



INVESTIGATION OF ACUTE TOXICITY OF CADMIUM ON SNAKEHEAD FISH *CHANNA PUNCTATUS*- A COMPARATIVE TOXICITY ANALYSIS ON MEDIAN LETHAL CONCENTRATION

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ABSTRACT

In the present investigation, the acute toxicity of cadmium compounds (-chloride, -sulphate, and -nitrate) and their toxicological effects on median lethal concentration, behaviour and morphology of widely consumed Indian snakehead fish *Channa punctatus* was observed for 24, 48, 72 and 96 hours. Specimens of *Channa punctatus* were exposed to different concentrations of cadmium in the form of cadmium chloride, cadmium sulphate and cadmium nitrate for 96 hours. The LC_{50} values for 96 h were for $CdSO_4$ (463.32 $\mu g/l$), $CdNO_3$ (711.05 $\mu g/l$) and $CdCl_2$ (561.11 $\mu g/l$). The LC_{50} values for 96 h shows remarkable changes in behaviour and morphology. Behavioural changes viz; increased respiration rate, schooling behavior, perpendicular swimming pattern were also studied. Among morphological changes white sedimentation of chemical on body, heavy mucous secretion. Frequency of occurrence of such changes was more pronounced in the beginning of treatment and decreased gradually by prolongation of time, but remained higher than control. The response of fish to cadmium was both time and dose dependent.

KEY WORDS: Cadmium toxicity, *Channa punctatus*, LC_{50} , Behavioural changes, Morphological changes.

INTRODUCTION

Among heavy metal pollutants, cadmium has been listed in "Black-list" of European community (Mason, 1996), and it is non-essential, non corrosive in nature and highly toxic metal which is distributed and released into the aquatic environment by industrial sources such as mining and refining of ores, Ni-Cd batteries, plating processes, the use of phosphate fertilizers and gasoline containing lead by fishery boats (U.S. ATSDR- 1999). Cadmium does not break down in the environment, remain in fish body for long periods of time and can bio-accumulate for many years after exposure to low levels of this metal. It has no known biological function (EMSL-1994). Pollution of freshwater reservoirs (water, sediment and fish) by cadmium and other heavy metals have been paid attention for considerable time (Srivastav and Srivastav, 1998; USATDR, 1999; Senthil Murugan *et al.*, 2008; Tripathi and Dubey, 2008; Ebrhimi and Taherianfared, 2008). Fish may absorb metal directly from contaminated water or indirectly from feeding on living organisms in the contaminated water (Suedel *et al.*, 1997; Javed, 2005). Fish may act as sentinel organisms for indicating the potential for exposure of human population to pollutants in water reservoir and recognized as major vectors for contaminant transfer to humans (Tripathi and Dubey, 2008; Senthil Murugan, 2008; Kasherwani *et al.*, 2009). Fishes are relatively sensitive to changes in their surrounding environment. Fish health may therefore reflect and give a good indication of the status of specific aquatic ecosystem (Gupta, 2009; Mokhtar *et al.*, 2009).

The toxic pollutant affects water quality and feeding, swimming behaviour of fish and also delays the hatching, maturation period (Atif *et al.*, 2005; Laovithayanggon,

2006 and Kumar, 2007; Srivastava and Srivastava, 1998). Toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystems and has been widely used to identify suitable organisms as a bio indicator and to derive water quality standards for chemicals. The purpose of toxicity test is to assess various abnormalities caused due to administration of a chemical or heavy metal to fish on occasion or other and to determine the order of lethality of the chemical (Shuhaimi-Othman, 2010).

Acute toxicity caused by different toxicant on freshwater fish can evaluate by quantitative parameters like survival and mortality of test animals and sensitivity of different fish species against metal's toxicity (Kausar and Javed, 2012, Azmat *et al.*, 2012, Ebrahimpoure *et al.*, 2010). Toxicity testing with a single chemical composition is inadequate identification of pollutant selective toxicity on aquatic biota and does not allow to evaluation pollutant hazard to the environment. 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. Toxicity in fish is the culmination of a series of events involving various physical, chemical and biological processes. LC_{50} : Estimation of median lethal concentration or dosage (LC_{50} and LD_{50} respectively) is very valuable. LC_{50} or LD_{50} is indicator to the level of resistance of population response to metals (Claude, 2005; Reda F. A. Bakr *et al.*, 2010). 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 (Das *et al.*, 2012). In order to obtain information on pollutant toxicity range, species with

generally different susceptibilities or metabolic activities should be used, including those easily available and common in the area where the toxicant may occur. Therefore, comparative toxicity studies should be developed to identify species that produce results suitable to the evaluation of eco toxicity of the pollutant under study (Svecevičius, 2010).

The snakehead air breathing fishes are exclusively fresh water with a wide distribution in the old world, extending from Amur river in Eastern Siberia in the North through China to India (Srivastav, 2007) since Asian people have long regarded snakehead fish as a valuable food fish. This fish is prized for its excellence in taste, even being eaten, smoked or dried. They are an important edible fish (Laovitthayangoon, 2006). Noted advantages of this fish, is that even the poor can buy them in quantities they can afford. The snakehead fish also provides livelihood opportunities and income to a large number of poor fishers. However such species have received sufficient attention in statistics, inland water fisheries policies and programmes in India, both at the national and state level. *Channa punctatus* fish can be useful bio-indicator organism of heavy metal contamination of water (Singh *et al.*, 2006).

In the present study in agreement with reports by others (Maruthanayagam *et al.*, 2002; Sobha *et al.*, 2007; Laovitthayangoon, 2006) morphological and behavioural attention was made with the increase of concentration of cadmium compounds on freshwater air breathing snakehead fish *Channa punctatus*. Further, more these parameters are easily measurable and provide an integrated measure of the physiological changes in organisms. However, information in this particular context is meagre around Lucknow region in India. The main objective of this study was to determine the lethal concentration (LC₅₀) of cadmium compounds at 96 hours and the behavioural and morphological alterations due to action of cadmium compounds on air breathing fish *Channa punctatus*, Bloch.

MATERIALS AND METHODS

Freshwater air breathing fish (SL 15±0.67 cm, TL 20.14±0.72 cm, Wt 40.35±1.06 g) *Channa punctatus* were collected from local fish markets of Lucknow region and confined to large plastic aquaria bearing tap water for 15 days in the laboratory for acclimatized in normal photoperiod & temperature and starved for 24 hour prior to experimentation. Fishes were fed daily with artificial feed, and water was renewed every day. For experiment analytical grade Cadmium compounds (viz., cadmium chloride, cadmium sulphate and cadmium nitrate) were used as toxicant and were purchased from Local scientific supplier. The solution prepared in tap water (having Dissolved Oxygen=16.0 ppm; pH=7.2; Carbon Dioxide=25.0 ppm and room temperature 30±2°C) for acute toxicity were performed over a period of 24, 48, 72 and 96 hours using various concentration of CdCl₂.H₂O (concentration in µg/l) 550, 560, 570, 580, 590, 600 were taken to study the behavioural, morphological and mortality study. CdSO₄. 8H₂O (concentration in µg/l) 425, 450, 475, 500, 550, 575 and Cd(NO₃)₂.4H₂O

(concentration in µg/l) 650, 675, 700, 725, 750, 775 were taken for study.

Experiments: The fish were divided into two groups, one is control and another is experimental group and each group contain ten fishes. The experiment was carried out according to guidelines of U. S. EPA (1994) and replicated thrice.

Lethal concentration of 50 percent was determined by following renewal bioassay and was calculated by Probit analysis method by Finney (1971). Behavioural and morphological alterations were recorded with respect to activity movement, mucous secretion, skin lesions, skin coloration and gill and fin damage. The mortality or dead fish were defined as “fishes that failed to respond even to strong tactile stimuli, non-motile, ceased all the opercular and body movement, white opaque (Vutukuru, 2005). No differentiation was made between sexes. After 24 hours each fish were examined and morphological changes were observed and compared with control group.

Statistical analysis for LC₅₀ by Probit (Finney 1971)

The acute toxic effect was determined as LC₅₀ values of cadmium composition for *C. punctatus* was determined using Finney's (1971) Probit analysis method by SPSS (version-17). Computation of 24, 48, 72 and 96 h LC₅₀ values and confidence limits, tests for parallelism of probit lines, and evaluation of potency ratios, where appropriate, were conducted as outlined in Finney (1971).

χ² value were computed to test whether the probit lines were adequate representations of data. To test the hypothesis that the LC₅₀ values computed from two or more time intervals are identical the assumption that the experiments are independent must be satisfied.

Therefore subjective comparisons of the 24, 48, 72 and 96 h LC₅₀ values within composition have been made in present study.

RESULTS

Behavioural changes

The behaviour and condition of fishes in both the control and experimental was noted at 24 hours. The fishes showed a marked change in their behaviour when exposed to various concentration of the chemical. The intensity of toxicity of cadmium chloride concentrations was most obvious than cadmium sulphate and cadmium nitrate in the first hour of exposure. Just after introduction to the test aquaria of chemical fishes showed interestingly try to jump out of aquarium to avoid the chemical followed by increased swimming, restlessness, surfacing and hyper activity. In lower concentration of cadmium compound the fishes showed slow swimming than the control group. Behavioural manifestation of acute toxicity like erratic swimming, restlessness and surfacing movement were observed in *Channa punctatus* (Bloch.) exposed to higher concentration of cadmium compound to the 24 hours. After 24 hours fishes exhibited lathery and erratic swimming suggesting loss of equilibrium at higher concentration. At the time of death transient hyper activity was also observed. The reaction and survival of aquatic animal depend not only the biological state of animals and physiochemical characteristics of water but also on the kind, toxicity, type of exposure to the toxicants.

Morphological changes

During this study, we documented the specific site of cadmium compounds action, among the exposed fish and examined the morphological changes. Among morphological changes, discoloration of skin, thin chemical deposition on skin, lesions started, less mucous secretion may cause the thin layer of chemical on aquarium bottom were observed at lower concentration of cadmium compounds. Among cadmium compounds, cadmium chloride caused maximum morphological changes than the cadmium sulphate and cadmium nitrate. The schooling is the characteristic of this fish was found weakened in this study. At higher chemical concentration, scale depletion start, skin lesion observed from dorsal to lateral side of the body of fish and these were deepens, copious mucous, clumping of gills increases with the increasing of concentration of toxicant. The skin lesions around the head region, base of caudal fins and pectoral fins were prominent in the 90 % of the fish (Picture-1). The fishes lost their natural coloration and become almost pale yellow in color.

Mortality and LC₅₀ Values

Channa punctatus weakened progressively with time before death; generally the pattern of mortality was similar for various concentrations of all the three composition of cadmium.

Results reveal that the test species, *C. punctatus* has shown differential toxicity level with the function of period. The mortality is both time as well as concentration dependent manner, and there is a significant negative correlation between LC₅₀ values and exposure periods and it also indicates a direct proportional relationship between mortality and concentration of cadmium composition. Thus increase with exposure period, LC₅₀ values was decreased. Theoretical Spontaneous Response Rate was zero (for control experiment). The 96 hours LC₅₀ concentration is less than the 24 hours, 48 hours and 72 hours concentration, which shows that the more is the duration period the less is the concentration required. Natural death rate one of the important factor was observed over 96 h period at all the three composition of cadmium.

Thus increase with exposure period, LC₅₀ values were decreased from 509.56 µg/l (24 h) to 463.32 µg/l (96 h) in cadmium sulphate, 578.71 µg/l (24 h) to 561.11 µg/l (96 h) in cadmium chloride and were 723.44 µg/l (24 h) to 711.05 µg/l (96 h) (table 1). The slope values given in toxicity were steep since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits may be because of higher natural. From the above results it was observed that the LC₅₀ values of 96 h were found lowest among all the exposure periods, of all the three compositions of a metal.

PLATE 1: Morphological alterations caused due to treatment of cadmium compounds after 96 hours exposure period



TABLE 1 : The LC₅₀ values (in µg/l) and with 95% confidence limits , chi-square p-values for goodness of fit in between cadmium composition for different duration viz., 24, 48, 72 and 96 h for freshwater fish *C. Punctatus*.

Exposure Time	Parameters			Cadmium sulphate	Cadmium chloride	Cadmium nitrate
24 h	LC ₅₀			509.56	578.71	723.44
	95% Confidence	Lower limit		466.74	558.16	694.31
	Limit	Upper limit		534.51	593.35	741.95
	χ ² (P-value)			14.98		
				(P<0.005)		
48 h	LC ₅₀			491.82	573.04	709.58
	95% Confidence	Lower limit		419.26	521.07	644.60
	Limit	Upper limit		525.62	599.95	740.10
	χ ² (P-value)		8.080 (3.92)			
72 h	LC ₅₀			454.91	551.00	683.03
	95% Confidence	Lower limit		226.28	370.13	469.20
	Limit	Upper limit		500.55	587.16	725.58
	χ ² P-value			6.564 (4.11)		
96 h	LC ₅₀			463.32	561.11	711.05
	95% Confidence	Lower limit		228.97	431.81	401.17
	Limit	Upper limit		483.09	574.00	732.08
	χ ² (P-value)			4.710 (2.93)		

DISCUSSION

Cadmium compounds poses toxic effects on the snakehead fish *Channa punctatus* which is evident by the findings of the present investigation and calculated LC₅₀ value observed in present study was nearer to the reporting of other workers on different fishes (Nilalohit *et al.*, 1981; Henary and Atchison, 1990; Brown *et al.*, 1994; Maruthayanagam *et al.*, 2002; Sobha *et al.*, 2007; Kasherwani *et al.*, 2009). The fish mortality may have resulted by absorption, bio-accumulation of cadmium compounds or greater activity of chemical in body of fish. The differences in the 24, 48, 72 and 96 hours LC₅₀ value between *Channa punctatus* and other fishes may be attributed to the fact that metal induced changes in physiology and survival of aquatic organisms under metallic stress is complicated because such changes differ from metal to metal, species to species and from one experimental condition to other. The exact causes of death due to heavy metal poisoning are multiple and depend mainly on time-concentration combination. Behavioural manifestation of acute toxicity in *Channa punctatus* were more or less similar to those reported in other fishes exposed to cadmium (Kasherwani *et al.*, 2009; Nagaraju *et al.*, 2011). Behavioural manifestations have been established as sensitive indicator of chemically induced stress in aquatic organisms (Suedel *et al.*, 1997; Vutukuru, 2005; Baltova and Velcheva, 2005; Remya *et al.*, 2008; Askari Hesni *et al.*, 2011). Behavioural alterations like erratic swimming, restlessness and surfacing may be an avoiding reaction to the heavy metal narcotic effects or to change in sensitivity of chemo-receptors (Nilalohit *et al.*, 1981; Sorenson, 1991; Maruthanayagam *et al.*, 2002; Atif *et al.*, 2005).

The skin together with the gills of fishes is the outer most defence against the surrounding environmental stress and toxicant (Ojha, 1993). Through respiration cadmium compounds circulate all over body and may become one of the causes of death of animal (due to hypoxia) (Nilalohit *et al.*, 1981; Maina, 1997). The injury, degenerative changes

in the skin and muscle have also been observed in fishes due to replacement by loose connective tissue elements (Banerjee, 1997). It has been observed that acute toxicity of cadmium compounds to fish *Channa punctatus* is mainly caused due to gill damage, enabling the fish to obtain oxygen from water and results due to anoxia (Hollis *et al.*, 1999; Eiser, Nilalohit *et al.*, 1981; Maruthanayangam *et al.*, 2002) during the study. Similarly, it has been stated that cadmium inhibits the action of acetyl-cholinesterase, causing death through paralysis of the respiratory muscle and/ or depression of respiratory system (Nilalohit *et al.*, 1981; Pratap and Bonga, 1990; Hollis *et al.*, 2000).

The differences in the 24, 48, 72 and 96 hours LC₅₀ value between *Channa punctatus* and other fishes may be attributed to the fact that metal induced changes in physiology and survival of aquatic organisms under metallic stress is complicated because such changes differ from metal to metal, species to species, chemical structure of metal compound and from one experimental condition (water temperature, salinity, oxygen content and pH) to the other. The exact causes of death due to heavy metal poisoning are multiple and depend mainly on time-concentration combination. Mortality was one of the important factors, observed over 96 hours for all three composition of cadmium for three seasons. Results reveal that the *C. punctatus* has shown different toxicity level with the function of period. The mortality is both time and concentration dependent, and there is a significant negative correlation between LC₅₀ values and exposure periods. The 96 hours LC₅₀ concentration is less than the 24, 48 and 72 h, which shows that the more is the duration period, the less is the concentration. Calculated LC₅₀ value observed in present study was nearer to the reporting of other studies on different fishes (Nilalohit *et al.*, 1981; Henary and Atchison, 1990; Brown *et al.*, 1994; Maruthayanagam *et al.*, 2002; Sobha *et al.*, 2007; Kasherwani *et al.*, 2009). The fish mortality may have

resulted by absorption, bio-accumulation of cadmium compounds or greater affect of chemical in body of fish.

The LC₅₀ values found for different fish species are the following: Cadmium chloride to *Clarius batrachus* 8.21 µg/l (Selvanthan *et al.*, 2011); to *Clarius batrachus* 103 µg/l (Bilal *et al.*, 2011); to *Channa* (*Ophiocephalus striatus*) 0.63 mg L⁻¹ (Bais and Lokhande); to *Tilapia mossambica* 209.34 mg l⁻¹ (Suresh *et al.*, 1993). Cadmium sulphate to *Ctenopharyngodon idellus* 9.42 mg/l (Emure and Ali; 2003). The 96 h LC₅₀ values obtained for *C. punctatus* in this study for cadmium sulphate, cadmium chloride and cadmium nitrate was 463.320 µg/l of 561.113 µg/l and 711.050 µg/l respectively.

CONCLUSION

The present findings suggest that the fishes showed a marked change in their behavioural and morphological changes when exposed to various concentration of the chemical. Mortality was one of the important factor, observed over 96 h period at all the three composition of cadmium in respect to all the three season. Results reveal that the test species, *C. punctatus* has shown differential toxicity level with the function of period. The mortality is both time as well as concentration dependent manner, and there is a significant negative correlation between LC₅₀ values and exposure periods. The 96 hours LC₅₀ concentration is less than the 24, 48 and 72 h, which shows that the more is the duration period, the less is the concentration required. Cadmium sulphate > cadmium chloride > cadmium nitrate.

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