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Bioaccumulation of Aluminium Chloride in Different Organs of Freshwater Fish *Labeo rohita* (Ham.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present research work was carried out to determine the bioaccumulation of aluminium chloride in the gills, liver, Kidney and muscles of *Labeo rohita*. Sub-lethal concentration (LC_{50}) for 96 hours of exposure was found to be 32.5 ppm. The accumulation of heavy metal gradually increases in liver during the heavy metal exposure period. In the present study the accumulation of aluminium chloride was not found in tissues like gill, liver, kidney and muscle in control fish, whereas in treated fish the accumulation was found in the following order of liver>kidney>gill>muscle. Changes in the aluminium chloride content of muscle tissue in treated fish against the control fish were statistically significant at P<0.05. In all heavy metal, the bioaccumulation of aluminium chloride proportion was

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significantly increased in the tissues of *Labeo rohita*. This study revealed the accumulation of heavy metals in fish *Labeo rohita* which may act as an indicative measure to address the production, bioaccumulation and health hazardous issues of heavy metals.

Keywords: Labeo rohita; aluminium chloride; liver; gill.

1. INTRODUCTION

"The environmental problems have always existed throughout human history but widespread recognition has yet to come. Pollution of environment by heavy metals is of prime importance and unrestrained release of heavy metals into environment via discharge of industrial effluents, sewage and agrochemicals into the water resources has not only rendered it unusable but at the same time resulted in great harm to fishes and related aquatic organisms" [1].

"Water is one of the most common and the most precious resources on earth, without which there would be no life on earth. Water is essential to all forms of life and makes up 50-97% of the weight of all plants and animals and about 70% of human body" [2]. Pollution is a serious problem as nearly 70% of India's surface water resources and ground water reserves have been contaminated by organic, inorganic and biological pollutants.

Heavy metal in the aquatic ecosystem occurs in the sediments and the suspended particulate matter [3,1] mainly because of increasing mining operations and industrial uses [4]. "Heavy metal concentration of the environment resulting from anthropogenic activities such as mining operations and industrial activities is of concern since they exhibit consistent behaviour with those of persistent chemicals" [5].

"Inhaling aluminium chloride can irritate the nose, throat and the lungs. It is corrosive and contact can severely irritate and burn the skin and eyes with possible eye damage. Higher exposures may cause a build-up of fluid in the lungs, a medical emergency. Repeated exposure to aluminium chloride may cause scarring of the lungs, reactive and a dangerous explosion hazard" [1].

"Due to deleterious effects of metals on aquatic organisms, it is important to monitor their bioaccumulation patterns. This will give an indication of temporal and spatial extant of metal accumulation, as well as assessment of potential impact on human health" [6]. "Fish being the top consumer in the aquatic food chain accumulate large amounts of heavy metals in their body" [7]. "In its early life stages rohu prefer zooplankton, mainly composed of rotifers and cladocerans, with phytoplankton forming the emergency food. In the fingerling stage, there is a strong positive selection for all the zooplanktonic organisms and for some smaller phytoplankters like desmids, phytoflagellates and algal spores. On the other hand, adults show a strong positive selection for most of the phytoplankton. In the juvenile and adult stages rohu is essentially an herbivorous column feeder, preferring algae and submerged vegetation. It is a mid-water feeder. The nibbling type of mouth with soft fringed lips, sharp cutting edges and absence of teeth in the bucco-pharyngeal region helps the fish to feed on soft aquatic vegetation which do not require seizure and crushing. The modified thin and hair-like gill rakers also suggest that the fish feed on minute plankton through sieving water. In ponds, the fry and fingerlings exhibit schooling behavior mainly for feeding" [1].

"The accumulation effect of some heavy metals, especially through the food chain, their bioavailability needs to be monitored. Through analysis of metal concentrations in living organisms, it is possible to deduce the bioavailability and by presumption, the level of environmental pollution by specific metals" [1]. Therefore, bioaccumulation of metals in fish can be considered as an index of metal pollution in the aquatic bodies that could be a useful tool to study the biological role of metals present at higher concentrations in fish organs gill, liver, kidney and muscle.

2. MATERIALS AND METHODS

2.1 Preparation of the Stock Solution

Aluminium chloride stock solution was prepared by dissolved 4.9 gram of aluminium chloride in 1 litre of distilled water, since 4.9 g of aluminium chloride = 1 g and this is obtained by dividing the molecular weight of aluminium chloride by

molecular weight of aluminium. The molecular weight of aluminium chloride = 133.341 and the molecular weight of aluminium is 26.982. 1ml of stock solution was made to 1 litre with distilled water to have the concentration 1 ml = 1ppm.

2.2 Experimental Methods

Labeo rohita weighing 6.5-7.5g were divided into three groups. Six fishes were included in each group. The following experimental groups were conducted in the freshwater fish *Labeo rohita* for the period of 28 days. In the present study two different concentrations of aluminium chloride has been used for the exposure and mention as lower and higher concentration. The 1/10th of the LC_{50} value of aluminium chloride given as lower concentration (3.3ppm), the 1/4th of the LC_{50} value of aluminium chloride given as higher concentration (8.25ppm).

Group I : The first group was maintained free from aluminium chloride and served as the control.

Group II : Fish exposed to 1/10th of the LC_{50} of lower sublethal concentration of aluminium chloride for the period of 7th, 14th, 21st and 28th days.

Group III : Fish exposed to 1/4th of the LC_{50} of higher sublethal concentration of aluminium chloride for the period of 7th, 14th, 21st and 28th days.

Fishes were exposed to sublethal concentrations of aluminium chloride separately in plastic troughs (10 L of water was filled in the respective troughs of 15 L capacity) and control fishes were also maintained separately. They were fed on *ad libitum* diet of rice bran and oil cake. The medium was renewed daily with sublethal concentration of the aluminium chloride. After the exposure period, *Labeo rohtia* were sacrificed and the gill, liver, kidney and muscle were removed for bioaccumulation examinations.

Bioaccumulation of aluminium chloride in the tissues was determined following the method of Kendall and Scanlon [8]. “The tissues were dried separately in hot air oven at a temperature of 60 °C for 24 hours. The dried materials were powdered using a pestle and mortar. 500ml of powdered samples from each tissue were digested with a mixture of nitric acid and perchloric acid in the ratio 3:1 until it was almost dry and colourless. The final products were made upto 25 ml with double distilled water and the concentration of aluminium chloride was analysed using an atomic absorption spectrophotometer (AAS)” (Perkin-Elmer Model 2380).

3. RESULTS

The bioaccumulation of aluminium chloride in the gill, liver, kidney and muscle tissues of the freshwater fish *Labeo rohita* increased with the duration of exposure periods and concentration (Table 1) The results are given in parts per million (ppm).

3.1 Gill

In the present investigation the accumulation of aluminium chloride was found in the gill, liver, kidney and muscle tissues in treated fish. In control no accumulation was found. On exposure to lower concentration of aluminium chloride, the gill tissues showed 1.18±0.01, 1.20±0.02, 1.34±0.01 and 1.53±0.02ppm at 7th, 14th, 21st and 28th days of exposure respectively (Fig. 1). During the high concentration exposure, it was observed to be increased as 1.34±0.12, 1.78±0.23, 1.84±0.61 and 2.67±0.07ppm at 7th, 14th, 21st and 28th days of exposures respectively (Fig. 2). Changes in the accumulation of aluminium chloride content of gill tissue in the treated fish against the control fish were statistically significant at $P<0.05$.

3.2 Liver

In liver tissue the aluminium chloride accumulation was found to be increased than the gill tissue. On the exposure of lower concentration of aluminium chloride on liver, the aluminium level was determined as 1.67±0.01, 1.79±0.00, 1.83±0.01and 1.99±0.02ppm at 7th, 14th, 21st and 28th days of exposure respectively (Fig. 1). During the high concentration exposure, it was observed to be as 2.17±0.15, 2.11±0.01, 2.68±0.34 and 2.76±0.56ppm at 7th, 14th, 21st and 28th days of exposure respectively (Fig. 2). Changes in the accumulation of aluminium content of liver tissue in the treated fish against the control fish were statistically significant at $P<0.05$.

3.3 Kidney

On the exposure of lower concentration of aluminium chloride, in the kidney tissues, aluminium chloride level was determined as 1.43±0.00, 1.47±0.01, 1.55±0.05 and 1.76±0.01ppm at 7th, 14th, 21st and 28th days of exposure respectively (Fig. 1). During the high

concentration exposure, it was observed to be as 2.36±0.04, 2.42±0.61, 2.51±0.55 and 2.64±0.13ppm at 7th, 14th, 21st and 28th days of exposure respectively (Fig. 2). Changes in the aluminium content of kidney tissue in treated fish against the control fish were statistically significant at P<0.05.

3.4 Muscle

In muscle tissue the accumulation of aluminium chloride was found in both concentrations of exposure. On the exposure of lower concentration of aluminium chloride, on muscle tissues, the aluminium level was determined as 0.27±0.01, 0.31±0.03, 0.33±0.01 and 0.35±0.05ppm at 7th, 14th, 21st and 28th days of exposure respectively (Fig. 1). During the high concentration exposure, it was observed to be as 0.42±0.21, 0.52±0.33, 0.63±0.23 and 0.87±0.26 ppm at 7, 14, 21 and 28th days of exposure respectively (Fig. 2). Changes in the aluminium chloride content of muscle tissue in treated fish against the control fish were statistically significant at P<0.05.

In the present study the accumulation of aluminium chloride was not found in tissues like gill, liver, kidney and muscle in control fish, whereas in treated fish the accumulation was found in the following order of liver>kidney>gill>muscle

Table 1. Bioaccumulation of aluminium chloride (ppm) in *Labeo rohita* exposed to lower and higher concentrations of aluminium chloride

S. No	Tissues	Control	Experimental concentration	Exposure period (Days)			
				7	14	21	28
1.	Gill	0.00±0.00	Lower	1.18±0.01 a	1.20±0.02 a	1.34±0.01 b	1.53±0.02 c
			Higher	1.34±0.12 a	1.78±0.23 b	1.84±0.61 b	2.67±0.07 c
2.	Liver	0.00±0.00	Lower	1.67±0.01 a	1.79±0.00 a	1.83±0.01 a b	1.99±0.02 b
			Higher	2.36±0.04 a	2.11±0.01 b	2.68±0.34 c	2.76±0.56 c
3.	Kidney	0.00±0.00	Lower	1.43±0.00 a	1.47±0.01 a	1.55±0.05 a	1.76±0.01 b
			Higher	2.17±0.15 a	2.42±0.61 b	2.51±0.55 b	2.64±0.13 b
4.	Muscle	0.00±0.00	Lower	0.27±0.01 a	0.31±0.03 b	0.33±0.01 bc	0.35±0.05 c
			Higher	0.42±0.21 a	0.52±0.33 b	0.63±0.23 c	0.87±0.26 d

Each value is mean ± SD for six fishes in each group. In each group, means with different superscript letter (a–d) differ significantly at p< 0.05 (DMRT

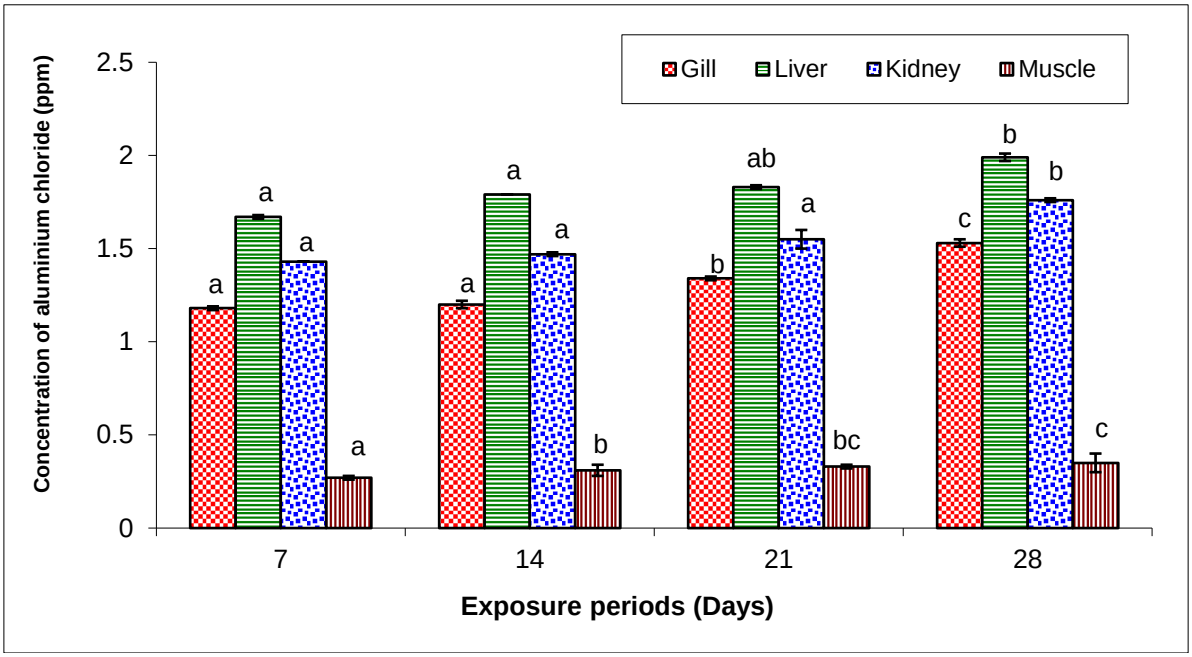

Fig. 1. Bioaccumulation of aluminium chloride (ppm) in *Labeo rohita* exposed to lower concentrations of aluminium chloride

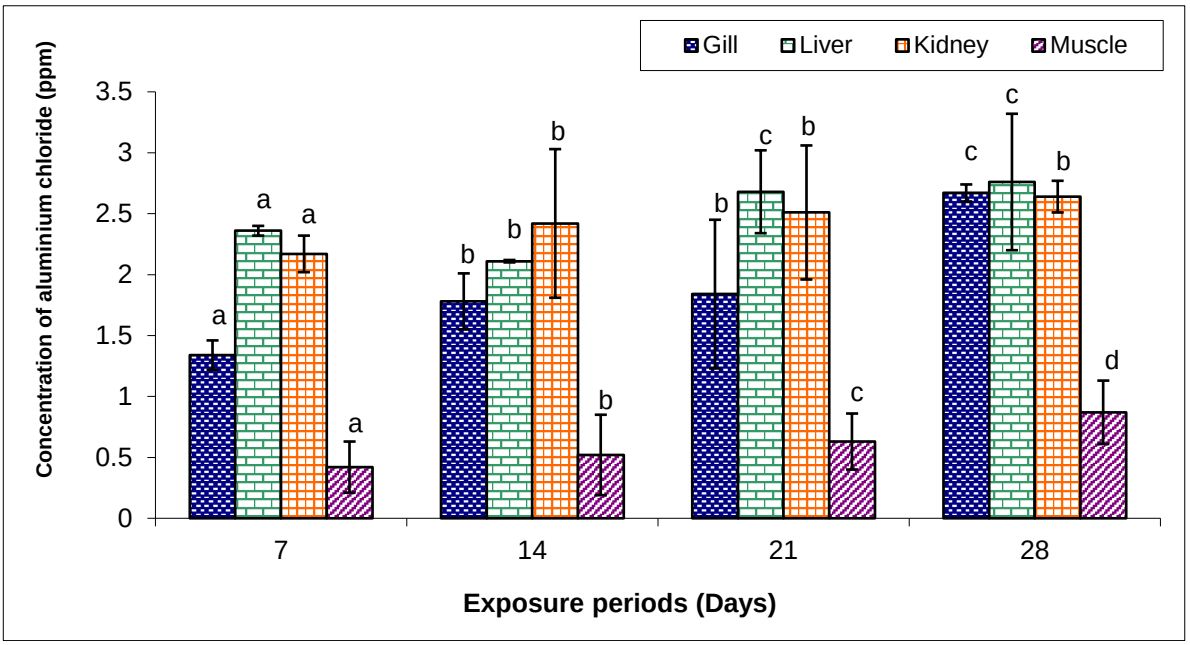

Fig. 2. Bioaccumulation of aluminium chloride (ppm) in *Labeo rohita* exposed to higher concentration of aluminium chloride

Each value is mean ± SD for six fishes in each group. In each group, means with different superscript letter (a–d) differ significantly at p< 0.05 (DMRT)

4. DISCUSSION

"Accumulation of metal in living organisms depends on the concentration of metal taken up by the organism from their surroundings and mechanism of metal's distribution in their body organs and the inherent ability of fish to concentrate metal" [9]. Azmat et al. [10] reported "the accumulation of Al, As, Ba, Cr, Ni and Zn that was significantly higher in liver and kidney while it was least in muscles and fats of fish". This has been confirmed in other studies assessing the toxic effect of aluminium on fish species [11].

"These variations among ability of different fish species to accumulate metals in their bodies appeared to be species specific" [12]. "Liver plays a central role in detoxification and accumulation of heavy metals" [13]. "Synthesis of metallothioneine, a metal binding protein, is induced in fish due to elevated levels of heavy metals. This protein helps in detoxification and accumulation of metal ions in liver" [14]. Fish exposed to water borne heavy metals generally showed higher metal load in gills than digestive tract because of their direct contact to the water-borne metals. Ahmed and Bibi [15] stated that "waterborne heavy metals exposure caused marked hypersensitivity in fish" [16]. "Metals may enter the fish through contaminated water and food intake and start accumulating in liver, kidney, gills, skin, fins, muscles and bones" [17].

Severe variations occurred in the pattern of aluminium chloride accumulation in different tissues of freshwater fish *Labeo rohita* after the exposure of lower and higher concentrations for a period of 7th, 14th, 21st and 28th days. Among four different organs studied liver was found to be the target organ of accumulation of aluminium chloride followed by liver, kidney, gill and muscle tissues respectively. Similar finding was reported by Murphy et al. [18]; Hofer et al. [19]; Seymore, [20] on exposure of zinc.

"Aluminium entered through gill and accumulated in liver tissue on longer period of exposure, which can be regarded as an indicator of cumulative contamination" [21]. Similar results were reported by Sivakumar and Khatiwada [22]. "The high accumulation of these metals in the liver may be related to the fact that the liver plays an important role in accumulation and detoxificaiton. Exposure of fish to elevated levels of heavy metals induces the synthesis of metallothioneins proteins (MT), which are metal binding proteins" [23,24,25].

The differences in the level of accumulation in the different organs of the test fish can primarily be attributed to the differences in the physiological role of each organ [26,12,27].

Azmat et al. [10] reported that for both 96hour LC_{50} and lethal concentrations, *Catla catla* were reported to be significantly more susceptible to aluminium toxicity, followed by that of *Labeo rohita* and *Cirrhina mrigala.*

After liver, kidney is one of the major excretory organs where aluminium chloride was highly accumulated. In the present study it was observed that as the exposure time increased the concentrations of aluminium chloride deposit increased in 28th day followed by 21st, 14th and 7th days.

5. CONCLUSION

Environmental pollution has become a global problem and heavy metals are considered as one of the most contaminants due to their bioaccumulation nature. Present results show that aluminium chloride accumulated in fish body organs varied significantly. *Labeo rohita* showed significantly higher tendency for Aluminium chloride accumulation. Fish liver and kidney exhibited significantly higher tendency while gill and muscle accumulated lesser Aluminium chloride. The bioaccumulation of these toxic metals has severely affected the normal physiology of fish, reducing the growth and reproductive of fish. This study revealed the accumulation of heavy metals in fish *Labeo rohita* which may act as an indicative measure to address the production, bioaccumulation and health hazardous issues of heavy metals.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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