Behavioural responses of freshwater Asian catfish, *Clarias batrachus* (Linn.) exposed to sodium fluoride.

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Abstract

Freshwater catfish *Clarias batrachus* were exposed to lethal and sub lethal concentrations of fluoride and allow to evaluating its behavioural alterations. The fish were observed to exhibit prompt and erectic behavioural responses at extremely low concentration of fluoride compared to high concentration. The abnormal behaviour observed in fish exposed to high concentration were characterized by hyperactivities, erratic swimming, frequent surfacing followed by sinking, loss of equilibrium, loss of coloration and gradual onset of inactivity. The behavioural responses were dependent on concentrations; concentration dependent increased behavioural responses were reported. Fish exposed to lethal concentrations of fluoride exhibited high degree of behavioural alterations when compared with those in exposed with sub lethal concentrations. No significant behavioural changes were observed in the control group.

Keywords: Freshwater Catfish, *Clarias batrachus*, fluoride toxicity, Bioassay, Behavioural responses, lethal responses, sublethal responses.

Introduction

Most of the countries in the world are facing the problem of drinking water. In India, drinking water is found to be contaminated at many places by different kinds of pollutants such as fluoride, iron and nitrates etc. Pure water is scarce and not easily available to all living beings including human beings, birds, animals as well as plants.[1] The water may be contaminated by natural resources or by industrial effluents. One such contaminant is fluoride. Fluoride is the most electronegative element and ionic form of fluorine. It is 17th most abundant element found in the Earth's crust. The main fluoride bearing minerals are cryolite (Na_3AlF_6), fluorspar (CaF_2) and fluorapatite ($Ca_{10}F_2(PO_4)_6$). Naturally, fluoride enters the aquatic system through the weathering of rocks, emission of volcanic eruption and anthropogenic activities results concentration of fluoride ion increases in water and sometimes it reached up to 50 mg/L in natural water[2]. Fluoride is highly toxic to aquatic organisms like fish, crabs and molluscans earlier reported by several workers.3[-9]

Behavioural alterations can be considered as sensitive indicators of environmental stress many workers have been recorded the behavioural changes in aquatic organism due to exposure of pollutants.[10-14] They all have also reported the behavioural abnormality include erratic jerky swimming, fast breathing, loss of schooling reflex and secretion of excessive mucus on the body. But very few reports are available for fish behavioural toxicity to

fluoride. Keeping this view in mind the present study have been planned to evaluate behavioural responses of freshwater catfish *Clarias batrachus* to exposed lethal and sublethal concentrations of fluoride.

MATERIALS AND METHODS

Fresh healthy live specimens of catfish, *Clarias batrachus* were collected from local freshwater resources and were transported to laboratory, treated with 0.5% KMnO4 solution to obviate dermal infection. There after acclimatized to standard laboratory conditions in large cement tank for 15 days, containing dechlorinated tap water at room temperature 28 ± 1° C and pH 7 ± 0.2. During the acclimatization fish were fed on goat liver twice in a day. The physiochemical parameters of water used for experimentation were maintained according to APHA guidelines.[15] The specimens weighing 50 ± 5 g and measuring 16 ± 2 cm were selected for experiments, experiment was conducted in large glass aquaria measuring 60 x 40 x 45.

The experiment was carried out in two stages, stage I for acute toxicity and stage II for sublethal responses. For acute toxicity, fish were exposed to various lethal concentrations of fluoride for 24, 48, 72 and 96 hours along with only one control (without toxicant). Fish were not given any food during experiment. Water with test concentration of fluoride was replaced after every 24 hour to 96 hours and resulting mortality was recorded in the range of 10 % to 100% for each concentration for the duration of 24, 48, 72 and 96 hours. The mortality data was statistically analyzed by Trimmed Spearman Karber Method[16] for calculation of LC_{50} and 95% confidence limits for each exposure period. Simultaneously behaviour changes of the fish were also observed and recorded during the exposure period.

In stage II, fish were exposed to three sublethal concentrations (35, 70 and 105 mg F/L) of fluoride along with a one control for 96 hours and observed behavioural responses, compared with control.

The test Chemical sodium fluoride (NaF) E- Merck, Mumbai, LTD., India was used as toxicant. The stock solution of toxicant was prepared by dissolving 22.11 g of NaF / L, containing 10 g fluoride ion per litre.

RESULTS

Fish exposed to lethal and sublethal concentrations of fluoride their behavioural responses were observed (Table 1).

Table 1: Behavioural responses of *Clarias batrachus* on fluoride exposure.

Parameters	Lethal concentration (mg F/L)					Sublethal concentration (mg F/L)		
	100	200	300	400	500	35	70	105
Erratic Jerky swimming	-	-	+	+	+	-	-	+
Loss of Reflexes	-	+	+	+	+	-	-	+
Discolouration	-	+	+	+	+	-	+	+
Change in schooling behaviour	-	+	+	+	+	-	+	+

Opercular movements	+	+	+	+	+	+	+	+
Frequent surfacing	+	+	+	+	+	-	+	+
Lethargy	-	+	+	+	+	-	+	+

Parameters compared with control (+ indicates increase, - indicates no response)

The abnormal behaviour observed in *Clarias batrachus* were characterized by hyperactivity, erratic swimming, frequent surfacing followed by sinking, loss of equilibrium, loss of coloration and gradual onset of inactivity. The concentration and duration dependent increased behavioural responses were observed in exposed fish. The observations from the bioassay revealed the *Clarias batrachus* exhibited increased in stress as evidenced by slow and uncoordinated movement. It was observed in the course of range finding tests the preliminary test that the least lethal concentration for fish within 2 to 3 hours of exposure. Hence the sub lethal concentrations of fluoride turn to lethal concentration of fish within 96 hours of exposure.

The mean lethal concentration (LC_{50}) of fluoride to fish, *Clarias batrachus* for 24, 48, 72 and 96 hours exposure periods were recorded (Table 2). No mortality was recorded in control group during experiment.

Table 2: **LC_{50} values of fluoride and their 95 % confidence limits to *Clarias batrachus*.**

Exposure duration (Hours)	LC_{50} Values (mg F ion/L)	Confidence limits	
		Lower limits (mg F/L)	Upper limits (mg F/L)
24	406.22	374.46	438.33
48	391.80	354.57	432.94
72	366.80	339.84	392.27
96	349.75	323.58	370.02

Fish exposed to lethal concentration and higher concentration of fluoride showed high degree of behavioural responses then the lower concentrations. However, the fishes in the control group don't show any behavioural abnormalities compared to different concentrations of fluoride exposed groups.

DISCUSSION

The concentration and duration dependent behavioural responses were observed in *Clarias batrachus* exposed to different lethal and sublethal concentrations. During the first two hours of the exposure of the test organisms to various concentrations of the fluoride, abnormal behavioural changes such as erratic with jerky swimming, frequent surfacing, discoloration, loss of schooling reflex, opercular movement and lethargy were exhibited. These responses are in consonance with observations of Tripathi et.al.[17] (2004), Bajpai et. al.[18](2009) and Sahu et.al [19](2014) that exposed *Channa punctatus*, *Hetropneustes* and, *Clarias batrachus* respectively to various concentrations of fluoride and fluoridated compounds. Oti [20] concluded that these behavioural changes are indication of death due to depletion of Oxygen and nervous disorder.

The study of effect of fluoride toxicity on *C. batrachus* at different concentrations revealed that fishes are more sensitive and responded promptly to the toxic effects of higher concentration in short duration. This was confirmed considering the erratic responses and death of the fishes exposed to lethal concentration. These observations were also reflected in the median lethal concentration (LC_{50}) recorded for *C. batrachus*. The trends observed in the behavioural pattern of fish could be traced to the variations in exposure duration and concentration level. The vulnerability of the *C. batrachus* to the toxic actions of fluoride could be attributed to the impairment and or seizure of the metabolic activities as a result of incomplete physiological systems.

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