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Assessment of toxic potential of three different heavy metals to *Clarias batrachus* (Linn.) utilizing static acute bioassay

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Abstract: Acute toxicity bioassay of three different heavy metals copper (Cu), lead (Pb) and cadmium (Cd) for the freshwater catfish, *Clarias batrachus* were conducted for evaluation of the toxicity. The 96h LC_{50} values recorded for Cu, Pb and Cd were 15 ppm, 29 ppm and 35 ppm respectively. The results of this study indicate that the order of toxicity of these heavy metals is Cu>Pb>Cd in short duration acute toxicity experiments. The toxicity was found to be dependent on dose and duration of experiment for all these heavy metals. Cu was found to be much more toxic than Pb and Cd to *C. batrachus.*

Keywords: Acute toxicity, Cadmium, Copper, LC_{50}, Lead

INTRODUCTION

The human efforts to increase production of food, energy and luxury things to enhance the standard of living resulted in increased industrialization, urbanization and rapid development. Heavy metals and other pollutants do not enter the aquatic environment as single entity, but are released in complex form (Olojo *et al*., 2005). The concern for environmental protection with respect to the aquatic environment arises from the fact that, various chemicals are discharged into surface water bodies which subsequently disturb their fragile ecology. As a result, several sensitive species are lost from the nature resulting in depletion of valuable biodiversity. Various non-biodegradable xenobiotics present in the industrial effluents get bound to sediments, animal and plant tissues and continue to exert their toxic effect due to slow release of toxicants (Adham *et al.,* 2002; Olojo *et al.*, 2005).

Aquatic ecosystems are sensitive to exposure of toxic contaminants and heavy metals. Among the aquatic organisms, fish is most vulnerable to these contaminants (Alinnor, 2005). Lead is a naturally occurring heavy metal present in the earth's crust, rocks, soil and water. Most water borne lead is derived from human activities such as mining, smelting, coal burning, cement manufacturing, use of gasoline, batteries and paint (Ramesh *et al*., 2009). Cadmium is one of the most toxic and widespread heavy metal and is a recognized carcinogen in mammals (Pruski and Dixon, 2002). Cadmium has become the focus of global research due to its toxicity to terrestrial and aquatic organisms while copper also acts as a potent toxin in aquatic organisms, when it enters into aquatic ecosystems. In this context toxicity of Cu, Pb and Cd to freshwater catfish *C. batrachus* was assessed in short duration acute toxicity experiments in order to conserve and protect the freshwater catfishes in natural aquatic ecosystems. The significance of the present study lies in the fact of conservation and protection of aquatic biota which is useful as a food factor for the man in long run which is to be protected on top priority.

MATERIALS AND METHODS

Study material: 100 healthy fish weighing about 40 - 45 gm. were procured from natural unpolluted sources reservoir and brought to the laboratory, examined for any pathological symptoms, treated with 0.1% $KMnO_4$ solution to avoid any dermal infection and acclimatized in dechlorinated tap water for 15 days. The fishes were fed on minced goat liver and tubifex worms once in a day in morning hours. The test fishes were selected and maintained in separate aquaria before actual experimentation.

Preparation of metal solution: The three different heavy metal salts viz. copper sulphate ($CuSO_4.5H_2O$), lead acetate ($Pb(C_2H_3O_2)_2$) and cadmium chloride ($CdCl_2$) (A.R.Grade) were selected for toxicological experiments. Dechlorinated tap water which was stored in a large overhead tank for about ten days was used for conducting the toxicity experiments. The physico-chemical characteristics of the dechlorinated tap water were determined as per standard methods

S.N.	Physico-chemical characteristics of dilution water	Range
1	pH	7.2-7.4
2	Temperature (^oC)	24-28
3	Dissolved Oxygen (DO) (mg/l)	6.9-7.1
4	Alkalinity (as $CaCO_3$) (mg/l)	160-170
5	Total hardness (as $CaCO_3$) (mg/l)	160-180

(APHA, 2012). The details of the physico-chemical characteristics of water are given below:

Stock solution of the three heavy metals (copper sulphate, lead acetate and cadmium chloride) and their dilutions were made according to standard guidelines (OECD, 1981; APHA, 2012).

Experimental design and toxicity bioassay: In order to determine the LC_{50} of $CuSO_4.5H_2O$, $Pb(C_2H_3O_2)_2$ and $CdCl_2$ (A.R.Grade) and their combination, method of Litchfield and Wilcoxon (1949) was followed. Successive batches of 10 fishes were exposed to different concentrations of heavy metals under standard laboratory conditions after 24,48,72 and 96 hrs. duration. After specified time interval, the number of fish dead and alive were noted (Sprague, 1969). The experiments were carried out in glass aquaria of 100 L capacity in static laboratory conditions.

RESULTS AND DISCUSSION

The percent mortality observed for each dose was calculated and converted to probits by means of a probit table. Dosage versus percent mortality was plotted on the graph. The LC_{50} values and the 95% confidence limits of heavy metals, copper sulphate, lead acetate and cadmium chloride for fish, *C. batrachus* during 24, 48, 72 and 96 hrs are presented in table 1 . The toxicity tolerance of freshwater fish *C. batrachus* to $CuSO_4.5H_2O$, $Pb(C_2H_3O_2)_2$ and $CdCl_2$ in the present investigation are dose and duration dependent. It was noticed that mortality increased with an increase in concentration. LC_{50} values of all the three heavy metals as well as their combination showed the susceptibility of catfish to lethal concentrations in acute short term exposure.

Higher percent mortality occurred in the fish *Clarias*

Table 1. LC_{50}, 95% confidence limits and slope function values of Cu to *C. batrachus* in acute toxicity bioassay.

Exposure Period (hrs.)	Parameter	Values		
		Copper	**Lead**	**Cadmium**
24	LC_{50} mg/L	25	42	54
	95% confidence interval	20.40 – 30.60	33.60 – 52.50	44.32 – 64.00
	Slope function	y=15.35x – 14.28	y=13.16x – 12.5	y=14.04x – 20.71
	Regression (R^2)	0.979	0.963	0.969
48	LC_{50} mg/L	20	37	44
	95% confidence interval	16.39 – 24.40	29.13 – 46.99	34.37 – 56.32
	Slope function	y=15.35x – 14.28	y=10.5x – 13.61	y=13.33x – 20
	Regression (R^2)	0.979	0.989	0.982
72	LC_{50} mg/L	17.50	34	40
	95% confidence interval	14.30 – 21.35	26.56 – 43.52	34.18 – 46.80
	Slope function	y=13.21x + 2.857	y=11.33x – 22.22	y=11.78x – 21.78
	Regression (R^2)	0.910	0.960	0.958
96	LC_{50} mg/L	15	29	35
	95% confidence interval	10.59 – 20.50	21.46 – 39.15	23.30 – 52.50
	Slope function	y=15.35x + 0.00	y = 9.66x – 21.66	y=11.78x – 21.78
	Regression (R^2)	0.932	0.934	0.958

batrachus, under investigation with increase in concentration and exposure period, which confirms the similar observations recorded by researchers world-wide such as in case of *Oncorhynchus mykiss*, *Salvelinus confluentus* and *Oncorhynchus tshawytscha* (Finalayson and Verrue, 1982; Hansen *et al.,* 2002), guppy, *Poecilia reticulata* (Yilmaz *et al*., 2004), *Cyprinus carpio* (Muley *et al.,* 2000; Dardenne *et al.,* 2007), Nile tilapia, *Oreochromis niloticus* (Garcia *et al.,* 2006) and Rohu, *Labeo rohita* (Dutta and Kaviraj, 2001).

In the present research work, Finney's Probit Analysis for evaluating the acute toxicity response gave 96 hour LC_{50} value for *C. batrachus* exposed to $CuSO_4.5H_2O$ as 15 ppm, $Pb(C_2H_3O_2)_2$ 29 ppm, $CdCl_2$ 35 ppm. From the derived LC_{50} values, it is quite clear that, the toxic effect increased with dose and duration. Our results are in agreement with Yilmaz *et al.* (2004) who reported 96 h LC_{50} value of cadmium to guppy *(P. reticulata).* The other supporting studies are of Oryan and Nejatkhah (1997), Woodal *et al*. (1988) and Muley *et al*. (2000).

Today, worldwide heavy metals are introduced into aquatic environment through industrial processes, soil leaching, mining activities, sewage disposal and rainfall. The heavy metals are relatively toxic, even at fairly low concentrations and affect the survival of all other aquatic organ-isms. Stebbing and Fandino (1983) reported that, the adverse biological effects of heavy metals in the aquatic environment are mainly due to their complex nature. When the toxicant concentration in the water body is very high, it results in the death of fish. So, the death of an organism was taken as the end point of toxicological studies previously (Jones and Reynolds, 1997). However, Adams (1990) stated that sublethal concentrations of toxicant also induce sub-stantial changes in the biological organization of fish.

The LC_{50} values derived from toxicological investiga-tions are highly useful in determining the sublethal concentration of a metal. Today, most of the information on the effects of heavy metals on aquatic organisms is focused on short duration experiments carried out at lethal concentrations. The information thus derived is not sufficient to assess the extent of damage. Hence, there is a need to carry out studies related to sublethal toxicity. Such studies are highly valuable in evaluating the sequence of events that involve the response of the test animal to sublethal concentrations (Nobbs and Pearu, 1976; Perkin, 1979). So, in order to derive sublethal concentrations and to evaluate the response of the fish at sublethal concentra-tions, LC_{50} values are of prime importance in today's changing scenario of environmental pollution.

Conclusion

The present toxicological investigation clearly showed that the toxicity is dose and duration dependent for all the three heavy metals. The heavy metal Cu was much more toxic than Pb and Cd. The mortality increased with an increase in concentration and LC_{50} values of all these heavy metals showed the susceptibility of catfish to lethal concentrations in acute short term exposure. Such type of toxicological studies will be useful for conservation and protection of aquatic organisms and ultimately safeguarding the interest of man in long run as a food supply.

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