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**Original Article**

# Toxicity Evaluation and Tissue Damaging Effects of Lead in *Labeo Rohita*

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## ABSTRACT

Heavy metal contamination of aquatic ecosystems due to industrialization and anthropogenic activities has become a serious global issue. Toxic effects of these heavy metals are posing a major threat to the aquatic organisms especially fish. Fishes exposed to lead (Pb), exhibit a wide-range of effects including muscular and neurological degeneration and destruction, growth inhibition, mortality, reproductive problems, and paralysis. Many fish species are used as food source by man. Bio magnification of these metals makes them hazardous for humans. **Objective:** To evaluate the acute toxicity (96-hr LC50 and lethal concentrations) of Pb in *Labeo rohita* and the effect of various sub-lethal doses of Pb on different organs (Gills, Liver, and Muscles) of fish. **Methods:** Live fish samples (150) were shifted from hatchery to laboratory. To measure metal (Pb), accumulation in fish body inductively coupled Plasma mass spectrometry (ICP-MS) was performed. **Results:** Acute toxicity (96-hr LC50) of Pb was observed as 52.20 mg/l, while Pb accumulation was noted more in gills as compared to liver and muscles. **Conclusions:** This study will provide baseline information for the control of aquatic pollution and conservation of aquatic organisms.

## INTRODUCTION

Water is essential element of life on earth. 90% of total water is used in agriculture, 6% is used for domestic purposes and 3% is used in industry. The main sources of soil and water pollution are domestic waste and untreated industrial sewage along with pesticides used in agriculture, heavy duty electric power generators etc. [1]. The volume of waste water generated from domestic and industrial zone has increased with increase in population, urbanization, improved lifestyles and economic conditions [2]. All liquid waste released from all industries may contain higher values of many physicochemical parameters like temperature, pH, conductivity, hardness, alkalinity, chemical oxygen demand, soluble salts, nitrates, nitrites and cations [3]. Waste waters from these main sources contain many types of organic and inorganic elements along with heavy metals (HMs). These heavy metals may be present in large amount in water which is harmful for water lives and also for humans and animals directly or indirectly. Industrial effluents are the most harmful water pollutants from both of these. This water most probably contains heavy metals like lead, chromium, cadmium, copper, zinc, manganese, nickel, arsenic and cobalt etc. Essential heavy metals are used by animals, plants and human bodies but non-essential heavy metals also get accumulated into the bodies causing harm. Increase in concentration of HMs in the environment increases certainly HMs enter the biogeochemical cycle causing disturbance [4]. Fish and other aquatic products like seaweeds and meat products

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are important for human diet all over the world. These products contribute to solve the global nutritional problems by providing important nutrients like proteins, minerals and vitamins [5]. To sustain normal aquatic life essential metals can also be taken from surroundings like sediments and rocks [6]. Lead (Pb) is a non-essential and toxic heavy metal. It is produced from lead ore called Galena. It belongs to carbon group with symbol Pb and atomic number 82. It is a soft and post-transition metal. Fresh lead is of bluish-white color that changes to grayish color when exposed to air. In liquid state it has shiny silver luster. Its physical and chemical properties make it extremely useful in many industries. Lead is an important part of waste materials which are discharged from industries to which aquatic animals especially fishes are exposed. It contaminates aquatic ecosystem through multiple ways like industries such as chemical and fertilizer industries, lead battery productions, Pb paint, colored inks, refining of ores, electroplating, and gasoline containing Pb that leaks from fishery boats. It can also get entry by erosion and leaching from soil, domestic waste, and combustion of petroleum products and fallout of Pb-dust [7]. Pb has different oxidation states (0, I, II, and IV). Among all of these Pb II is most stable ionic state. This is the ionic specie of Pb present in aquatic environment and gets accumulated in water animal bodies [8]. When animal have high level of pollutants in its surrounding environment, it begins to absorb these particles in its body. Same is for fishes, when fishes are exposed to extreme level of metal ions in surrounding environment, their body tissues begin to take up these metal ions by different ways. In general, there are two main ways of metal gaining i.e. either from water or from food [9]. Pb gets entry into fish body via gills. It can also be the part of food taken up by fish [10], and get accumulated in different organs of fish like gills, kidneys, liver and scales [11]. Lead effects fishes in many ways including muscular and neurological destruction growth inhibition, mortality, reproductive problems, and paralysis [12].

The goal of this study was to evaluate the toxicity of Pb and damage caused in *Labeo rohita.* The research was conducted with the objective for comparing toxicity of various doses of lead and the tissue damage of different organs in *Labeo rohita.*

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## METHODS

The proposed research work was conducted in Physiology Lab, Institute of Zoology, University of the Punjab, Lahore, Pakistan. Liver samples (fingerlings of *Labeo rohita*) were taken from Himalaya Fish Hatchery Muridke, which were of 8-9 inches long and 120 days old. For acclimatization fish was kept in dechlorinated tap water in cemented tanks of 1000 liter water capacity, equipped with filters and aerators. After every 24 hours water was changed. Fishes were given 2-3 minutes bath in 0.1% KMnO4 solution for disease prevention. After acclimatization dead fishes were immediately removed from the stock. Out of 150 fishes, 30 fishes were found dead. After acclimatization, fish stock was divided into 4 groups (three treatments and one control (without metal stress), each treatment having 3 replicas. Heaters were also used to protect fingerlings from cold. Fingerlings were fed with artificial commercial diet equal to 1/10th of their body weight [13]. Food debris and fecal material were removed from the test tank on daily basis. Composition of food is given in table 1.

**Table 1:** Nutritional Composition of Artificial Commercial Food

| Nutrients | Percentage Composition |
|---|---|
| Crude Protein | 35% (Min) |
| Crude Fat | 4% (Min) |
| Crude Fiber | 5% (Max) |
| Moisture | 12% (Max) |

Fishes were not fed for 96 hours during acute trial. Water was also changed regularly. Laboratory tests were performed in aquaria with 40-liter water capacity. Each group of 10 fishes was shifted to glass aquaria for acute toxicity experiments. Acute toxicity test was performed in terms of 96-hr LC50 and lethal concentrations. Chemically pure chloride compound of lead i.e. lead chloride (PbCl2) was used to dissolve in deionized water. Stock solution was also prepared for required metal on metallic ion equivalence basis. Stock solution was freshly prepared and renewed after every 24 hours. The concentration of metal in aquaria was increased slowly to meet the desired concentration within seven hours. Toxicity trail was performed according to APHA, 2017 [14]. All physicochemical variables of water viz. carbon dioxide, dissolved oxygen, pH, temperature and hardness were checked after every 12-hour. Fish mortality data was analyzed through the probit analysis by using Minitab [15, 16]. To collect samples for the investigation of accumulation of Pb in fish organs by ICP-MS, the fish was placed on the dissecting board, in such a way that ventral side remains upwards and dorsal side downwards for dissection. By using scalpel blade, fish was cut from vent up to the operculum and fixed the body muscle of ventral side on dissecting board with the help of pins. To expose the internal organs its muscular wall was cut. With the help of forceps and hands desired organs were exposed. Abdominal muscles, liver and gills were taken from the

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abdomen and to make small pieces for further process. Finally, the samples were placed in aluminum foil and put them in ultra-low freezer. By using digital weight balance (Shimadzu), sample was weighted 0.5 to 2 grams and put into conical flask. Conical flask was already containing 10 ml mixture of Merck concentrated Nitric acid ($HNO3$) and a small amount of hydrochloric acid (HCl). Water bath was given to contents for about 4-6 minutes with constant shaking until complete digestion. Measurements were done by measuring cylinders. By using Whatman's filter (0.45µm membrane thickness) paper digested samples were filtered. Filter paper was also washed with 50ml de-ionized water. At the end filtered samples were stored in glass jar which were also washed with 70% alcohol. The samples were then forwarded for ICP-MS for metal determination [17]. It is a special technique for the determination of low (ppb= parts per billion) and ultra-low concentrations (ppt= parts per trillion) of metals and elements. Atomic elements are usually pass through a plasma source for their conversion to ionized form. Then such ions are separated on the basis of their mass. All statistical analyses were performed by using Microsoft Excel 2019, SPSS version 21.0 and Probit method.

## RESULTS

In this study total 150 fish fingerlings were taken from Himalaya hatchery Muridke. Acute toxicity test was performed and 96-hr $LC_{50}$ for lead was found to be 52.20 mg/l (Figure 1). Further treatments were prepared 1/2 (13 mg/l), 1/3rd (17 mg/l) and 1/4th (26 mg/l) of 96-h $Lc_{50}$.

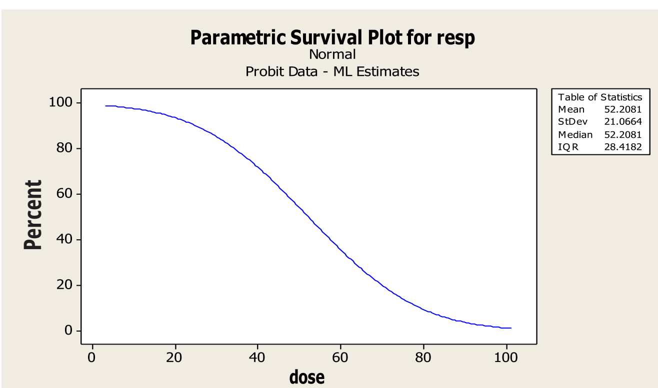


**Figure 1:** Regression Line between the Probit Kill of Labeo Rohita and Log Concentration of Lead Chloride ($PbCl2$).

Lead accumulation values using three sub-lethal doses in three replicates are shown in table 2.

**Table 2:** Lead Accumulation in Gills, Liver and Muscles (µg/g)

| Doses | Liver | Gills | Liver | Muscles |
|---|---|---|---|---|
| None | Control | ND | ND | ND |
| 13 mg/l | T1, R1 | 3.86 | 2.32 | 0.88 |
| | T1, R2 | 3.89 | 2.31 | 0.83 |
| | T1, R3 | 3.87 | 2.35 | 0.85 |
| 17 mg/l | T2, R1 | 4.78 | 3.08 | 1.66 |
| | T2, R2 | 4.69 | 3.07 | 1.66 |
| | T2, R3 | 4.77 | 3.08 | 1.63 |
| 26 mg/l | T3, R1 | 5.69 | 3.24 | 1.99 |
| | T3, R2 | 5.67 | 3.27 | 2.03 |
| | T3, R3 | 5.73 | 3.25 | 2.07 |

T= Treatments, R= Replicas, ND= Not detected

Figure 2 shows comparative effect of different doses on specified organ tissues of *Labeo rohita*. According to it, bioaccumulation of heavy metal (Pb) in all specified organ tissues increased by increasing the doses of heavy metal. Although, Pb accumulation was noted more in gills as compared to liver and muscles because gills remain in direct contact with water all the time in comparison to any other organ.

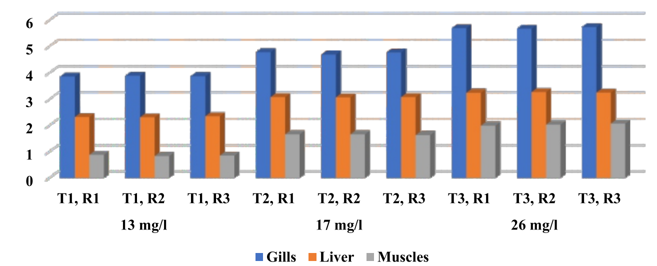


**Figure 2:** Comparative Effect of Different Doses of Pb on Different Organ Tissues of *Labeo rohita* (Gills, Liver, and Muscles)

## DISCUSSION

During the present study the 96hr LC50 of Lead Chloride ($PbCl2$) for the fish, *Labeo rohita* was found to be 52.20 mg/l. Although this value was not in line with previous studies, as 96-h LC50 value of lead for *Labeo rohita* were reported 30 mg/l and 34.20 mg/l by previous authors [11, 18]. But acute toxicity was found dependent on different factors like amount of dose provided, age, sex, feeding habit, size of fish and duration of exposure of metal to animal [19, 20]. Finney's Probit Analysis was used for the evaluation of acute toxicity response given 96hr-LC50 value for *Labeo rohita* exposed to $PbCl2$ [15]. Bio-accumulation of lead varied in different organ tissues of *Labeo rohita*. Various factors (physicochemical properties of water, species, developmental stage, sex, metal and its concentration) control the accumulation of heavy metals and their toxic effects in fish tissues [21]. During present study as the concentrations of metal in test medium increased the bio-accumulation pattern of Pb also increased in all organ tissues of fish. This pattern is also reported by previous researchers [22]. The gills of fish showed more lead accumulation, whereas, muscles exhibited least accumulation of metal. These findings were also reported by previous researchers [23, 24]. In previous studies liver and gills of the *Labeo rohita* were the main organs which

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showed greater tendency for the metal accumulation as compared to muscle tissues. So, our findings were in accordance with previous research work [25, 26]. Gills remained in direct association of metal (Pb) might be the reason of the elevated level of lead accumulation in the gill's tissues. According to earlier authors fish gills might be the main site for the intake of water borne contaminants [27, 28]. High accumulation of lead in liver might be due to the fact that liver plays a significant role in detoxification. Metal concentrations are normally lowest in muscle tissues as compared to liver and gill tissues because during physiological activities in fish metabolism heavy metals usually target metabolically active tissues. Metabolic activity in muscles is relatively low and there is no risk involve regarding Pb for human consumption of flesh [29].

## CONCLUSIONS

$LC_{50}$ values are very useful in finding sub lethal concentrations of a metal. Gills, liver and muscles are evaluated for bioaccumulation of heavy metal lead (Pb). Among the three organs the levels of Pb followed the order: Gill> liver> muscles. In conclusion, by considering the hazardous effects of heavy metals on freshwater ecosystems and human health it is pertinent to record the levels of heavy metals in edible fish species such as *Labeo rohita* to understand and manage the alarming levels of pollution.

## Authors Contribution

Conceptualization: MAR
Methodology: MAR
Formal analysis: AH
Writing, review and editing: SAH

All authors have read and agreed to the published version of the manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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