-made discharges eventually find their way into aquatic ecosystems. With rapid global economic development, the large-scale emission of heavy metals has become particularly concerning. The frequent release of these excessive pollutants into water bodies has detrimental effects on aquatic life. To assess fish toxicity, contemporary and standardized acute toxicity tests are frequently employed, proving highly effective in environmental risk assessment. For example, the lethal concentration (LC₅₀) is widely recognized worldwide as a valuable tool for studying environmental toxicology. The test for quantifying the diversified effects of this toxicant is thus immensely essential to maintain the water quality standard and to assume the effects of toxicant to the aquatic organism.

Methods: In this study, two ornamental fishes i.e., *Pethia*, commonly called as Rosy Barb that belongs to order- cypriniformes and *Hyphessobrycon* commonly known as Buenos Aires Tetra that belongs to order-characiformes were used as test organisms. They were exposed to different concentrations of lead. Rosy Barb was exposed to 250, 275, 300, 325, 350 mg/L of lead and 365, 375, 385, 395 and 405 mg/l in case of tetra along with control in both the species having no metal toxicant and with all the conditions constant.

Result: The 50% lethal concentration (LC₅₀) was 565 mg/l after 24 h, 525 mg/l after 48 h and 389 mg/l after 72 h for Rosy Barb and 380 mg/l after 24 h, 355 mg/l after 48 h and 347 mg/l after 72 h for BA Tetra. Rosy Barb was detected with higher LC₅₀ values and showed higher resistance than BA Tetra. Presence of more keratinized structure and having larger body size than tetras may be the possible cause behind this.

Key words: Characiformes, Cypriniformes, Heavy metal, LC₅₀, Toxicity.

INTRODUCTION

Pollution caused by metals in the aquatic system has been marked as a big health concern as well as serious threat to the environment that happens through channels like agricultural pesticide run-off, domestic garbage dumps, industrial effluents and wastes and mining activities (Kumar *et al.*, 2018; Merian and Clarkson, 1991). Contamination is a potential threat to the aquatic organisms produced from being exposed to significant amounts of heavy metals, which at high concentrations can cause harmful effects on physiological, biochemical and metabolic systems of fishes (Canli and Atli, 2003; Folmar, 1993, 1993; Heath, 1995; Shao *et al.*, 2014). It may be of short term or long-term effects. Hence, these metals are the serious issues as it's have the potentiality to get fixed, accumulated and magnified to the food chain (Elnimr, 2011).

The development of water quality standards for pollutants and the selection of appropriate organisms as bio-indicators have both benefited by the widespread use of toxicity testing. It is also regarded as a crucial way for determining the outcomes of toxicants in aquatic ecosystems (Shuhaimi-othman *et al.*, 2010) as toxicity test depicts the responses of a bio-active compound in the environment and are useful to quantify the water quality. Fishes are the top carnivores in the aquatic food chain. So, they can exhibit the reliable indication of metal contamination in the aquatic body.

¹Department of Biological Sciences, Aliah University, Kolkata-700 160, West Bengal, India.

Corresponding Author: Sk. Kabita, Department of Biological Sciences, Aliah University, Kolkata-700 160, West Bengal, India. Email: kabita@aliah.ac.in

ORCID: <https://orcid.org/0000-0002-0776-8368>

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Owing to their toxicity, heavy metal is considered to be environmental contaminants, persistence in the ecosystem and bio accumulative existence (Ali *et al.*, 2019). It is characterized as a metal with density is bigger than 5 g/cm³ and their atomic weight is 63.5-200.5 g/mol (Koller and Saleh, 2018). Metals like Zn, Co, Cu, Ni, Mn, Se, Fe and Cr are essential for the growth of organisms, they are called essential heavy metals. Non-essential heavy metals like Pb, As, Hg and Cd are not only biologically non-essential, but beyond the optimum threshold levels they found to be seriously hazardous and environmentally toxic. These metals may participate in aquatic life, infiltrate onto solid

surfaces, stay soluble or gliding in water, or be ingested by flora after entering a watery environment. Metals' tendency to accumulate in animal tissues is their most significant biological trait. Some metals are essential for fish to maintain normal body physiology, but they are harmful when they build up in their bodily tissues and are not metabolised in larger than optimum amount (Kumari and Chand, 2021).

Additionally, fish with much higher metal concentrations may experience changes in physiological processes that result in high mortality and ultimately a loss of the fish biota (Avenant-oldewage, 2000).

Lead is reported to the highly toxic metal that cause retardation of growth, sub-lethal concentration in reproduction, behavioural changes and finally to death (Dash *et al.*, 2019; Ramsdorf *et al.*, 2009). Pb²⁺, the most prevalent and stable ion in aquatic environments, is most likely to bio-accumulate in fish organs such the gills, kidney, liver, muscles, scales and skin (Ahmed and Bibi, 2010, 2010; Spokas *et al.*, 2006).

Aquatic toxicology is the study of how environmental pollutants, including agrotoxins, hazardous industrial residue, toxic metabolites, *etc.*, harm fish and other aquatic organisms' health. Stated in distinct ways, aquatic toxicology is the study of how artificial chemicals, as well as other man-made and natural materials and activities, affect aquatic creatures at different levels of organization, spanning from subcellular units to individual animals to communities and ecosystems (Rand *et al.*, 2020). Acute toxicity is phrased as a negative or unfavourable effect occurring within 24 hours of the toxicant administration (No, 2021; OECD, 2024; Saganuwan, 2016). The median lethal concentration (LC₅₀) has been employed as a screening method to determine a substance's toxicity as well as a crucial parameter to assess acute toxicity (Agbohessi *et al.*, 2023; Priyadarshi *et al.*, 2023; Subburaj *et al.*, 2020).

The study aims to calculate the LC₅₀ (lethal concentration for 50% of the population) for lead in these two fish species, *i.e.*, Rosy Barb and Buenos Aires Tetra and investigate why their responses to lead exposure might differ. Both the fishes used in this study were ornamental fish. They offer many advantages, including high fecundity, tiny size, ease of breeding in a lab, quick generation time, rapid development, translucent embryos and ease of maintenance in a lab. Understanding the LC₅₀ values and the reasons behind the differences in toxicity responses can provide insights into the environmental risks posed by lead contamination and help in designing safer aquatic environments for ornamental fish.

MATERIALS AND METHODS

Fish collection, acclimatization and water quality monitoring

In this experiment two fishes were selected by reviewing previous literature; first one is *Pethia* (average weight- 1.895g; average length- 5.20 cm) commonly called as Rosy

Barb that belongs to order- cypriniformes and second one is *Hyphessobrycon* (average weight-2.36 g; average length- 5.49 cm) commonly known as Buenos Aires Tetra that belongs to order-characiformes. Fishes were collected from local fish market, Galiff street, Kolkata; brought them to the laboratory. Prior to start the experiment, they were housed, stocked in 80L glass aquarium and were acclimatized for 2 weeks in dechlorinated water. The aquarium water was aerated through stone diffusers connected to a mechanical air compressor continuously. Throughout the study period water temperature was maintained between 25±2°C. Water quality parameters were kept sustained according to American Public Health Association (APHA) standards (Clesceri *et al.*, 1989). Fishes were fed with commercial fish pellets during the acclimatized period.

Toxicant solution preparation

The standard stock solution of lead was prepared from Lead acetate (C₄H₆O₄Pb. 3H₂O) in distilled water. All the chemicals used were analytical grade (Sisco Research Laboratories Pvt. Ltd.).

Experiment design

Test was performed to determine the toxicity of lead by calculating 24-, 48-, 72- and 96-hour LC₅₀ values using five concentrations of Pb 250, 275, 300, 325, 350 mg/L in case of Rosy Barb and 365, 375, 385, 395 and 405 mg/l in case of tetra along with control having no metal toxicant and with all the conditions constant. The study follows by randomization of the fish in test aquaria to the method suggested by the U.S. Federal Water Pollution Control Administration, 1968. Ten fishes were picked up from store fishes to use for each concentration. Fresh treatment of toxicants of metals were changed daily along with water. Food was stopped during the experiment. A fish was examined dead after halting its gill and operculum movement. Dead fishes were taken out of aquaria immediately after death to prevent contamination.

Data analysis

The mortality was recorded at each 24 hours. The mortality percentage was calculated after 24 h, 48 h, 72 h and 96 h by using Abbott's formula (Abbott, 1925) in Table 1 and 2. Collected data were analysed and LC₅₀ values were derived by Finney's Probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

10 individuals of Rosy Barb and Buenos Aires Tetra in each concentration were tested against lead concentration for determination of 96 hours LC₅₀ at room temperature. After exposure, both the fishes are exhibited deformed behavioural nature like gathering in the corner, disbalancing, loss in equilibrium, strong opercular movement to the surface of water, less swimming activity.

Obtained data from this toxicity test is increased to dose and exposed time, hence proportional. Calculated

lethal concentration values are presented in table. The 24 h, 48 h, 72 h, 96 h LC₅₀ of lead acetate upon Rosy Barb are 565, 525, 389, 275 mg/L and for Buenos Aires Tetra are 380, 355, 347, 339 mg/L (Table 3).

In aquatic environment presence of toxicant is becoming more hazardous for aquatic flora and fauna. It gets magnified upon used by terrestrial fauna. High concentration of this toxicants in aquatic environment is a serious threat because of its long resistance, bioaccumulation, biomagnification and obviously its toxicity. Since fish can absorb agricultural and industrial wash outs directly from the water through respiration and also through their dietary intake as well as it has been suggested that fish could potentially be used as indicators to monitor pesticides and fungicides used on land (Subaramaniyam *et al.*, 2023; Плынаров *et al.*, 2023).

Heavy metals are produced from natural as well as anthropogenic sources (Bauvais *et al.*, 2015). Heavy metal pollution in aquatic environments occurs as a result of direct geologic weathering, air deposition, or the discharge of industrial, municipal, household, or agricultural waste, as well as wastewater treatment plants (Campos-Garcia *et al.*, 2015; Demirak *et al.*, 2006a; Dhanakumar *et al.*, 2015; Maier *et al.*, 2015a). Because of their toxicity, lengthy persistence, bioaccumulation and biomagnification in the food chain heavy metals and metalloid contaminate in water and sediment, when it occurs in higher amounts creates a major danger (Demirak *et al.*, 2006b; Maier *et al.*, 2015b, 2015b). The ratio of the amount of heavy metals collected in fish tissue to the amount of heavy metals present in the surrounding water and any suspended food is known as the bioaccumulation factor. Fish, which are at the top of the aquatic food chain, may accumulate metals and transmit them to people through their diet, leading to chronic or acute disorders. The fact that metals are not biodegradable and can build up in the environment makes them harmful to aquatic organisms and, as a result, to people who eat fish (Eisler, 1993). Aquatic creatures may acquire heavy metals from the aquatic environment through a variety of pathways, including direct water intake by gills or body surface.

The goal of the current study is to determine the vulnerability of the potentially dangerous heavy metal, lead to the Rosy Barb and Buenos Aires Tetra. Fishes, which are restricted to aquatic environments, are the group most susceptible to heavy metal toxicity (Rand and Petrocelli, 1985). To assess the possible toxicological impacts of environmental pollutants on aquatic biota, aquatic toxicity tests are utilised. The effects of waterborne heavy metals on the most vulnerable biomarkers of aquatic pollution, particularly fish, must be studied (Gautam and Lall, 1989). Lead is a naturally occurring heavy metal that is considered to be harmful to health. Anthropogenic factors such as the burning of fossil fuels, mining, the production of batteries, the use of metal products like solder and water supply pipes, X-ray shielding devices, leaded petrol and the use

of glass food and beverage containers all greatly increase the concentration of lead in the environment (Monteiro *et al.*, 2009). The natural permissible concentration of lead in ground water has been estimated at 0.02 mg/l. Exposure to heavy load of lead levels in the aquatic system may cause alteration in blood and nerves cells and generative damage in fish and other aquatic organisms (Kalay *et al.*, 1999; McCoy *et al.*, 1995).

Ullah *et al.* (2016) carried out lethal concentration of toxicant Lead Nitrate for the fish *Oreochromis niloticus* 44 mg/L for 96 hours. (Batoool and Javed, 2015) reported 96-hour LC50 of cobalt for the fishes *Catla catla*, *Cirrhina mrigala* and *Labeo rohita*, were 86.32±0.37, 117.39±0.36 and 106.12±0.38 mg/L while 96-hour LC50 showed for the lead with *Catla catla*, *Cirrhina mrigala* and *Labeo rohita* were 31.25±0.22, 40.54±0.32 and 36.72±0.37 mg/L respectively. (Ferrer *et al.*, 2006) has demonstrated the 96-h LC50 values of different toxicants *i.e.*, 1093.40 µg/L for Pb and 172.10 µg/L for Zn in the early life stage of the crab *Chasmagnathus granulata*. (Shuhaimi-Othman *et al.*, 2010) performed acute toxicity test on *Penaues indicus* post larvae with metals (Pb, Zn, Cd and Cu). (Al-Kshab and Yehya, 2021) demonstrated that the mean lethal concentration (LC₅₀) of lead for the mosquito fish *Gambusia affinis i.e.*, 24, 48, 72 and 96 hours LC₅₀ were 59.443, 55.978, 53.256 and 500.514 mg/L respectively. Fish are potential of acquiring and absorb active ingredients like metals from water by both active and passive procedures in their bodies.

Table 1: Number of mortalities at 24-hour interval of Rosy Barb.

Dose	24 h	48 h	72 h	96 h
Control	0	0	0	0
250	0	0	0	2
275	0	1	0	3
300	0	1	1	7
325	0	1	0	8
350	1	1	2	6

Table 2: Number of mortalities at 24-hour interval of BA Tetra.

Dose	24 h	48 h	72 h	96 h
Control	0	0	0	0
365	4	3	0	0
375	5	4	0	0
385	3	6	1	0
395	5	3	0	2
405	9	1	0	0

Table 3: LC₅₀ value of Rosy Barb and Buenos Aires Tetra.

Time	LC ₅₀ value of Rosy Barb	LC ₅₀ value of Buenos Aires Tetra
24	565 mg/l	380 mg/l
48	525 mg/l	355 mg/l
72	389 mg/l	347 mg/l
96	275 mg/l	339 mg/l

Hence, metal absorption, distribution and deposition in tissue contribute to the accumulation of metals in fish tissues (Al-Kshab and Yehya, 2021).

Different factors affect the toxicity of the chemicals. Presence of scales is one of the most important factors on toxic responses of fishes. Scaled fish *Cyprinus carpio* is more tolerant to lead, chromium and cadmium load in comparison with scaleless *Pangasius hypophthalmus* (Abedi *et al.*, 2012). Keratinization in fish species may affect on accumulation of toxicants in fish body. Additionally, El-Sheikh and Sweileh, 2008; Mustafiz, 2003; Varanasi and Markey, 1978, 1978) have been reported the reduced toxicity of 27 metals after filtration *via* scales and they concluded that the keratin in scales may be the most major ectodermal secretions in metal absorption and protection from hazardous levels. Similarly, study was also been conducted on the sorption and removal of heavy metals by fish scales. Characiformes possess less keratinization relative to cypriniformes (Menezes and Marinho, 2019; Torres-Mejia and Vari, 2005, 2005). A few species only do possess keratinization. In case of cypriniformes, organs like gill, teeth, epidermis show keratin cover (Agarwal *et al.*, 1979; Pinky *et al.*, 2004).

CONCLUSION

The LC₅₀ is a commonly used metric in toxicity research for aquatic animals. The current value of 24 h LC₅₀ of lead is 565 mg/l for Rosy Barb, which differ with the previous investigations where it was noted 4.45ppt from other species of Order: Cypriniformes. In contrast, lower values of LC₅₀ after 24 h (380 mg/l) for BA Tetra have been documented. The process and use of toxicity tests implies the data regarding effects of toxicants on fishes that could be used effectively to predict the risk and effects of the chemical and their risk managements. In this study, Rosy Barb with higher LC₅₀ value appears to be more tolerant to lead than BA Tetra. These wide variations indicate that different species have varying levels of sensitivity to Pb. This suggests that Barb's body, with its protective keratinization, exhibits greater resistance to lead compared to Tetra's less keratinized body. Additionally, the larger body size of Barb contributes significantly to its metal resistance. However, Research on the metal resistance of fish, particularly comparing species like Barb and Tetra, often highlights how factors like keratinization and body size play crucial roles. Barbs, are noted for their relatively larger body size and more extensive keratinization. These characteristics could contribute to better protection against environmental stressors, including metal pollutants, when compared to smaller and less keratinized species like Tetras.

Additionally, higher mortality rates recorded in the species in BA Tetra than Rosy Barb in first 48h due to Pb-induced lethality. Larger body size can dilute the concentration of metals within the body, reducing toxic effects, while thicker keratin layers act as a physical barrier, preventing metals from penetrating deeper tissues.

However, more targeted studies are necessary to conclusively establish how these factors interact in different environments.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the committee of experimental animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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