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Evaluation of acute toxicity levels and ethological responses under heavy metal cadmium exposure in freshwater teleost, *Channa punctata* (Bloch)

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Abstract: The present study was undertaken to investigate the acute toxicity of cadmium, a heavy metal widely detected in the aquatic environment due to natural effects and anthropogenic activities, in freshwater teleost, *Channa punctata* (Bloch). The experiments for the bioassay were performed in semi-static test condition according to the standard guidelines. The behavioural changes in the fish were observed for all tested concentrations of the metal. The data obtained for bioassay were analyzed for median lethal concentrations (LC_{50}) of the metal by SPSS computer software, Finney's Probit analysis and Trimmed Spearman-Karber's method. The LC_{50} values, estimated by SPSS, with 95% confidence level were found to be: 26.88 (21.69-71.68), 18.76 (17.13-20.81), 16.70 (14.77-17.96) and 14.95 (13.13-15.88) mg l^{-1} for dissolved metal concentrations, at 24, 48, 72 and 96 h exposure durations respectively. All three methods showed good agreement among the estimates. Furthermore, the exposed specimens showed dose and duration dependent abnormal behaviour and hyperactivity.

Keywords: Acute toxicity, Behavioural changes, Cadmium, LC_{50}.

Introduction:

Effluents of agricultural and industrial processes contain highly toxic chemicals that may pollute the aquatic environments. The heavy metals found in these effluents have been considered as serious pollutants of the aquatic systems. Such heavy metal like cadmium (Cd), a relatively rare, odourless, white element found in nature, is non-essential for any biological processes. It is

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one of the PBT (persistent, bioaccumulative and toxic) chemicals identified as a primary toxicant by EPA (2000). Cadmium is considered to have toxic effects on the prostate, kidney, lung and testes. The itai-itai disease, detected in Japan, is the most severe form of chronic Cd poisoning induced by prolonged oral uptake of the metal (Takaki et al., 2004).

In natural freshwater, Cd occurs at concentrations of less than 0.01 μg l^{-1}, but in environments impaired by human, the concentrations may be higher (U.S. EPA, 2000). Cadmium is used in producing of fungicides, in textiles dyeing and printing and in metal finishing baths. Cadmium is released into the environment from various anthropogenic sources, such as by-products from zinc refining, coal combustion, mine wastes, electroplating processes, iron and steel production, fertilizers and pesticides (ATSDR, 2008).

Apart from detecting a threshold above which fish are likely to be killed, data obtained on the concentration of selected individual pollutants which are lethal to fish can also provide very necessary information. One of the commonly used measures of the toxicity is the TL_m or LC_{50} (the median Lethal Concentration). The knowledge obtained from dose response studies in animals is used to set standards for human exposure and the amount of chemical residue that is allowed in the environment.

Aquatic pollution is significant in fisheries and aquaculture industries. Discharge of industrial wastewater produce serious consequences in fish which results in impairment of important functions such as respiration and osmoregulation (Kumaraguru, 1995). The changes in physical, chemical and biological parameters of water may even alter the behaviour of fish besides causing mortality (Yadav et al., 2007). The ethological changes in fish have been considered to be sensitive indicator of toxicity and the fish, as vertebrates are closely related to mammals (and humans) and tend to be similar in sensitivity.

The present study was conducted to evaluate the toxicity of heavy metal cadmium and its effects on behavioural responses on common edible murrel, *Channa punctata* (Bloch). This species was selected for bioassay because of its availability throughout the year, easy raisability under laboratory conditions, sensitivity to xenobiotics and easy handling. Thus, it meets most of the requirements for a model test species.

Materials and Methods:

Healthy specimens of freshwater murrel, *Channa punctata* (Bloch, Family: Channidae, Order: Perciformes) were obtained from local fish market. The specimens had wet weight and length (mean ± SE) of 21.34 ± 2.79 g and 12.05 ± 0.56 cm respectively. Fish specimens were subjected to prophylactic treatment by bathing them in 0.05% (w/v)

potassium permanganate ($KMnO_4$) solution for 2 min. The fish were then acclimatized for 15 days under laboratory conditions prior to cadmium exposure. No mortality occurred during this period. The fishes were fed, *ad libitum,* with boiled chicken eggs, goat liver and poultry waste materials. Acclimated fish were not fed 24 h before the start of the test to maintain their catabolic and anabolic physiological state as suggested by APHA (2005).

For the present study, analytical-grade cadmium chloride ($CdCl_2.H_2O$) (98%), manufactured by Himedia Lab. Ltd., Mumbai, India was used as the test compound.

Behavioral responses

During the acute toxicity bioassay, behavioural responses of fish such as convulsions, equilibrium status, fin movement, hyperactivity, somersaulting activity and swimming rate in exposed as well as the control group were observed as suggested by Rand (1985).

Determination of median lethal concentration

Stock solution (10 mg ml^{-1}) was prepared by dissolving cadmium chloride in ultrapure water (Milli-Q Synthesis H_2O Purification System, Millipore. MA, USA). Test concentrations were prepared by diluting appropriate aliquots of the stock solution with tap water. Water quality of the test solution was determined according to the standard procedures (APHA, 2005).

The exploratory range of concentrations of test solution was determined with a series of range finding experiments. Thereafter, definitive acute toxicity bioassay was conducted by exposing fish to eight different concentrations of test chemical, i.e. 11.16, 12.28, 13.39, 14.51, 15.62, 16.74, 17.85 and 18.97 mg l^{-1}, in 20 l aquaria (20" x 10" x 18") along with one control. The control group was kept in experimental water without adding metal salt, keeping all other conditions constant. The bioassay was conducted in semi static system in three set/ triplicate, with 10 specimens exposed per concentration per set, following the standard methods of acute toxicity bioassay procedures (APHA, 2005). During the experiment, the test solution was changed every 24 h to maintain the appropriate concentration of the metal in the test aquaria. The experiments were conducted under natural photoperiod (13L: 11D) for 96 h. No food was given to the fish during the experiments.

Mortality was recorded on 24, 48, 72 and 96 h of exposure. Fish were considered dead if there was no visible movement (e.g. opercular movements) and if touching of the caudal peduncle produced no reaction. The dead fish was removed immediately from the aquaria. The fish exposed to various concentrations of Cd and control were evaluated for behavioural changes as suggested by Kumari et al. (1997).

The safety level estimations, at 96 h

exposure, for Cd were based on an "Application Factor" (AF) proposed by Sprague (1971), Committee on Water Quality Criteria (CWQC, 1972), National Academy of Science/National Academy of Engineering (NAS/NAE, 1973) and International Joint Commission (IJC, 1977).

Cadmium analysis

At 96 h, cadmium level for all tested concentrations was analysed using an Atomic Absorption Spectrophotometer (AAnalyst 300 Spectrometer, Perkin Elmer USA). Three water samples from each set of concentration were taken for analysis of Cd concentration. The sample digestion and analysis was done following the standard methods (APHA, 2005).

Data analysis

The acute toxic effect was determined as LC_{50} values using Finney's (1971) Probit analysis method and SPSS (version-16.0.2, 2008) software and 'Trimmed Spearman-Karber Method' (version-1.5) (Hamilton et al., 1977). The 95% confidence levels for the LC_{50} values were obtained by Finney's method were calculated using the formula given by Mohapatra and Rengarajan (1995). The LC_{50} values estimated by three methods were compared using chi-square test (α = 0.05). Multiple comparisons between the effects of exposure duration on fish mortality was done using Tukey's HSD post hoc test (α = 0.01).

Results:

During the experiment the temperature of the test water ranged from 19.3 to 22.5 °C (21.1 ± 0.40), dissolved oxygen from 6.72 to 8.13 mg l^{-1} (7.51 ± 0.16), and pH from 7.14 to 7.95 (7.45 ± 0.08) respectively. The conductivity of the test water ranged from 239-303 µS cm^{-1}. During the experimental period, the total hardness and total alkalinity of the test water varied from 169 to 198 mg l^{-1} (182.8 ± 2.96) and 242 to 278 mg l^{-1} (261.1 ± 4.04) as $CaCO_3$ respectively.

The alterations in the behavioural pattern are the most sensitive indication of potential toxic effects. The behavioural changes observed in fish increased with the dose and duration (Table 1). The fish exposed to 11.16 to 18.97 mg l^{-1} metal concentrations exhibited abnormal behaviour in the form of erratic swimming, loss of equilibrium and enhanced surfacing behaviour. At the start of the exposure, the fish exposed to the Cd became alert; with the progression of the experiment, the fish stopped swimming and remained in static position in response to the sudden changes in the surrounding environment. There were no behavioural changes observed in the control group.

Median lethal concentration (LC_{50}) values and their corresponding 95% confidence levels for 24, 48, 72 and 96 h exposure duration to *C. punctata,* calculated by three methods for the dissolved and measured

concentrations of Cd in the test solution at 96 h exposure duration are presented in Tables 2A and B respectively. In the present study, the 96 h LC_{50} value of Cd was determined to be 14.95 mg l^{-1} (based on dissolved concentration) and 13.65 mg l^{-1} (based on measured concentration) respectively. In both the dissolved and measured concentrations, all the three methods were in good agreement with LC_{50} estimates during all exposure durations and the difference was non-significant ($P > 0.05$). For pair wise comparison, all differences were found significant ($P < 0.01$). No mortality occurred in the control group during the experimentation.

Table 1: Impact of heavy metal cadmium on the behavioural parameters of a teleost fish, *Channa punctata* at various durations and concentrations (none: -; mild: +; moderate: ++; strong: +++; *Toxicant Concentrations)

(1A)

Exposure duration (h) 24						
T.C.* **(mg l^{-1})**	**Hyperactivity**	**Equilibrium status**	**Swimming rate**	**Convulsions**	**Somersaulting activity**	**Fin movement**
0.00	-	+++	+	-	+++	+++
11.16	-	+++	+	-	+++	+++
12.28	-	+++	+	-	+++	+++
13.39	-	+++	+	-	++	++
14.51	-	+++	+	-	++	++
15.62	-	+++	+	-	++	++
16.74	+	++	++	-	+++	++
17.85	+	+	++	+	++	+
18.97	+	+	++	+	++	++

(1B)

Exposure duration (h) 48						
T.C.* (mg l^{-1})	Hyperactivity	Equilibrium status	Swimming rate	Convulsions	Somersaulting activity	Fin movement
0.00	-	+++	+	-	+++	+++
11.16	-	+++	+	-	+++	+++
12.28	-	++	+	-	++	++
13.39	+	++	++	+	++	++
14.51	+	++	++	+	++	++
15.62	+	++	++	+	++	++
16.74	+	++	++	+	+++	+
17.85	++	++	+++	++	++	+
18.97	++	+	+++	++	++	+

(1C)

Exposure duration (h) 72						
T.C.* (mg l^{-1})	Hyperactivity	Equilibrium status	Swimming rate	Convulsions	Somersaulting activity	Fin movement
0.00	-	+++	+	-	+++	+++
11.16	+	+++	+	-	++	++
12.28	+	++	+	+	++	++
13.39	+	++	++	+	+	+
14.51	++	+	++	+	++	++
15.62	++	++	++	++	++	+
16.74	++	++	+++	++	+	+
17.85	+++	+	+++	++	+	+
18.97	+++	+	+++	+++	+	+

(1D)

Exposure duration (h) 96						
T.C.* (mg l^{-1})	Hyperactivity	Equilibrium status	Swimming rate	Convulsions	Somersaulting activity	Fin movement
0.00	-	+++	+	-	+++	+++
11.16	+	++	++	-	+++	++
12.28	++	+	++	+	+	+
13.39	++	+	++	++	+	+
14.51	++	-	+++	++	+	+
15.62	+++	+	++	+++	+	+
16.74	+++	-	++	+++	-	+
17.85	+++	-	+	+++	-	+
18.97	-	-	-	+++	-	-

Table -2: LC_{50} values with 95% confidence limits (in parentheses) for Cd (based on dissolved concentrations, **A**; measured concentrations at 96 h, **B**) estimated by SPSS, Finney and Trimmed Spearman-Karber methods

(2A)

Method	Estimated Concentrations LC_{50} (mg l^{-1})			
	24 h	**48 h**	**72 h**	**96 h**
SPSS	26.88[a]	18.76[b]	16.70[c]	14.95[d]
	(21.69- 71.68)	(17.67-20.80)	(16.00-17.63)	(14.50-15.41)
Finney's Probit	26.15[a]	18.62[b]	16.60[c]	14.80[d]
analysis	(18.81-36.35)	(15.27-22.71)	(14.69-18.76)	(13.58-16.13)
(Slope)	1.9278	0.6654	1.2737	1.1884
Trimmed	Not calculable	Not calculable	16.56[c]	14.92[d]
Spearman-Karber			(15.56-17.62)	(14.49-15.37)

Values with different alphabet superscript differ non-significantly ($P > 0.05$) between test methods within exposure duration

(2B)

Method	Estimated Concentrations LC_{50} (mg l^{-1})			
	24 h	**48 h**	**72 h**	**96 h**
SPSS	25.79[a]	17.47[b]	15.39[c]	13.65[d]
	(20.44-74.75)	(16.36-19.54)	(14.69-16.32)	(13.20-14.10)
Finney's Probit	25.70[a]	17.78[b]	15.49[c]	13.49[d]
analysis (Slope)	(17.02-38.81)	(15.07-20.98)	(13.47-17.81)	(12.26-14.84)
Trimmed	Not calculable	Not calculable	15.26[c]	13.61[d]
Spearman-Karber			(14.27-16.31)	(13.18-14.06)

values with different alphabet superscript differ non-significantly ($P > 0.05$) between test methods within exposure duration

Discussion:

The toxicity tests are needed to evaluate the nature and degree of adverse effects of toxic compounds on the organisms. The main aim behind any toxicity test is to determine the concentration of test material that can influence a group of test organisms during a period of exposure. The information generated on toxicity of the compound may be useful in protecting aquatic ecosystem

and valuable aquatic life. In the present study, the acute toxicity effect of heavy metal Cd was studied on the alterations in behavioural pattern of a freshwater teleost, *C. punctata*.

On initial exposure at higher concentration (15.62 mg l^{-1} and onwards), the fish exhibited characteristic avoidance behaviour by rapid and erratic swimming with jerky movements and hyper-excitability. Enzymatic as well as ionic disturbances in blood and tissues may be associated with such abnormal behaviour and altered movements (Larsson et al., 1981), while heavy exudation of mucus over the body and dispigmentation is attributed to dysfunction of the endocrine (pituitary) gland under toxic stress (Pandey et al., 1990). The behavioural alterations of fish and subsequent death imply that the toxic effect is mediated through the disturbed nervous system, which involves control of almost all vital activities.

Inactivation of acetylcholinesterase leads to accumulation of acetylcholine at synaptic junctions which in fish results in hyperactivity, swimming in imbalanced manner lethargy and stimulation of peripheral nervous system which induces increased metabolic activities and more oxygen utilization. Likewise, the faster opercular movement in fish has been reported to increase considerably following the exposure of toxins (Pandey et al., 2005). The deficiency of oxygen causes hypoxic condition in fish results in increased in the breathing rate, and to cope with the condition fish gulp air by frequent surfacing.

The mortality of an organism is an easily detectable deleterious response to a toxicant, and thus, the most common acute toxicity test is acute lethality test (Parrish, 1985; FAO, 1987). The mortality of fish increased with the increase in the concentration of the metal, depicting a positive correlation between the mortality and the concentration of test chemical (Figs 1 and 2). Significant ($P < 0.01$) effect of duration of metal exposure on fish mortality was observed.

The 96 h LC_{50} values of Cd have been reported in a number of fish species as: 5.4 mg l^{-1} in *Garra mullya* (Wani and Latey, 1983), 16.71 mg l^{-1} in *Oreochromis mossambicus* (James et al., 1991), 173.78 mg l^{-1} in *Rita rita* (Ghosh and Mukhopadhyay, 2000), 121.8 mg l^{-1} in *Cyprinius carpio* (Muley et al., 2000), and 17.9 mg l^{-1} in *Cherax tenuimanus* (Chambers, 1995). The differences in LC_{50} values of toxicant to different fishes and sometimes to the same fish is attributed to individual specific characteristics such as size, weight, sex and biological behaviour. To an extent, physico-chemical properties of test water also influence the toxicity, and thus, the LC_{50} value. The toxicity of Cd is greater in soft water as compared to hard water because it precipitates in the latter. Sprague (1969) observed variability in acute toxicity even in a single species and single toxicant depending on the size, age and condition of the test species along with experimental factors. The decrease in the concentration

after metal analysis may be attributed to the precipitation of the metal in test water. Besides, the hardness and relative higher pH of test solution decreases the solubility of Cd (Adhikari, 2003).

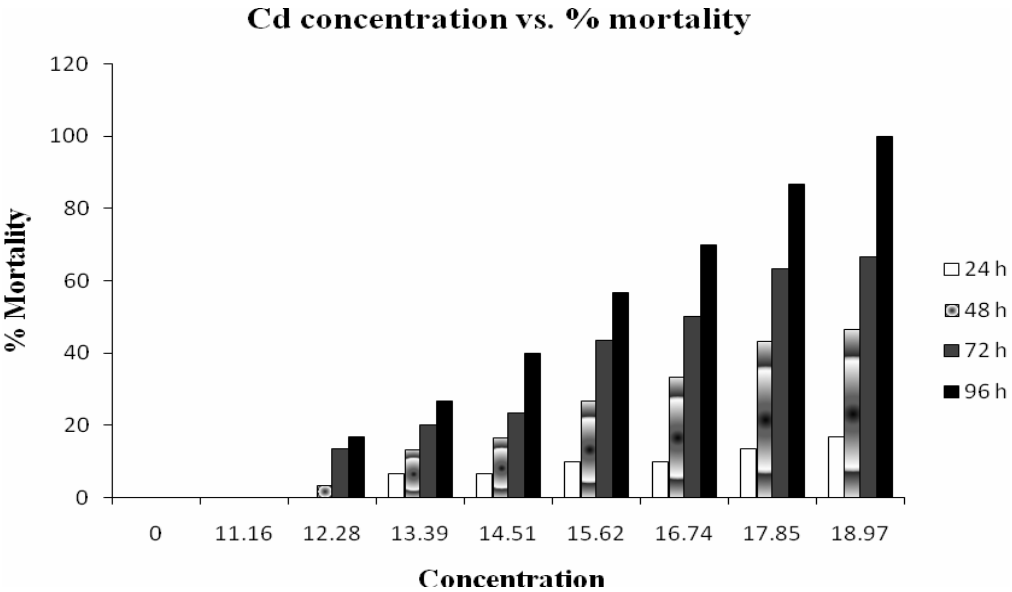

Fig. 1 Toxicity evaluation of heavy metal, cadmium to freshwater fish, *C. punctata* (n=30). The graph shows proportional relationship between concentration of Cd and percentage mortality

Application factors are used to establish acceptable toxicant concentration ranges depending on water quality, species under study and life stage. Large variation was found in safety levels determined by different methods (Tab. 3), which is why the estimates of safety levels cannot be guaranteed. Mount and Stephan (1967) underscored the fact that extrapolation of laboratory data to the field is not always meaningful, and hence it is difficult to decide an acceptable concentration based on the laboratory experiments that may be considered "safe" in the field. In another study it was also emphasized that the major weakness in calculation of the application factor (AF) is its dependence on the LC_{50} value (Kenaga, 1979).

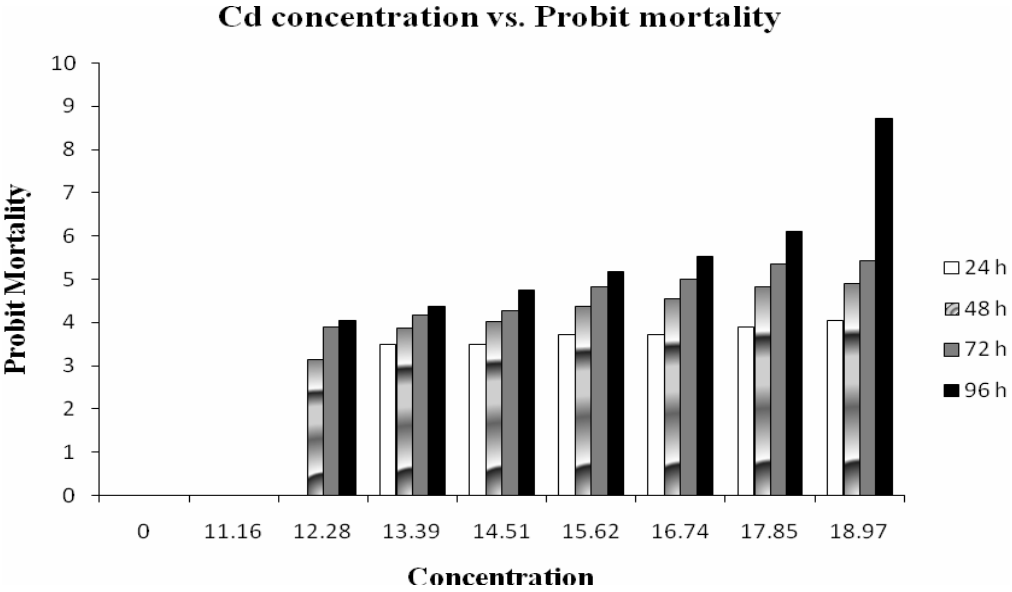

Fig. 2 Toxicity evaluation of heavy metal, cadmium to freshwater fish, *C. punctata* (n= 30). The graph shows proportional relationship between concentration of Cd and probit mortality

Table- 3: Estimates of safety level of cadmium at 96 h exposure time

96 h LC_{50} (mg l^{-1})		Method	Application Factor (AF)	Safe level (mg l^{-1})
Dissolved[a]	14.95	Sprague (1971)	0.1	1.495
		CWQC (1972)	0.01	14.95×10^{-2}
		NAS/NAE (1973)	0.1 to 0.0001	$1.495\text{-}14.95 \times 10^{-4}$
		IJC (1977)	5% of $^{96}LC_{50}$	0.7475
Measured[b]	13.65	Sprague (1971)	0.1	1.365
		CWQC (1972)	0.01	13.65×10^{-2}
		NAS/NAE (1973)	0.1 to 0.0001	$1.365\text{-}13.65 \times 10^{-4}$
		IJC (1977)	5% of $^{96}LC_{50}$	0.6825

a- value determined on the basis of metal concentration dissolved; b- value determined on the basis of metal concentration measured at 96 h

Acute toxicity studies are among the first steps in determining the water quality requirements of fish. These studies reveal the toxicant concentrations (*viz*. LC_{50}) that cause fish mortality even at short time exposure. Biological monitoring using a series of assays having different endpoints in a "key species" could allow a sensitive approach to predict the potential risk of persistent contaminants like heavy metals,

which is helpful in formulating the "safe levels" of such bioaccumulative toxins. The results obtained in this study indicated that cadmium is moderately toxic to freshwater murrel, *C. punctata* and in general the toxic response in the fish was dose and duration dependent.

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