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Cadmium Toxicity to Freshwater Catfish, *Heteropneustes fossilis* (Bloch)

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Abstract : Static bioassay tests were carried out in order to evaluate LC_{50} values of Cadmium chloride for freshwater stinging cat fish, *Heteropneustes fossilis* as well as to observe behavioural alterations posed by Cadmium. The LC_{50} values for 24h, 48h, 72h and 96h were found 434.74, 409.88, 401.31 and 392.92 mg/l, respectively. The major behavioural alterations observed during the experiments were erratic swimming, restlessness, muscle spasm, profuse mucous secretion, discolouration of the integument and cutaneous ulcerations in exposed animals. Overall increase in opercular beats in exposed fishes was also recorded throughout the experiments.

Key words : *Heteropneustes fossilis*, Cadmium chloride, $LC_{50,}$ behaviour.

Introduction

Increased industrialization, urbanization, population growth and overall man's greed to overexploit Mother Nature has created a serious threat to all kinds of life in the form of pollution which has now become a global problem. Among all types of pollution, aquatic pollution is of greater concern as each and every kind of the life depends on water. Among all types of aquatic pollutants, heavy metals are of greatest concern. Heavy metals when reach the aquatic bodies deteriorate the life sustaining quality of water and cause damages to both flora and fauna (Nriagu and Sprague, 1987; Nriagu, 1996; Mason, 1996; Kotsanis and Georgudaki, 1999; Zyadah and Abdel-Bakey, 2000; Lliopoulou-Georguadaki and Kotsanis, 2001; Verma *et al.*, 2005; Samanta *et al.*, 2005; Sharma and Agarwal, 2005). Being intrinsic components of the earth crust, nature has not provided effective control mechanisms for these metals. The problem increases many folds due to their long half-life period and properties of non-biodegradability, bioaccumulation and biomagnifications (Burman and Lal, 1994; Sanders, 1997; Pitter, 1999; Lodhi *et al.,* 2006).

Cadmium, a non-essential heavy metal has been listed in the "Black list" of European community (Mason, 1996). It has also been classified as b-class (soft) metal (da-Silva and Williums, 1991). It is a non-biodegradable element with no known biological function and is reported to be a major contaminant of aquatic ecosystems causing adverse effects on aquatic organisms (Hollis *et al.,* 1999). It enters into aquatic ecosystems through diverse sources including both natural and anthropogenic activities. The major sources of contamination include electroplating, paper, PVC plastic, pigments and ceramic industries, battery, mining and smoldering units and many

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other modern industries (Gupta *et al.,* 2003). It also enters into aquatic bodies through sewage sludge and with the run off from agricultural lands as it is one of the major components of the phosphate fertilizers (Cherian and Goyer, 1989), where it produces deleterious effects on aquatic flora and fauna by affecting various physiological, biochemical and cellular processes (Gill *et al*., 1988; Venugopal and Reddy, 1992; Faverney *et al*., 2001; Drastichova *et al.*, 2004; Patro, 2006).

The freshwater, air-breathing, stinging catfish, *Heteropneustes fossilis* (Order: Siluriformes; Family: Heteropneustidae) is a cherished table fish in India and is distributed throughout the Indian sub-continent in various fresh water ecosystems including muddy, marshy and derelicts ponds having low levels of water and dissolved oxygen. They are seen even in contaminated water bodies also. Various fishes have been investigated in relation to heavy metal toxicity (Agarwal *et al.*, 1985; Singh and Reddy, 1990; Khangarot, 1992; Kotsanis and Georgudaki, 1999; Subramanian and Ramalingam, 2003; Rabitto *et al*., 2005; Gupta and Rajbanshi, 1982, 2006; Susithra *et al*., 2007). Many workers have reported the manifestation of toxic effects of cadmium on fishes (Randi *et al*., 1996; Hollis *et al*., 1999). However, effects of Cadmium on freshwater catfishes, especially on *H. fossilis* have been documented less (Srivastava and Srivastava, 2002; Nath, 2003; Kuruppasamy *et al*., 2005). The present work has been taken into consideration to evaluate LC_{50} values of Cadmium chloride for freshwater stinging cat fish, *Heteropneustes fossilis* (Bloch), a fish having nutritive value as well as which can serve as a better bio-indicator of freshwater streams, lakes and ponds and also to observe behavioural alterations posed by Cadmium.

Materials And Methods

The freshwater, air-breathing, stinging catfish, *Heteropneustes fossilis* Bloch (Order: Siluriformes; Family: Heteropneustidae) commonly known as "Singhi" in vernacular "Hindi" are the test organisms for the present study. These were collected from river Gomti and water reservoirs in and around Lucknow, U.P. (India) with the help of local fisherman, brought to laboratory (N-26º51'59'', E-80º56'17'') and acclimatized to laboratory conditions for 15days before the experiments.

Stock solution of Cadmium chloride ($CdCl_2$.21/2 H_2O, M.W. = 228.35AR Grade, Manufactured by Thomas baker chemicals ltd. Mumbai, India) was prepared by dissolving weighed amount of salt in double distilled water. For toxicity test six aquaria of 50 liter capacity were taken having 30 liters of dechlorinated tap water (Physico-chemical properties, pH = 7.6±0.2; Temp. = 26±2^{0}C; Alkalinity = 65±4.5 mg/L; Total Hardness = 265±2.5 mg/L; D. O. = 7.0±0.2 mg/L). Series of five concentrations of Cadmium chloride *viz*. 180, 240,320, 420, 560 mg/l (Toxic range was predetermined by exploratory tests) was prepared by adding calculated amount of stock solution. One aquarium having diluent water without Cadmium chloride served as control. Adult 10 fishes of similar size and weight (average length 15± 1.5cm and weight 26.5± 2.0gm) were introduced to each test as well as control aquaria from stocking tank. Feeding was suspended 24 hour before start and throughout the experiment to avoid dissolved Cadmium losses due to particulate adsorption. Proper aeration was maintained in test as well as control aquaria by air pumps and stone diffusers throughout the experiments. Mortality was recorded after 24 hrs. interval each day at the same time up to 96 hrs. Experiment was carried out according to guidelines of APHA *et al.* (1998) and replicated thrice. The LC_{50} values of various intervals were calculated according to Trimmed Spearman Karber's Method (Hamilton *et al*., 1977) on PC.

Modifications of behavioural characteristics were also recorded with respect to activity, movement, mucous secretion, skin colouration and opercular beats. The data obtained for opercular beats were statistically analyzed for student t-test and ANOVA using MINITAB software on PC.

Results

The LC_{50} values and their confidence limits of Cadmium chloride for *Heteropneustes fossilis* are summarized in Table-1. The 24, 48, 72 and 96h LC_{50} values of cadmium chloride were 434.74, 409.88, 401.31 and 392.92 mg/l, respectively. An inverse relationship of exposure duration and concentration was clearly evident.

Just after introduction to test solution fishes showed increased swimming, surfacing and hyperactivity. Restlessness, rapid surfacing, peeling of skin and colour fading were prominent after 24 hrs. exposure. After 48h exposure the fishes showed slightly reduced activity and gradual increase in colour fading. Gill adhesion and a thin film of mucous were noticed on gills, operculum and general body surface at this stage. After 72h exposure increased surfacing and gulping of air was observed. At this stage fishes showed loss of balance and jerky movements during swimming. The school formation, a characteristic of this fish, was found weakened in test animals as compared to controls at this stage. After 96h ulceration on trunk, base of caudal and pectoral fins were prominent in 95% of the animals. The fin hemorrhage was also noticed in some fishes. A thick film of mucous on whole body and gills was observed in almost all test fishes. Test fishes lost there natural colouration and become almost reddish in colour.

Cadmium chloride induced marked effects on opercular beats/minute of *H. fossilis* in the test fishes which were found significantly higher throughout the experiment (Table-2; Graph-1). Though a decline was noticed from 24 to 48 hrs. exposure but the values were still significantly higher than the controls. The

Table 1 : LC_{50} values of Cadmium chloride and their 95% confidence limits to *Heteropneustes fossilis*

Exposure Duration (hrs.)	LC_{50} (mg/l)	Lower confidence limit (mg/l)	Upper confidence limit (mg/l)
24	434.74	390.67	483.78
48	409.88	380.04	442.06
72	401.31	380.29	423.49
96	392.92	372.34	414.64

Table 2 : Opercular beats/minute of *Heteropneustes fossilis* after acute exposure of Cadmium chloride

Exposure Duration (hrs.)	Control	Exposed
24	62 ± 3.78	96 ± 3.47***
48	65 ± 4.75	87 ± 3.64**
72	71 ± 3.51	97 ± 4.14***
96	75 ± 3.87	102 ± 4.19***

Values are Mean ± SE, N=10

** denotes differences in means to be significant at $P<0.001$.

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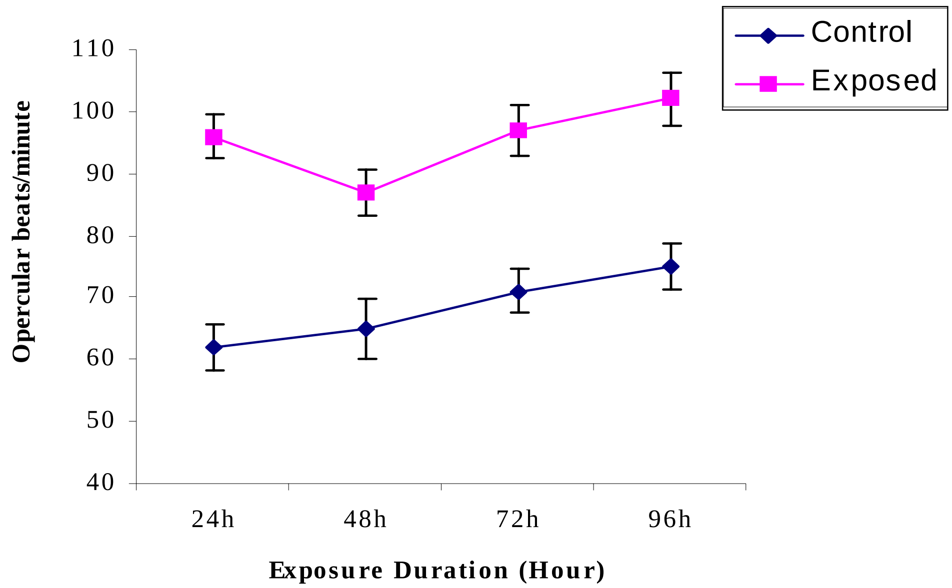

Fig. 1 : Opercular beats/minute of *Heteropneustes fossilis* after acute exposure of Cadmium chloride

differences between means of opercular beats of test animals and controls animals were highly significant at 24 hrs. (t=12.50; P<0.0001), 48 hrs. (t=6.55; P<0.001), 72 hrs. (t= 10.86; P<0.0001) and 96 hrs. exposure (t=11.55, P<0.0001).

The overall fluctuations in means of opercular beats from 24 hrs. to 96 hrs. were found significant in test animals (F=15.29; P <0.001) as well as in control animals (F=12.75; P <0.001) using One Way ANOVA.

Discussion

Cadmium chloride poses toxic effects on the fish *Heteropneustes fossilis* which is evident by the findings of present investigations and LC_{50} values observed in present study are nearer to the reporting of other workers on different fishes (Pundir and Sexena, 1992; Vankhede and Dhande, 1999; Srivastava and Srivastava, 2002; Dutta *et al.*, 1993, 1996; Drastichova *et al.*, 2004).

Behavioural alterations have been established as sensitive indicators of chemically induced stress in aquatic organisms (Olla, 1980; Sharma and Shukla, 1990; Agarwal, 1991). Behavioural alterations like erratic swimming, restlessness and surfacing, observed in present study may be an avoiding reaction to the heavy metals as also observed by various workers (Sprague, 1969; Giattina *et al.*, 1982; Black and Bing, 1989; Svecevicus, 2001). The avoidance reaction may be related to narcotic effects or to change in sensitivity of chemo receptors (Suterlin, 1974).

The immediate physiological consequence of the lamellar fusion is the reduction in the surface area available for gaseous exchange, which could adversely affect respiratory physiology of the fish. A wide range of functions has been attributed to fish mucous including protection against environmental contaminants and UV radiation (McKim and Lien, 2001; Häkkinen *et al.*, 2003). Many workers are of the opinion that some components of the fish mucous, probably the acidic and/or sulphated glycoprotein moieties have a metal binding (Pärt and Lock, 1983) and ameliorative effect against ambient toxicants (Arillo and Melodia, 1990). Daoust *et al.* (1984) suggested that the lamellar adhesion in gills might be result of contact stress, which causes erosion of mucous coating and epithelial lining leading to alterations in the chemical composition and thickness of the mucous layer due to interaction with xenobiotics. Erosion of the epithelial lining and alteration in mucous coating has also been observed in the present study also.

It is well known that the presence of glycoproteins in the mucous is indicative of its metal binding capacity as cadmium specially binds –SH groups (Moore and Ramamoorthy, 1984). Mucous coating on one hand prevents the further metal entry by precipitation and on other hand can also eliminate the accumulated Cd during its sloughing.

Loss of balance during swimming might be due to some neurological impairment in central nervous system as evident by inhibition of e.g. ACHE by cadmium and other metals (Devi and Fingerman, 1995; Lata *et al.,* 2001; Patro, 2006). Skin ulcerations as observed in present study are similar to the reporting of other workers on fishes due to cadmium exposure (lger, 1992; lger *et al.,* 1994).

The overall increase in opercular beats as observed in present study is in good conformity with earlier reports on different fishes in relation to various toxicants (Konar, 1968, 1969; Varma *et al.,* 1975; Varma and Dalela, 1976; Srivastava *et al.,* 1979; Rathore and Kothari, 1986). The increased opercular activity may be due to shock received by the fish in new toxic environment along with sensory stimulus to increase the opercular movement for proper ventilation of the gills to cope with hypoxia (Joseph *et al.,* 1987; Lata *et al.,* 2001).

Cadmium induced gill necrosis in fishes 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. The respiratory distress is further compounded due to the decreased hemoglobin, hematocrit and erythrocytes as observed in cadmium exposed fish (Gill and Pant, 1985; Lorson, 1975; Vosyline, 1999). Surfacing and gulping of air might be a compensatory device to cope with the oxygen deficiency as observed in *Channa punctatus* after cadmium exposure (Saxena and Parashari, 1982).

The increased gulping activity and opercular movement by the exposed fish may be the reflection of an attempt by the fish to extract more oxygen to meet the increased energy demand to with stand the cadmium toxicity. It may also be correlated to the formation of a hypoxic condition due to the interference in gaseous exchange caused by the accumulation of mucous on the gill epithelium.

The present findings suggest that Cadmium produces respiratory distress in fishes and opercular beats per minute can be considered as good bio-marker to access the health status of these valuable and cherished fishes as well as worsening status of aquatic bodies in relation to metallic contaminants, particularly Cadmium.

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