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VALIDATION OF MEDIAN LETHAL CONCENTRATION OF LEAD CHLORIDE IN BANDED GOURAMI, *Trichogaster (Colisa) fasciata*, BY ACUTE TOXICITY BIOASSAY

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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

Industrial revolution in India has been useful in terms of national economy, but it has one major deleterious effect on the environment, as heavy metals that are toxic and utilized in industries in enormous scale, are alarmingly being discharged into aquatic environment and are found to cause serious adversities to the biotic organisms living in water like fish, molluscs. Lead chloride, an inorganic salt used in industries like batteries, petroleum, dye, chemical, mining in a major scale, is chosen as the toxicant for this study. *Trichogaster (Colisa) fasciata* (common name banded gourami), a fresh water fish of air-breathing habit having ornamental as well as nutritional values, is chosen as the test organism for this study. Acute toxicity bioassay was done in this study following the guidelines of United States Environment Protection Agency, in which a static test of 96 hours' duration was performed in 4 replicates, each containing 1 control and 5 different concentrations (100, 125, 150, 175 and 200 mg/lit respectively) of lead chloride. Total number of fish used in the study was 192, distributed in 4 series, with each series having eight fish for each concentration of the salt. During the entire study, the temperature of water was kept at 21℃ to 23℃. Finney's Probit Analysis was the method used to analyse the observed data statistically by the help of SPSS Software. The median lethal concentration (LC_{50}) value of lead chloride at 96 hours for the chosen fish species was estimated to be 145.3 mg/lit. From this toxicity assay, it can be concluded that increasing concentrations of toxic heavy metals like lead in water can be detrimental and even fatal for aquatic organisms like fish. This study also helps in validating a permissible level of lead in water for the selected fish species.

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1. INTRODUCTION

In India, rapid industrial progression of the twentieth century provided multiple beneficial effects on the country's economy. However, environmental impact of that revolution was not much rewarding. Most adverse impact of industries on the environment, particularly the aquatic environment, was attributed to the manifold use of heavy metals most of which are toxic. Waste waters from industries containing thick concentrations of such harmful heavy metals like lead, mercury, zinc, cadmium, copper were reported to be directly discharged into the waterbodies, resulting in serious toxic effects on aquatic community, most commonly on fish [1-3].

Lead (Pb) is not essential for biological organisms, as it bears no role in cellular metabolism. Its natural concentration in water is too low [4]. Several industries like batteries, petroleum, dye, chemical, mining however discharge huge concentrations of lead into aquatic bodies of the environment. As lead is non-biodegradable in nature, hence release of such huge concentrations of lead into water causes its bio accumulation in tissues of aquatic organisms, like aquatic invertebrates and fish, as reported from ecotoxicological researches all over the world [5,6]. Huge mortality of aquatic organisms like fish exposed to lead was reported by Sorensen [7] and Health [8]. It was proven that lead is a potent xenobiotic, that causes haematological, neurological, histopathological and immunological alterations in fish [9-11]. Fish tissues like gills, liver, kidney, bones and scales exhibit highest accumulations of lead on exposure. Lead exposed fishes had shown cirrhosis of liver, vacuolization of hepatocyte, degeneration of parenchyma, extension of sinusoidal spaces within hepatic lobules, curling of the lamellae in gills, reduction of RBCs and WBCs in blood [12-14]. Lead exposure caused reduction in antioxidants and enzymes in the cell, leading to excess formation of Reactive Oxygen Species (ROS), and subsequent 'oxidative stress' [15]. Moreover, Lead exposed fishes suffered from impaired nerve impulse transmission due to reduced functioning of acetylcholinesterase and mono amino oxidase enzymes. Exposure to huge concentrations of lead caused reduced growth, decreased reproductive output and increased fish mortality [16].

Trichogaster (Colisa) fasciata Bloch and Schneider, [17] is commonly known as banded gourami. It is a fish of fresh water commonly found in weedy bentho-pelagic niche of rivers, lakes, ponds and flooded paddy fields. It is an air-breather due to having labyrinthine organs in their supra-branchial chambers. The fish is characterised by its compressed, elongated body and small protrusible mouth. Body is greenish in colour with prominent blue or orange bars running obliquely downward along the body [18]. A steady market demand for this species exists throughout the year for its delectable taste. Moreover, its hardy nature and prominent colouration has made it a good ornamental fish. It shows good adaptability in community aquaria and can breed easily in captive conditions. Off late, a decline in the availability of this fish species is seen owing to several anthropogenic stresses.

From ecotoxicological aspect, evaluation of the deleterious impacts of a toxic substance on an organism like fish can be done by acute toxicity tests [19,20]. By these tests, ecotoxicologists can estimate a dose response correlation between various concentrations of a chosen toxicant and their corresponding adverse effects on the chosen test organism. LC_{50} or median lethal concentration is that concentration of a chosen toxicant, that results in 50% mortality of a population of test organisms. It is a standard way that gives an idea of acute or short term toxic potential of a substance. As of now, not enough data on LC_{50} value of lead chloride in *Trichogaster (Colisa) fasciata* is available. The objective of the present study is to determine the LC_{50} of lead chloride in this chosen fish.

2. MATERIALS AND METHODS

This present work of acute toxicity was done in the laboratory of P G Department of Zoology, Vidyasagar College, University of Calcutta. Collection of healthy disease free samples of *Trichogaster (Colisa) fasciata* was done from Subhas Sarobar, at Beleghata, Kolkata. From the collected samples, fish of almost equal length (63 ± 11 mm) and weight (7 ± 2.6 g) were chosen for the test. In the laboratory, the fish samples were given 0.05% potassium permanganate bath twice as a prophylactic control against possible dermal infections and then they were immediately released into a cement tank filled with natural water and kept for fifteen days. For feeding the fish, Organic Valueman Aqua Fish Feed Floating Pellets produced by Valueman Organic Agritech Private Limited were used twice daily and water was changed at every 24 hours' interval. For oxygenation of the water, an air compressor was used. The parameters of water quality in the cement tank were recorded periodically. After the fish were acclimatized of 15 days in the cement tank, healthy disease free fish were selected for the toxicity test in the laboratory. The lengths and weights of the chosen fish were noted beforehand. Various principal water quality parameters like temperature, dissolved O_2 (DO_2), salinity and pH were periodically determined both before as well as during the test following standard protocols earlier published by APHA [21]. The temperature of test water used in the test was maintained between 21℃ - 23℃ with a mean value of ~22℃.

2.1. Validation of LC_{50}

The chosen healthy test organisms were distributed equally and randomly in 6 separate 50 lit aquaria, at the rate of eight test organisms per aquarium. Lead chloride ($PbCl_2$, Merck) was used as the toxicant for the static bioassay study and mixed with distilled water to prepare the test solutions. Five separate concentrations of lead chloride solution viz. 100, 125, 150, 175 and 200 ppm $PbCl_2$ were prepared and added in 5 separate aquaria respectively, each containing 8 fish. Recordings of fish mortality in each of the aquaria were done after 24, 48, 72 and 96 hours. 4 replicates of the total sets were conducted. In each replicate, simultaneous to the exposed groups, a control group of 8 fish was maintained in an aquarium, containing the experimental water only, but not the toxicant, keeping other parameters same. Each replicate was performed for a duration of 96 hours, and mortality of fish was recorded after every 24 hours' duration followed by immediate removal of dead fish from each aquarium, if any. Statistical analysis of the observed data was done to find out whether fish mortality was influenced by different concentrations of the test substance. Statistical software SPSS version 20 was applied for the analysis of the data at P = .05. Finney's Probit Analysis method [22] was used for the estimation of lethal concentration values (LC) and their lower and upper confidence limits (95%) of the toxicant. Probit regression line was also generated against log of concentration values of the toxicant using the same software.

3. RESULTS

The test containers were well aerated to maintain an average dissolved O_2 of 8.4 mg/lit. Salinity values slightly increased with the duration of the test, its average value on first day of trial was 0.133% and it increased to 0.310% on the final day of trial. The pH of water during the entire test remained 8 on average.

In Table 1, the correlation between different concentrations of lead chloride and the corresponding numbers of mortality of *Trichogaster (Colisa) fasciata* at different intervals of the test is shown. Zero mortality was observed in control group of each replicate, making the lower and upper confidence limits for LC_{50} acceptable and fit enough for regression equation.

In Table 2, probit analysis of *Trichogaster (Colisa) fasciata* mortality at different concentrations of lead chloride is shown. There is not much deviation between observed and expected responses as evident from the probit analysis in Table 2.

In Table 3, the calculated values of lethal concentration and their corresponding 95% lower and upper limits of confidence at 96 hours is shown. The Table 3 provides us the estimated LC_{50} value of 145.3 mg/lit for lead chloride at 96 hours in *Trichogaster (Colisa) fasciata* at 95% confidence limit, between the lower limit of 132.9 mg/lit and upper limit of 158 mg/lit.

Table 1. Mortality data of banded gourami, *Trichogaster (Colisa) fasciata* at different concentrations of lead chloride

Concentration	Number of Fish Exposed	Number of Mortality				Percentage Mortality at 96 Hours
		24 hours	48 hours	72 hours	96 hours	
Control (0 ppm)	8	0	0	0	0	0
100 ppm	8	0	0	0	0	0
125 ppm	8	0	0	0	1	12.5
150 ppm	8	0	1	2	4	50
175 ppm	8	1	2	3	8	100
200 ppm	8	8	8	8	8	100

In Fig. 1, a regression line generated by the software between log values of the lead chloride concentrations and probit mortality values of *Trichogaster (Colisa) fasciata* is shown. R^2 linear = 1 implies perfect fitting of data with the predicted regression.

4. DISCUSSION

As mortality of *Trichogaster (Colisa) fasciata* increased with gradual increase of both time of exposure and concentration of lead chloride (Table 1 and Table 2), therefore, a prominent positive correlation exists between the surge in fish mortality and increase of both time of exposure and concentrations of lead chloride.

A gradual rise in the estimated values of lethal concentrations at 96 hours' duration (Table 3) can be explained as increase in accumulation of a toxicant in the aquatic media makes more and more individuals of an aquatic population susceptible to that toxicant, thereby increasing lethality.

Table 2. Probit Analysis of banded gourami, *Trichogaster (Colisa) fasciata* mortality at different concentrations of lead chloride

	Number	Concentration (mg/lit)	Number of ubjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	100	8	0	.002	-.002	.000
	2	125	8	1	.646	.354	.081
	3	150	8	4	4.926	-.926	.616
	4	175	8	8	7.663	.337	.958
	5	200	8	8	7.988	.012	.998

Table 3 Data of calculated values of lethal concentration of lead chloride for *Trichogaster (Colisa) fasciata* at 96 hours with lower and upper confidence limits

LC_{50} Values	95% Confidence Limits for Concentration at 96 Hours		
	Estimate	Lower Limit	Upper Limit
LC_{10}	126.600	100.286	137.029
LC_{20}	132.739	111.544	142.518
LC_{30}	137.349	119.896	147.273
LC_{40}	141.414	126.870	152.247
LC_{50}	145.323	132.932	158.018
LC_{60}	149.340	138.318	165.155
LC_{70}	153.760	143.294	174.389
LC_{80}	159.100	148.316	187.143

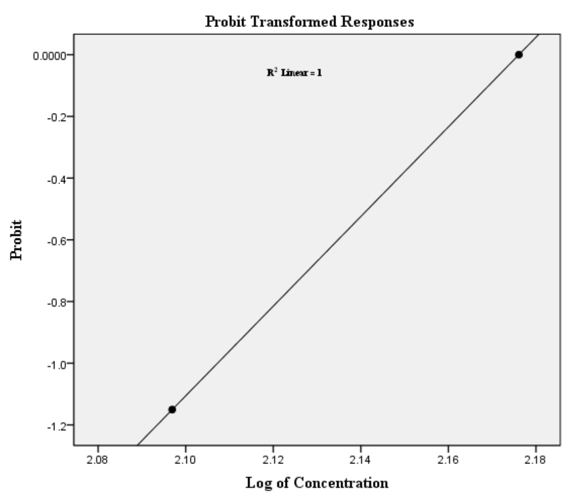

Fig. 1. Regression line plot between the log values of concentrations of lead chloride and probit mortality of banded gourami, *Trichogaster (Colisa) fasciata* [R^2= Coefficient of determination]

Finally, the scatter diagram of Fig. 1 gives a linear line of regression, indicating a linear positive correlation between the probit values of the mortality of *Trichogaster (Colisa) fasciata* and log of concentrations of lead chloride. The observations of this assay comply with bioassays of lead in other species of fish performed by various other workers like Olaifa *et al*. [23] in African magur, *Clarias gariepinus*, Falayi and Amatosero [24] in African magur, *Clarias gariepinus*, Verep *et al*. [25] in rainbow trout, *Oncorhynchus mykiss*.

Other works of lead toxicity in fish showed variations in the values of LC_{50} in different fish species. Tabche *et al*. [26] estimated the LC_{50} values of lead chloride in tilapia, *Oreochromis hornorum* as 202 mg/lit. Martinez *et al*. [27] estimated the LC_{50} values of lead chloride in streaked prochilod, *Prochilodus lineatus* as 95 mg/lit. Sadeghi and Imanpoor [28] estimated the LC_{50} values of lead chloride in silver dollar fish, *Metynnis fasciatus* as 86.8 mg/lit. When the result of the present assay is compared with the results of those other studies, it clearly suggests that variations in lethality of a toxicant like lead in fish are species-specific, as supported in the report of Environmental Protection Act of Canada, 1994.

5. CONCLUSION

From the above discussion, it may be concluded that increased accumulation of heavy metal like lead in water beyond natural level, mainly due to anthropogenic reasons, can make the fish species more susceptible with increased mortality over prolonged exposure. Heavy metals accumulated in excess content in fish tissues affect their activities ultimately leading to mass mortality of fish. Moreover, lead also poses a major threat of bio magnification in the animals of higher trophic levels through the food chain. Thus, such bioassays allow us validations of permissible limits of various toxicants in water for different fish species. Similar studies of acute toxicity of other heavy metals can help us measure the tolerable levels of those metals in water for the survival of fish and the other aquatic biota. Finally, the damage to the aquatic ecosystems done by contamination of heavy metals can be analysed by these studies, and subsequent policies for the conservation of those ecosystems can be properly implemented by regulating the direct discharge of industrial effluents to water.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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