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Preliminary Evaluation of the Acute Toxicity of Cadmium to *Channa punctatus* (Bloch)

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Abstract: Cadmium poses a serious environmental threat to the aquatic ecosystems. In the present study, the acute toxicity test for cadmium chloride was conducted for 96 h period using freshwater fish *Channa punctatus* as experimental model. The LC_{50} value was determined by using two different methods. According to Trimmed Spearman Karber method it was 368.23 mg/L, but when the same data was analyzed according to the arithmetic method of Karber as adopted by Dede and Kaglo (2001), it came out to be 351mg/L. There was difference of 17.23 mg/L between the two LC_{50} values. The physicochemical parameters were also taken into consideration. Results suggested that cadmium exerted toxic effects on fish behavioural pattern. Dose- and duration-dependent increase in the mortality of fish was recorded with increased concentrations.

Keywords: Cadmium chloride, *Channa punctatus*, Acute toxicity, Behavioural changes

Introduction

Natural, geochemical and anthropogenic factors are responsible for the infiltration of toxic heavy metals into aquatic ecosystem and cause environmental pollution. The fish are intimately associated with water and constitute an important food item in human diet. From the surrounding water, the fish may absorb dissolved heavy metals which may accumulate in various tissues and organs and biomagnified in the food chain. For this reason fishes may be one of the sources of xenobiotics for human beings. Non-degradable heavy metals are regarded as hazardous to aquatic ecosystems because of their environmental persistence and their tendency for bioaccumulation (Burman and Lal, 1994; Sanders, 1997; Shukla *et al*., 2007). Cadmium, a non-essential heavy metal, is highly toxic to aquatic organisms even at minute concentrations (Leland and Kuwabara,

1985; McKim, 1985; Sorenson, 1991; Javed and Mahmood, 2001). Thus toxicity testing becomes an essential tool for accessing the action and fate of toxicants in aquatic ecosystems, to derive water quality standards for chemicals and to identify suitable organisms as bioindicators. Toxicity in fish is a result of a series of events involving various physical, chemical and biological processes.

There are several reports regarding the toxicity evaluation of this non-essential heavy metal cadmium (Mc Cahon *et al*., 1988; Brown and Pascoe, 1989; Stuhlbacher and Maltby, 1992; Shyong and Chen, 2000; MeiWu *et al*., 2002 ;Untersteiner *et al*., 2005; Cicik and Engin, 2005; Jayakumar and Paul, 2006; Prato *et al*., 2006). Similarly the LC_{50} values reported by different workers have been found to vary (Dalal and Bhattacharya, 1994; Chinni and Yallapragda, 2000; Subathra and Karrupasamy, 2003; Gupta and Rajbanshi, 2006; Kawade and Khillare, 2014; Ullah *et al*., 2016). There may be several factors responsible for this variation such as temperature, pH and water hardness (Wepener *et al*., 1992; Abdullah *et al*, 2007).

In the present study, an attempt has been made to observe the LC_{50} value of cadmium chloride for the fish *Channa punctatus* by using two analytical techniques. In the acute toxicity test, fish are exposed to a range of toxicant concentrations in a static system for 96 h. Toxic effect is determined by a statistically significant decrease in the survival rate of fish exposed to toxicant in comparison to relative survival of fish in the control (i.e. without toxicant). Fish can serve as a better bioindicator of freshwater streams, lakes and ponds and also to observe behavioural alterations induced by cadmium.

Materials and Methods

The present investigation was carried out on fresh water fish *Channa punctatus* (Bloch) which were collected from the local resources. They were bathed in 0.1% $KMnO_4$ solution to remove any dermal adherent and acclimatized for two weeks. The morphometric parameters of fish included weight (25 ± 2 g) and length (15-18 cm). *C. punctatus* was chosen for this study because of its availability, survival for longer period under laboratory conditions, easy handling etc. Fish were maintained following standard maintenance procedure (APHA *et al*., 2005) in glass aquaria containing 40 L of dechlorinated water at room temperature (27±1.5 C) with food *ad libitum*. Water was renewed every 24 h and fish were fed on dried prawns. Feeding was stopped two days before the commencement of the experiment to minimize the contamination from the metabolic wastes. A stock solution of cadmium ($CdCl_2.H_20$) was prepared by dissolving weighed amount in deionized water to give a concentration of 1gm/ml. The physicochemical analysis of the test water was done according to standard methods of APHA *et al.* (2005), which showed the mean values as : temperature 27±1.5 C, pH 7.1±0.2, DO 6.2±1 mg/L, total hardness 290-300 mg/L, alkalinity 200 mg/L, phosphate 0.30-0.33 mg/L, Nitrite 0.10-0.11 mg/L and Nitrate 7.0-7.1 mg/L.

A static bioassay test was conducted to determine 96 h median lethal concentration (LC_{50}) of cadmium. Initial range finding experiments were performed to derive the suitable concentrations for LC_{50} determination by using five different concentrations along with control group. In each test concentration ten adult fish were introduced. Definitive tests

were subsequently performed using various concentrations of cadmium chloride solution. Test was carried out for 96 h treatment period. Fishes that failed to respond even to strong tactile stimuli were considered dead and removed immediately. No differentiation was made between sexes. The number of dead fish was recorded against the time of their death in a tabular form as specified by Sprague (1969). The 96 h LC_{50} value of cadmium chloride monohydrate was calculated using arithmetic method of Karber as adopted by Dede and Kaglo (2001).

The obtained data was also statistically analyzed for LC_{50} values and 95% confidence limit using the Trimmed Spearman Karber method (Hamilton *et al*., 1977). During the experiment behavioural changes were also critically observed.

Results

The fish in the control aquarium were observed to be healthy, normal and no mortality was recorded within 96 h. At concentrations 320, 340, 360, 380 and 400 mg/L the per cent mortality was found to be 10%, 20%, 40%, 50% and 100%, respectively (Table 1). After 96 h treatment period the LC_{50} value of cadmium chloride monohydrate was calculated and it was found to be 351mg/L for C. punctatus (Table 2) according to arithmetic method of Karber as adopted by Dede and Kaglo (2001). But according to Trimmed Spearman Karber method (Hamilton et al., 1977) the LC_{50} values for 24, 48, 72 and 96 h were 393.22, 379.55, 369.17 and 368.23 mg/L, respectively (Table 3). Marked alterations in the behavioural patterns were found such as disturbed opercular beating, irregular surfacing activity, loss in equilibrium, excess mucus secretion, erratic swimming in the exposed group of fish in comparison to control (Table 4).

Discussion

Present findings suggest that the mortality of fish was duration- and dose-dependent reflecting the effects of cadmium chloride

Table 1: Toxicity test of cadmium chloride monohydrate on *Channa punctatus* for 96 h

Concentrations mg/L	No of alive fish				% survival at 96 h	% mortality at 96 h
	24 h	48 h	72 h	96 h		
0	10	10	10	10	100	-
320	9	9	9	9	90	10
340	9	8	8	8	80	20
360	8	7	6	6	60	40
380	7	6	5	5	50	50
400	4	2	1	0	-	100

Table 2: Determination of LC_{50} value of cadmium chloride monohydrate for 96 h based on arithmetic method of Karber (adapted by Dede and Kaglo, 2001)

Concentrations mg/L	Concentration difference	Number of alive fish	Number of dead fish	Mean death	Mean death x concentration difference
0	-	10	0	0	0
320	320	9	1	0.5	160
340	20	8	2	1.5	30
360	20	6	4	3	60
380	20	5	5	4.5	90
400	20	0	10	7.5	150
					∑=490

Summation indicates sum of Mean death x concentration difference. LC_{50} value of cadmium chloride monohydrate = LC_{100}-∑ (Mean death x concentration difference)/number of organisms per group=400-490/10=400-49=351 mg/L

Table 3: LC_{50} values with their 95% confidence limits for *C. punctatus* exposed to cadmium chloride monohydrate according to Trimmed Spearman Karber method (Hamilton *et al.*, 1977)

Exposure period (h)	LC_{50} value (mg/L)	95% confidence limits (mg/L)	
		Lower limit	Upper limit
24	393.22	378.18	408.85
48	379.55	364.12	395.64
72	369.17	355.94	382.90
96	368.23	355.29	381.63

Table 4: Impact of Cadmium chloride on behavioural parameters in *Channa punctatus* upto 96 h

Durationof exposure	Opercular Beats/min	Surfacing	Loss of balance	Swimming	Mucus secretion
24 h	+++	+++	-	+++	+
48 h	+	++	++	+++	++
72 h	++	++	+++	++	+++
96 h	+++	+	+++	+	+++

- = Nil; + =Less; ++ =More; +++ = Prominent

which may be due to bioaccumulation of cadmium causing toxicity that lead to fish death. Shah and Altindag (2005) have also suggested that the accumulation of heavy metal has a direct effect on LC_{50} values of the respective metal in fish. The findings of the present work is strongly in concurrence with the findings of other workers who have worked on cadmium (Guven *et al*., 1995; Shyong and Chen, 2000; Subathra and Karrupasamy, 2003; Puvaneswari and Karrupasamy, 2007; Kasherwani *et al*., 2009; Kawade and Khillare, 2014).

The acute toxicity of cadmium has been studied by several workers for different durations in various animals. For 96 h treatment period, LC_{50} values of cadmium in different fish species have been reported different by various workers. In *C. punctatus*, LC_{50} was reported as 405.0 ppm by Dalal and Bhattacharya (1994) and 102 mg/L by Subathra and Karrupasamy (2003) which is different from LC_{50} value observed in the present study. James and Sampath (1999) have reported LC_{50} value as12.0 ppm for *Oreochromis mossambicus. Witeska et al.* (2006) reported LC_{50} value as 4.5 mg dm^{-3} for *Tinca tinca.* In water with calcium hardness of 100 mg/L, 96 h LC_{50} for carp (*Cyprinus carpio*) fry and fingerlings have been reported as 4.3 and 17.10 mg/L, respectively (Suresh *et al*., 1993). Benjamin and Thatheyus (2012) have reported 96 h LC_{50} as 96.57 ppm for *Oreochromis mossambicus*. Kawade and Khillare (2014) have reported 96 h LC_{50} value for *Channa punctatus* as 360 ppm. In mosquito fish *Gambusia holbrooki* the LC_{50} was reported 37.298 µg/L by Ali *et al*. (2016). Ullah *et al.* (2016) reported 96 h LC_{50} as 24 mg/L for the fish *Labeo rohita.*

The variations in LC_{50} values may be due to the differences of physicochemical properties of test water as well as biological factors. Effects of temperature, pH, hardness on the toxicity of metals are well known (Wepener *et al*., 1992; Abdullah *et al*., 2007). According to Sprague (1969) variability in toxicity in single species and single toxicant may also be found depending on the size, age and condition of the test species apart from the experimental factors.

The alterations in behaviour such as hyperactivity, loss of balance, rapid swimming, increased surfacing activity, enhanced rate of opercular activity and convulsions in the treated fish were observed with the increasing concentrations of cadmium as compared to the control fish. Behavioural alterations observed in the present study may be an avoiding reaction to heavy metals as also reported by various workers (Sprague, 1969; Giattina *et al*., 1982; Black and Bing, 1989; Svecevius, 2001). Various mechanisms have been proposed for the process of cadmium poisoning such as disrupted respiratory process, kidney damage and whole body burden (Eisler, 1971). Cadmium induced gill necrosis in fish has been reported by various workers (Voyer, 1975; Gill *et al*., 1988) which may also be a cause of decrease in oxygen consumption and increase in ventilation frequency. This is evident by the increase in rate of opercular beat. Acute cadmium toxicity in cat fish is characterized by gill damage and hypersecretion of mucus (Mallat, 1985) which supports the findings of the present study. The observed behavioural changes in fish may be as a consequence of several changes for more oxygen in order to provide energy to cope with stress condition. It may also be due to choking of gills by excess secretion of mucus.

Thus it can be concluded from the present study that fish are highly sensitive to cadmium and their mortality is duration- and dose-dependent.

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