

Toxicity of two heavy metals Lead and Cobalt on Zebrafish, *Danio rerio*Chandra Bhushan Singh¹, Badre Alam Ansari^{1*}¹Zebrafish Laboratory, Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur - 273009 (U.P.), India***Corresponding author**

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Abstract: Heavy metals are considered as the most important pollutants, since they are present throughout the ecosystem and are detectable in critical amounts. Heavy metals, such as lead (Pb), copper (Cu), arsenic (As), zinc (Zn), chromium (Cr), nickel (Ni), cobalt (Co), cadmium (Cd), mercury (Hg), manganese (Mn) are the pollutants which affect aquatic environment and fish. The aim of the present study was to determine and compare the acute toxicity of two different heavy metals viz. Lead and Cobalt on zebrafish, *Danio rerio*. The mortality rate of zebrafish was monitored under laboratory conditions for the time period of 24, 48, 72 and 96-h. Data obtained from the toxicity test were evaluated using by the Stat plus[®] computer program. The 96-h LC₅₀ of lead is 21.63mg/L and for cobalt 69.83mg/L, it was found that lead is more toxic to zebrafish as compared to cobalt. Zebrafish can be used as bio-indicator to assess the heavy metals pollution in aquatic environment.

Keywords: Bioassay, Zebrafish, Heavy metals, LC₅₀, Toxicity, Bio-indicators

INTRODUCTION

Environmental pollution and its hazards are the most important problems of societies and living creatures. On the other hand, increased population with the development of technology and production can cause a lack of attention to environmental safety. Industrialization leads to the pollution of ecosystems. Industrial effluents are discharged into rivers and lakes from where it leaches into the soil and ground water, or is emitted

into the air as particulate matter [1, 2]. Heavy metals are critical in this regard because of their easy uptake into the food chain and of bioaccumulation process [2, 3]. Metals tend to accumulate in water [4, 5], but they may be released under certain physicochemical conditions, moving up through the food chain [6]. Some metals are essential for normal physiological functioning of fish but become toxic when they accumulate in their body tissues and are not metabolized. In addition, significantly higher concentrations of metals in fish can change its physiological functioning that could lead to high mortality and ultimately loss of fish biota [7]. The river system may be extremely contaminated with heavy metals released from domestic, industrial, mining and agricultural effluents. Heavy metal contamination may have disturbing effects on the ecological balance of the recipient environment and a diversity of aquatic organisms [8, 9, 10]. Among animal species, fishes are inhabitants that cannot escape from the detrimental effects of these pollutants [11]. The impact of metals, as well as other pollutants, on aquatic biota can be evaluated by toxicity test, which are used to detect and evaluate the potential toxicological effects of chemicals on aquatic organisms. However, little research has been done on the impact of contaminations on tropical ecosystems [12]. Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build

up in the food chain and are responsible for adverse effects and death in the aquatic systems. Fish can obtain their trace elements, either directly from the water through the gills or indirectly from food through the alimentary tract [13].

Heavy metals like Co, Zn, Ni, Cu, Se, Mn, Fe, and Cr are essential for the growth of organisms, while Pb, As, Hg and Cd are not only biologically non essential, but these heavy metals beyond optimum threshold levels found to be hazardous and toxic. After entering the water body these metals may participate, get absorbed on solid surface, remain soluble or suspended in water or taken up by fauna. The most important biological property of metal is their tendency to accumulate in the animal tissues.

Cobalt is a toxic element that exists in special fertilizers and waste water following the evolution of cobalt mines. Metals such as cobalt may cause environmental risk when occurring at raised levels [14,15], although cobalt is of relatively low abundance in the earth's crust and in natural waters.

Lead is highly toxic metal as it is reported to be responsible for death or sub-lethal changes in reproduction, growth and behavior of the fish [16].

Lead in the form of Pb^{2+} is most common and stable ion in aquatic environments and has strong tendency to get bio-accumulated in fish organs like gills, kidney, liver, muscles, scales and skin [17, 18]. Under the exposure of waterborne lead, fish exhibits a wide range of neurological and muscular abnormalities, growth inhibition, reproductive problems and mortality [19].

The term bioaccumulation refers to the wastes, which have been re-concentrated in organisms often having undergone initial dilution in environment producing toxic effects in fishes. Among the heavy metal pollutants, lead and cobalt receives a special attention due to its potential health hazard to aquatic fauna accumulation in freshwater fishes and human life in particular.

The present study was undertaken to investigate the toxicity of lead and cobalt on zebrafish, *Danio rerio*. The zebrafish is selected for this experiment because it has great benefits, with regards to high fecundity, small size approx 2-5 cm long, easy to breed in laboratory, short generation time, rapid development, translucent embryos and easy to maintain under laboratory conditions. *Danio rerio* was selected for the present study because they are model organism for toxicological research and also recommended by the Organization for Economic Co-operation and Development (OECD, 1992) [20].

MATERIALS AND METHODS

Zebrafish, *Danio rerio* were reported from Uttar Pradesh [21]. They were collected from the local ponds, stocked and acclimatized for a time period of 10-15 days under the laboratory conditions in glass aquaria containing dechlorinated water. The water of the aquarium was aerated continuously through stone diffusers connected to a mechanical air compressor. Water temperature maintained between 25 ± 2 °C. These conditions were sustained during the entire length of the experiments and the fishes were fed with commercial fish pellets, goat liver, tubifex worm and brine shrimps. The standard stock solution of lead was prepared from $C_4H_6O_4Pb \cdot 3H_2O$ (Lead acetate) and cobalt was prepared from $CoCl_2 \cdot 6H_2O$ (Cobalt chloride) in distilled water. All the chemicals used were analytical grade (S d Fine Chemicals Limited, Mumbai) using adult zebrafish about 3 to 3.5 cm body length.

Toxicity test was performed in laboratory to determine the 24, 48, 72 and 96 hour LC_{50} values using five concentrations of Pb 10, 15, 20, 25 and 30 mg/L and Co 60, 65, 70, 75 and 80 mg/L., both the solution were prepared in distilled water. The randomization of the fish in test aquaria was done according to the method prescribed by the U.S. Federal Water Pollution Control Administration, 1968. Ten fishes were used for each concentration. Water was changed daily with fresh

treatment of metals. Six replicates for each concentration were used accompanied by a control having no treatment. The control group was kept in water without adding the metals, keeping all the other conditions constant. The mortality was recorded at the end of 24, 48, 72 and 96 h. The feeding was stopped during the experiments. A fish was considered dead when its gill movement ceased and it did not respond to gentle prodding. Dead fishes were removed carefully from aquarium to avoid deterioration. The result were computed by Stat plus® 2009 computer software purchased from Analystsoft Vancouver, Canada. The LC_{50} values, upper and lower confidence limits (UCL and LCL), slope, Chi-square values were calculated. The behavioral changes in treated zebrafish were also observed during the entire exposure period of the heavy metals.

RESULTS AND DISCUSSION

After the exposure of both heavy metals, the zebrafish showed some behavioral changes, like erratic swimming and loss of equilibrium. They aggregated at one corner of the aquarium, resting at the bottom and frequently come to the surface followed by the heavy breathing with stronger opercular movement and over secretion of mucus from the body surface was observed. The zebrafish in control aquarium were observed to be healthy active, normal and no mortality was recorded.

The data obtained from the toxicity test of Pb and Co for zebrafish shows that the fish mortality increased with increasing concentration and exposure time. The estimated lethal concentration values for 24, 48, 72 and 96-h and their upper and lower confidence limits, slope values, and chi-square values on each metal are presented in table 1. The 24-h LC_{50} for cobalt was 90.04 mg/L while it was 69.83mg/L after 96-h of exposure. On the other hand the 24-h LC_{50} for lead was 41.27 mg/L and after 96-h it was 21.63 mg/L.

Heavy metals are produced from a variety of natural and anthropogenic sources [22]. In aquatic environments, heavy metal pollution results from direct atmospheric deposition, geologic weathering or through the discharge of agricultural, municipal, domestic or industrial waste products, also via wastewater treatment plants [23, 24, 25, 26]. The contamination of heavy metals and metalloids in water and sediment, when occurring in higher concentrations, is a serious threat because of their toxicity, long persistence, and bioaccumulation and bio magnification in the food chain [27, 28]. Bioaccumulation factor is the ratio of concentration of heavy metals accumulated in the tissue of fish with respect to the concentration of heavy metal in surrounding water and suspended food materials. Fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human beings through food causing chronic or acute diseases [29].

The fact is that metals are non biodegradable and can accumulate in the environment, make them deleterious to the aquatic organisms and consequently to human being who consume fish as a food source. The heavy metals in the aquatic system may accumulate in aquatic organisms through different routes such as direct uptake from water by gills or body surface [30].

The present study was initiated to find the susceptibility of the Zebrafish, *Danio rerio* to potentially hazardous heavy metals Lead and Cobalt. Fishes are confined exclusively to aquatic habitats, constitute the group most vulnerable to heavy metal toxicity. Aquatic toxicity test are used to evaluate the potential toxicological effects of environmental contaminants on aquatic biota [31]. It is necessary to study the presence of water borne heavy metals on tolerance of the most sensitive bio-indicators of aquatic pollution, particularly fish [32].

Lead is a naturally occurring heavy metal characterized as health hazardous substance [33]. In environment, concentration of Pb is significantly increased by anthropogenic sources such as fossil fuel burning, mining, battery manufacturing, metal product like solder and pipes for water supply, X-ray shielding devices, leaded gasoline, glass containers of food and beverages [34]. Uses of lead in the chemical industry for preparing paints, pigments and colored inks was widespread but many countries have now restricted their use [35]. The natural concentration of lead in surface water has been estimated at 0.02µg/l and it rarely exceeds a few micrograms/l. However, high levels of lead are associated with areas in the vicinity of lead mines and battery-producing industries.

Exposure to high lead levels in the aquatic system can cause generative damage and alteration in blood and nerves cells in fish and other aquatic organisms [36, 37]. Study of lead toxicity in the present day is very important because of its effect on human health [38, 39].

Ullah *et al.* [40] reported that 96-hr LC₅₀ of Lead Nitrate for the fish *Oreochromis niloticus* 44 mg/L. Batool & Javed [41] reported that with *Catla catla*, *Cirrhina mrigala* and *Labeo rohita*, 96-hour LC₅₀ of cobalt were 86.32±0.37, 117.39±0.36 and 106.12±0.38 mg/L. while for the lead with *Catla catla*, *Cirrhina mrigala* and *Labeo rohita*, 96-hour LC₅₀ were 31.25±0.22, 40.54±0.32 and 36.72±0.37 mg/L respectively. Cobalt appeared least toxic metal in comparison to lead. Ferrer *et al.* [42] has reported the 96-h LC₅₀ values 1093.40 µg/L for Pb, 219.20 µg/L for Cu and 172.10 µg/L for Zn in the early life stage of the crab *Chasmagnathus granulata*. Chinni & Yallapragda, [43] carried out acute toxicity test with metals (Pb, Zn, Cd and Cu) on *Penaeus indicus* post larvae. The

resulting 96-h LC₅₀ values showed that copper was the most toxic metal followed by that of cadmium, zinc and lead. LC₅₀ values were 2.535, 3.119, 6.223 and 7.283 mg/L respectively.

Similar results were also found by other researchers with different heavy metals for the same fish. The value of LC₅₀ may vary in the same fish for the same heavy metals determined by some scientists. This is attributed to the fact that several factors including differences in the test species, age, feeding habit, sex, composition of toxicant and also the experimental conditions under which the tests are performed.

Cobalt is an essential nutrient for man and is an integral part of vitamin B₁₂. It performs important biochemical function but its higher concentration in aquatic ecosystems becomes toxic to fish as it interferes with the enzyme systems [44]. It is reported to be a potential carcinogenic compound and has been included recently in group 2A carcinogens (*i.e.*, probably carcinogenic to humans). The maximum of authorized cobalt in the air, water and earth are 1-5 mg/m³, 1 mg/L and 1 mg/m², respectively. Cobalt concentration in rivers are low at about 0.2 mg/L [45]. In human the average daily intake of cobalt in all forms ranges from 0.30 to 1.77 mg/day [46]. It has also been implicated in blood pressure regulation [47], and has been found to be necessary for proper thyroid function [48]. Excessive ingestion of cobalt is reported to cause congestive heart failure, polycythemia and anemia [49].

Results of acute-toxicity test on marine fish have shown that cobalt has a low toxicity, with 96-h LC₅₀ ranging from 52.5 to more than 1000 mg/L [36]. Several studies reported the 96-h LC₅₀ values of CoCl₂ on *Pimephales promelas*, and *Carassius auratus*, rainbow trout (*Oncorhynchus mykiss*) were 21.8 mg/L by Ewell *et al.* [50], and 333 mg/L by Das & Kaviraj [51], and 1.4 mg/L by Marr *et al.* [52] respectively.

Nath & Kumar [53] found that Co at 2.18 mmol/L was lethal to 50% of *Colisa fasciatus* in 24 hour. In a study Jaseen, [54] found that 100% mortality on blind Mexican cave fish at very low concentration of cobalt *i.e.* 2 mmol/L within 14 hours of exposure period. Toxicity of cobalt to fish has been little studied because it is not a common contaminant in waterways. Naseem *et al.* [55] carried out acute toxicity test with metals (Cu and Co) on *Tilapia nilotica*. The resulting 96-hour LC₅₀ values showed that copper was the most toxic metal followed by that of cobalt. LC₅₀ values of copper and cobalt were 25±0.53 and 95±1.48 mg/L respectively. However, lethal concentrations of copper and cobalt for *Tilapia nilotica*, were 48±0.25 and 178±1.31 mg/L. In separate studies with *Cyprinus carpio*, Naji *et al.* [56] reported 96-H LC₅₀ of cobalt as

327.5 mg/L. Yaqub & Javed [44] reported that Indian major carps viz. *Labio rohita*, *Cirrhinus mrigala* and *Catla catla* showed more sensitivity towards cobalt than cadmium.

The toxicity of lead was significantly higher when compared to cobalt, after 96-h of exposure in zebrafish. It is therefore biologically reactive and gives rise to acute poisoning. This difference could be due to the biological diversity and functional variability of cells and tissues to chemical pollutants. Toxicity of metals may vary depending upon their permeability and detoxification mechanisms [57]. The results obtained in

this study clearly indicate that it is necessary to control the use of heavy metals especially lead.

The slope values are steep, which indicate that the test animals are very sensitive to even the minor change in concentration of the toxicant. The LC₅₀ values of metals showed a significant ($p < 0.05$) negative correlation with exposure time. The chi-square values are not significant indicating that the fish population used in the experiment was homogeneous (Table- 1). In the present study it was found that the *Danio rerio* was highly sensitive to lead than cobalt.

Table 1: Toxic effect of two different heavy metals lead and cobalt against zebrafish, *Danio rerio* (Cyprinidae)

Heavy metals	Exposure Period (h)	LC ₅₀ values (mg/L)	Confidence limits		Slope	Chi-square values
			LCL	UCL		
Lead	24	41.27	32.87	49.68	1.64	3.03
	48	35.78	29.02	42.53	1.90	1.35
	72	24.43	19.25	29.60	2.09	1.79
	96	21.63	15.89	27.37	2.16	0.89
Cobalt	24	90.04	89.92	98.17	1.28	0.15
	48	81.67	69.20	96.79	1.24	0.47
	72	75.44	68.92	81.96	1.21	0.09
	96	69.83	65.95	73.72	1.12	0.03

The slope value shown in the table one is steep. The LC₅₀ values of the heavy metals showed a significant ($p < 0.05$) negative correlation with exposure time. The chi-square values were not significant, indicating that the fish population used in the experiment was homogeneous.

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