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ORIGINAL ARTICLE

Assessment of Behavioral Alterations in *Labeo rohita* Due to Acute Exposure of Heavy Metals

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ABSTRACT

Various pollutants are being introduced into aquatic ecosystems both directly or indirectly as a result of industrialisation and urbanisation. Heavy metal contamination in freshwater bodies is of great concern owing to their toxicity, persistence and bioaccumulation. In toxicology, behavioural bioassays are used as a tool to assess heavy metal toxicity. Even at very low doses, chemicals can cause organisms to exhibit rapid behavioural responses. The current study deals with the acute toxicity of cadmium, zinc and their mixture to freshwater fish, *Labeo rohita*. Static bioassay tests were carried out to evaluate LC_{50} value of Cadmium (Cd), Zinc (Zn) and a mixture of both metals (Cd+Zn) for freshwater fish, *L. rohita* as well as the behavioural responses were also observed. Fishes were treated with various concentrations of metals for different exposure periods and per cent mortality was recorded. The objective of this study was to grasp the link between mortality and abnormal behavioural alterations of *L. rohita* exposed to $CdCl_2$, $ZnCl_2$ and a mixture of both metals ($CdCl_2$+ $ZnCl_2$). The major behavioural responses observed during the experiment were restlessness, jumping, erratic swimming, gulping of air at the surface, loss of equilibrium, sluggishness, opercular movements and fishes lying on the water surface before death etc. were observed in exposed fishes. The observed data showed that *L. rohita* can be used as a good bio-indicator for heavy metal contamination in freshwater bodies. Thus, a behavioural bioassay is more promising than lethality evaluating bioassays, which are currently employed for toxicant risk evaluation.

***Key words*:** Behavioral Alterations; Behavioral Bioassay; Heavy Metal Contamination; *Labeo rohita*

1) INTRODUCTION

Now a days an increasing ecological and public health concern has been associated with contamination of food and water by heavy metals. An aquatic ecosystem is the ultimate recipient of almost every pollutant coming from different anthropogenic sources like dumping of hospital remains, idol immersion, accidental spillage of chemical wastes, recreational activities, sewage drainage etc. [1], due to which the aquatic organisms are affected by numerous pollutants such as heavy metals and are always at risk. The contamination of these heavy metals has devastating effects on the diversity of aquatic organisms and ecological balance of the recipient water bodies. Because of their long persistence, toxicity, non-biodegradable properties and bioaccumulation in the food chains, the heavy metals comprise a core group of the aquatic pollutants. Aquatic ecosystems contained these metals in combined form due to diversified sources of their discharge [2]. Metals in combined form showed much varied effects than in single form [3]. The complex mixtures of toxicants in water bodies may cause injurious effects on the water living animals [4]. Water pollution mainly affects the aquatic animals in various ways such as biochemical or physiological disturbance in response to toxicity caused by those pollutants [5]. The application of cadmium-containing fertilizer, agricultural chemicals, herbicides, and sewage sludge to farmland may also contribute to water contamination [6]. Cadmium is thought to have the ability to change aquatic trophic levels over centuries [7]. Zinc is an essential element and is one of the most common heavy metal pollutants. Zinc is a possible toxicant to fish [8] that disrupts acid-base and ion balance, disrupts gill tissue and causes hypoxia [9]. Zinc can cause sub-acute effects that alter fish behavior [10]. Fishes are an easy test subject for determining the health of an ecosystem since they are more susceptible to many toxicants than invertebrates [11]. The fish as a bioindicator species plays an increasing important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment [12,13].

Fish can adapt to disruptions by evoking nonspecific responses in response to physical, chemical, and perceived

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stressors. It is necessary to establish a relationship between subcellular reactions and behaviour, chemical stress, and higher levels of biological organisation. Because behaviour is influenced by both internal and external processes, modifications in these variables can provide insight into the health and viability of naturally occurring populations exposed to xenobiotics. Fish respond in an unspecific way to stressors in order to adapt or deal with the disturbance. But if stress persists for a long time, fish health may be in danger [14]. Any modification in fish behaviour provides knowledge and information on behavioural changes that may be connected to physiological biomarkers in aquatic species [15]. Behavior connects ecological processes and physiologic function together and it is susceptible to chemical exposure and environmental stressors. The use of behavioural changes in organisms in response to pollutant to improve the estimation of ecologically appropriate risk end points are progressively investigating in ecotoxicology.

It has been observed and reported that most of the fishes are competent of accumulating heavy metals in their tissues many times higher than their current levels in water through consumption of contaminated sediments and foods and by absorption through their gills. Consuming such fishes that are contaminated with heavy metals can lead to devastating effects by causing many health hazards such as neurodegenerative diseases, chromate nephropathy, hepatic failure, cardiovascular diseases etc. When the animal is exposed to a chemical concentration lower than that which can result in mortality, changes in behaviour can be seen, indicated that multidisciplinary research is needed to improve the value and importance of behavioural indicators. The main objectives of the present study were to determine behavioural alterations and 96 hrs LC_{50} values. The animal used for this study was *Labeo rohita* fish. *Labeo rohita* (Rohu), is a species of carp family, widely distributed in Asian countries, like in India, Pakistan, Sri Lanka, Bangladesh, Myanmar, and Nepal [16].

2) MATERIALS AND METHODS

Fish Handling and Acclimatization

Labeo rohita were collected from the Ganga River at Kachchla, Badaun (U.P.) and brought to the laboratory. Fishes transferred to the glass aquariums of 40 litres of capacity containing tap water for acclimatization period of 15 days in the laboratory. Proper aeration was maintained in experimental as well as control aquaria by air pumps throughout the experiments. The fishes were fed with fish food and water in the aquaria was changed at every 24hrs, leaving no fecal matter, unconsumed food or dead fish.

Experimental Design

Fishes selected for the experiment were 12-16 cm in length and 100-120 gm of weight and divided into four equal groups each comprising 10 fish. The first group was kept as control and was maintained in normal tap water without any treatment. Treatment groups were exposed to sublethal concentration of metals for acute exposure i.e. 96 hrs. The second and third group was exposed to sublethal concentration of cadmium and zinc respectively while the fourth group was exposed to sublethal concentration of mixture of both metals i.e., cadmium+zinc. Water was replaced once in 48hrs exposure period.

Preparation of Metal Solution:

Chemically pure chloride compounds of metals, Cadmium and Zinc were dissolved, separately, in deionized water and stock solutions were prepared for required metals and their mixture concentrations (1:1 ratio) on metallic ion equivalence basis.

Estimation of 96hr LC_{50}:

The 96 h LC_{50} value of Cadmium Chloride, Zinc Chloride and mixture of both metals was determined by static renewal bioassay following probit analysis [17]. Different concentrations of Cadmium Chloride and Zinc Chloride were prepared by dissolving weighed amount of $CdCl_2$, $ZnCl_2$ and mixture of both metals in tap water. In a gradual manner the concentration of each test media was increased and as per mortality rate from 0 % to 100%, the amount of metal concentration was maintained. Fishes were exposed to metal concentrations to know the acute toxicity at different exposure period i.e., 24, 48, 72 and 96 hrs. The number of dead fishes were counted every 24 hr and removed immediately from the test media to avoid any organic decomposition and oxygen depletion.

Mortality rate at different concentrations and at different times of exposure was analyzed by using the computer software SPSS 21. The actual LC_{50} data was analyzed using Probit analysis with SPSS 21 statistical analysis software. In the parametric procedure and long history of statistical applications, Probit analysis is based on linear regression technique following transformation of toxicity data. The LC50 value (with 95% confidence limits), the correlation between mortality (Probit) against the $\log_{10}$ concentrations and the regression line equation were also obtained through best-fit line.

For studying the behavioral alterations, fishes were divided into two groups i.e., control and experimental group. Experimental group was exposed to LC_{50} dose. The behavioral alterations were recorded simultaneously at different exposure. In order to maintain the concentration of metal and their mixture, the water in the aquaria was changed every 24 hrs during the acute exposure.

3) RESULTS AND DISCUSSION

Due to anthropogenic activities, fishes are one of the vertebrates' groups that respond firstly when the environment is contaminated with pollutant [18]. Fishes are an important indicator of water pollution as it remains in direct water for food and oxygen and thus is highly susceptible to any change in aquatic environment. Cadmium does not break down in the environment and persist in the fish body for long periods and can bio-accumulate for many years after exposure to low levels of this metal [20]. As, fishes are the part of human nutrition as well as important source of protein. So, if human consume heavy metal contaminated fishes, they can cause heavy metal accumulation in the human body. Therefore, heavy metal contaminated fish need to be carefully screened before consumed by humans.

96 hrs LC_{50} Estimation

In present study, acute toxicity test was conducted. The test fish (*Labeo rohita*) was exposed to heavy metals i.e., Cadmium as cadmium chloride, Zinc as zinc chloride and mixture of both metals upto 96 hrs. The percent mortality rate for different concentrations of cadmium chloride, zinc chloride and mixture of both metals are given in Fig.1-3 and Table 1-3. No mortality was observed over 96 hrs in control group fishes. The lowest cadmium chloride concentration at which mortality observed was 55 mg/l (Table.1) and the first death of experimental fish was recorded in 70 mg/l at 24 hrs exposure period. 96 hrs LC_{50} value was found to be 58.88 mg/l for cadmium chloride (Fig.1). The lowest zinc chloride concentration was 30 mg/l (Table. 2) and the first death of experimental fish was recorded in 50 mg/l at 24 hrs exposure. 96 hrs LC_{50} value was found to be 37.15 mg/l for zinc chloride (Fig.2). The lowest concentration for mixture of both metals at which mortality observed was 40 mg/l (Table. 3) and the first death of experimental fish was recorded in 80 mg/l at 24 hrs exposure. 96 hrs LC_{50} value was found to be 50.11 mg/l for mixture of both metals (Fig.3). The evaluation of LC_{50} was done by using Finney's Probit analysis (1971) method. This variation in LC_{50} value may be due to change of fish species, geographical area as well as metal toxicity [21]. The mortality of the fish, *Labeo rohita*, increased with increasing concentrations of $CdCl_2$, $ZnCl_2$ and mixture of metals ($CdCl_2$+$ZnCl_2$) and duration of exposure. The LC_{50} and lethal value (96 h) for *C. mrigala* was reported as 47.56±0.04 and 93.89±0.07 mg/l, respectively by [22]. The 96 h LC_{50} value for *C. mrigala* (90-day old) was 32.68, 40.58, 23.96 and 71.24 mg/l of Pb, Zn, Ni and Mn respectively, reported by [23]. [24] found the 96 h LC_{50} and lethal concentrations of Fe+Zn+Pb+Ni+Mn mixture for *C. mrigala* as 43.35±0.78 and 75.22±0.45 mg/l, respectively.

Behavioural Alterations

Behavioral abnormalities have been found most responsive signs of chemically induced stress in aquatic organisms [22]. In current study, when *Labeo rohita* was subjected to different heavy metal concentrations for acute toxicity test period (96 hrs), it was reported that exposure to increasing amounts of metals resulted in increased mortality and caused various behavioral changes. In both the control and the test aquaria, the behavior and condition of fishes were observed during the whole experiment. When *L. rohita* exposed to sublethal concentration of Cd, Zn and mixture of both metals, they showed marked changes. In control group, no behavioral alterations were reported. After introducing the fishes to the test aquaria, they showed alter behavior such as hyperactivity, restlessness, increased swimming rate, loss of equilibrium, mucous secretion, drowning, hitting against the wall of test aquaria and trying to jump out of the test aquaria to avoid the chemical (Table 4-6). These activities were increased with increasing the concentration of Cd, Zn and mixture of both metals. Lastly fish became settled at the bottom and before death all activities were stopped. Behavioral activities increased upto 96 hrs of exposure period. After few hours of the introduction of the fishes to the test aquaria, they became lethargic and settle down at the bottom of the tank. The discomfort seems to be higher upto 96 h of exposure. However, it was observed that whenever water was changed in the test aquaria, the fishes showed normal behavior for a few minutes to an hour and after some time they became lethargic. The relation of avoidance reaction may be related to change in sensitivity of chemoreceptors [25]. The survival and reactance of aquatic organism mainly depends on the physico-chemical parameters of water and biological conditions of organisms [22]. In addition, it also depends upon toxicity, type and nature of toxicants [26]. According to [27] modifications in behavioral patterns of organisms is the major responsive sign of anxiety caused by contact to chemicals.

Loss of balance during swimming, observed during this study, might be due to some neurological impairment in the central nervous system. Similar results were reported by [28]. Fast opercular movements, gulping of air and surfacing was also observed in present study. Surfacing or gulping of air might occur due to a demand of higher oxygen level after exposure [29]. [30] and [31] also reported that with the increase of concentration of cadmium compounds on freshwater air breathing snakeheaded fish *C. punctatus*, it showed various behavioral responses and morphological changes.

The acute impacts of cadmium and copper on behavioral responses of *C. marulius and W. attu* were studied by [26]. Fishes showed hyperactivity, increased surface behavior and erratic swimming, during the acute exposure of both metals. These results are also similar to the findings of [32], under the exposure of high concentration of cadmium chloride, fish (*Chanos chanos*) showed behavioral changes such as swimming disorder and fin movements[33] also reported exposure of fenvalerate change the behavior such as swimming at the water surface, increased mucus production and hyperexcitation of *C. catla*, *L. rohita* and *C. mrigala*. Hyperactivity, irregular swimming, restlessness and loss of balance are common behavioral responses in various species of fishes exposed to a variety of toxicants as recorded in *L. rohita* exposed to cypermethrin [34] and in *Rasbora daniconius* exposed to zinc [35]. The abnormal behavior like hyperactivity was dose and duration dependent [36]. In present study, other changes observed were hyper excitability, disturbed schooling, disrupted behavior, reduced feeding behavior and after few hours became lethargic. Sluggishness observed at the end of exposure periods may be due to loss of energy as a result of erratic swimming, jumping and restlessness. Behavioral alteration is considered as a sensitive biomarker to evaluate the effect of toxicant [37].

Table 1: Mortality of *L. rohita* in different concentration of Cadmium Chloride at 96 h exposure period.

Concentration of $CdCl_2$ (mg/l)	Log_{10} Conc.	Mortality	Probit Value
Control	-	-	-
50	1.69897	00	00
55	1.740363	40	4.75
60	1.778151	60	5.25
65	1.812913	90	6.28
70	1.845098	99.9~100	8.09

Table 2: Mortality of *Labeo rohita* in different concentration of Zinc Chloride at 96 h exposure period.

Concentration of $ZnCl_2$ (mg/l)	Log_{10} Conc.	Mortality	Probit Value
Control	-	-	-
30	1.477121	10	3.72
35	1.544068	20	4.16
40	1.60206	50	5.00
45	1.653213	70	5.52
50	1.69897	99.9~100	8.09

Table 3. Mortality of *L. rohita* in different concentration of Mixture ($CdCl_2$+$ZnCl_2$) at 96 h exposure period.

Concentration of Mixture ($CdCl_2$+$ZnCl_2$) (mg/l)	Log_{10} Conc.	Mortality	Probit Value
Control	-	-	-
40	1.60206	20	4.16
50	1.69897	50	5
60	1.778151	60	5.25
70	1.845098	80	5.84
80	1.90309	99.9~100	8.09

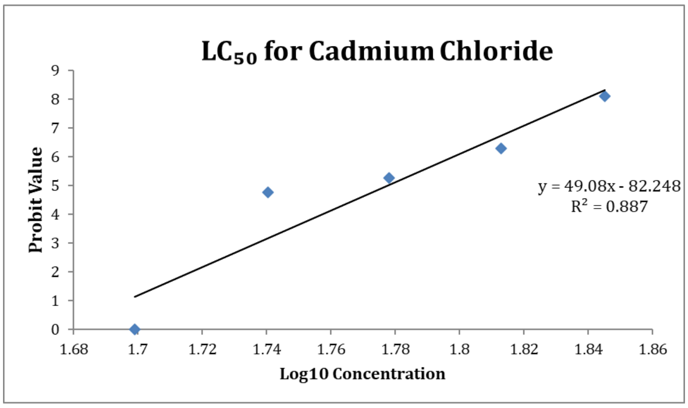

Fig. 1: Regression line between the Probit kill value of *L. rohita* at different log_{10} concentrations of Cadmium Chloride

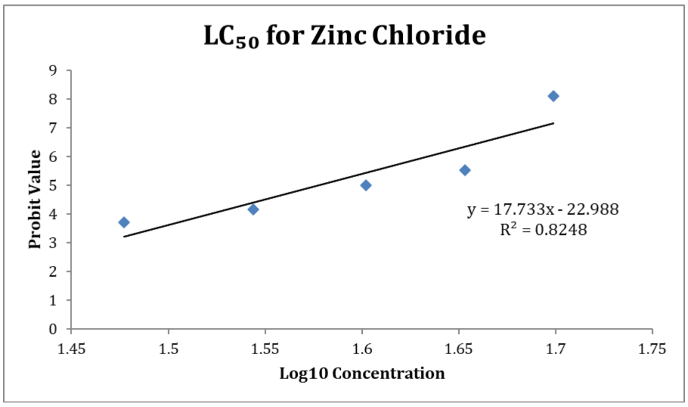

Fig. 2: Regression line between the Probit kill value of *L. rohita* at different log_{10} concentrations of Zinc Chloride.

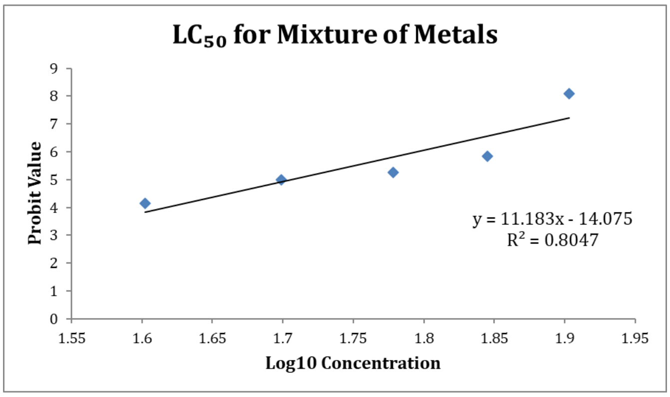

Fig. 3: Regression line between the Probit kill value of *L. rohita* at different log_{10} concentrations of Mixture of metals ($CdCl_2$ + $ZnCl_2$).

Table 4: Effect of sublethal dose of Cadmium Chloride ($CdCl_2$) exposure on Behavioural responses of freshwater fish *L. rohita*.

S. No.	Behavioral Changes	Control	Acute Exposure of $CdCl_2$			
			24 hrs	48 hrs	72 hrs	96 hrs
1.	Jumping	-	++++	++++	+++	+++
2.	Restlessness	-	++++	++++	+++	+++
3.	Erratic Swimming	-	++++	++++	+++	+++
4.	Loss of Equilibrium	-	+++	+++	+++	++
5.	Sluggishness	-	++++	+++	+++	++
6.	Gulping air at Surface	-	++++	++++	+++	+++

(-) Normal, (+++) Moderate Change and (++++) Prominent Change

Table 5: Effect of sublethal dose of Zinc Chloride ($ZnCl_2$) exposure on Behavioural responses of freshwater fish *L. rohita*.

S. No.	Behavioral Changes	Control	Acute Exposure of $ZnCl_2$			
			24 hrs	48 hrs	72 hrs	96 hrs
7.	Jumping	-	++++	++++	+++	+++
8.	Restlessness	-	++++	++++	+++	+++
9.	Erratic Swimming	-	++++	++++	+++	+++
10.	Loss of Equilibrium	-	++++	++++	+++	+++
11.	Sluggishness	-	++++	++++	+++	+++
12.	Gulping air at Surface	-	++++	++++	++++	+++

(-) Normal, (+++) Moderate Change and (++++) Prominent Change

Table 6: Effect of sublethal dose of mixture ($CdCl_2$+ $ZnCl_2$) exposure on Behavioural responses of fresh water fish *L. rohita*

S. No.	Behavioral Changes	Control	Acute Exposure of Mixture ($CdCl_2$+ $ZnCl_2$)			
			24 hrs	48 hrs	72 hrs	96 hrs
1.	Jumping	-	++++	+++	++	++
2.	Restlesness	-	++++	++++	+++	++
3.	Erratic Swimming	-	++++	++++	+++	++
4.	Loss of Equilibrium	-	+++	+++	++	++
5.	Sluggishness	-	+++	+++	+++	++
6.	Gulping air at Surface	-	++++	+++	+++	++

(-) Normal, (++) Less Change, (+++) Moderate Change and (++++) Prominent Change

4) CONCLUSION

In present study, the acute toxicity of heavy metals and mixture of metals affects the behavior of fish. When compared to other standard testing, behavioural changes can provide early warning signs regarding the health of an exposed population. It was also concluded that behavior of fish can be taken as a sensitive biomarker in eco-toxicology and fish can be used as a good indicator of water contamination.

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