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Acute toxicity of fluoride and aluminium on the freshwater fish, *Cyprinus carpio* L.

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Fluoride is a trace element which has beneficial effect at lower concentration but detrimental at higher concentration. The fluoride contamination in ground water is a major global problem. Similarly, aluminium is the most abundant metal of the earth, highly toxic to all organisms. In the present study, we investigated the acute toxicity of fluoride and aluminium on three month old fingerlings of *Cyprinus carpio* L. in hard water (Hardness 125 mg/L of $CaCO_3$) under static renewal bioassay. The 96 h LC_{50} value of the fluoride and aluminium were 675.615 and 224.214 mg/L, respectively. There was a significant correlation (P <0.01) between mortality rate of *C. carpio* with all concentrations of fluoride as well as aluminium. The exposure time (24, 48, 72 and 96 h) and different concentration of fluoride (680, 684, 686 and 692 mg/L) and aluminium (228, 229, 231 and 232 mg/L) was also significantly correlated (P <0.05). The different abnormal behaviour displayed by the fluoride and aluminium exposed fishes were erratic swimming movements, rapid opercular activity and excessive secretion of mucous. The safe level of concentrations of fluoride and aluminium were 6.75 and 2.24 µg/L, respectively. The LC_{50} values of fluoride and aluminium of the present study may be useful in deriving water quality standards in West Bengal.

Keywords: Aquatic pollution, Dental fluorosis, Indian major carp, Opercula, Sodium fluoride, Water toxicity

The fluoride ion is derived from the element fluorine, reacts with the cations of other elements to forms fluoride compound which together represent about 0.06-0.09% of the earth crust[1,2]. Fluoride at low concentration (0.5-1.0 mg/L) in drinking water, is beneficial for human health preventing dental decay by remineralization. But it causes dental fluorosis, a hypo mineralization disorder of ameloblasts and a crippling bone disease called skeletal fluorosis when drinking water contains a higher concentration of fluoride (>1.5 mg/L)[3] . The permissible limit of fluoride by World Health Organization (WHO) and Bureau of Indian Standard (BSI) is 1.5 mg/L and according to Indian Council of Medical Research (ICMR), and the Committee on Public Health Engineering Manual and Code of Practice, Govt. of India it is 1.0 mg/L[4,5]. Ground water with more than 1.5 mg/L of fluoride is reported in 21 out of 29 states of India[6,7], including West Bengal[8,9]. Fluoride contamination in ground water has also been reported from Africa, China, Japan and Sri Lanka[10].

Aluminium is the most abundant metal in the lithosphere, exist in the form of silicates, oxides and hydroxide[11]. Despite its low abundance in the water, aluminium is acutely toxic to fish but the toxicity of aluminium is depends on pH, temperature and presence of inorganic and organic ligands[12]. The toxicity of aluminium is most severe between pH 5.0 and 6.0 and the toxicity is increase when pH rises in the water due to polymerization of aluminium[13]. Aluminium induces hypoxia in fishes due to accumulation on the gill surface after polymerization[14]. Several studies reported the neuro- as well as cardio-toxicity of aluminium to fish[15,16]. The permissible limit of aluminium in drinking water by Bureau of Indian Standard is 0.2 mg/mL[5].

Both, the fluoride as well as aluminium, exert oxidative stress in animals through excess production of reactive oxygen species via the fentom reaction and inhibition of antioxidant enzymes[1,17,18]. Also, fluoride and aluminium damage the gill of fishes, result in ionoregulatory, osmoregulatory and respiratory dysfunction, and lead to fish death[17,19]. The growth[20], biochemical[20,21], hematological[11], and histopathological[22] parameters of fish are also severely affected by fluoride and aluminium.

The common carp, *Cyprinus carpio* L. is the 4th major species (7.7% of the total) produced in world aquaculture in 2018[23]. With its importance in commercial aquaculture and wide geographical distribution, the common carp has been selected for the present study [18,24,25]. Though extensive studies are available on the impact of fluoride and aluminium on *C. carpio*, detailed report on the acute toxic effects are lacking. Hence, in the present study, we investigated the acute toxic effect of fluoride and aluminium on *C. carpio* fingerlings under static renewal bioassay.

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Materials and Methods

Test animals

The fingerlings of *C. carpio* L. (Cyprinidae: Cypriniformes) (mean length: 6.171±0.145 cm, mean weight: 2.602±0.552 g) of three months old, were used for the bioassay. The fingerlings of *C. carpio* were collected from a fish market located at Naihati, North 24 Parganas, West Bengal, India and transported to the laboratory in a large plastic container with sufficient oxygen. Collected fingerlings of *C. carpio* were stored in a large glass aquarium with proper aeration for a period of four week to acclimate gradually in the test media. The experimental study was conducted as per the internationally accepted laboratory animal use and care, and guidelines (guiding principles in the use of animals in toxicology, adopted by the Society of Toxicology in 1999), and as per the guidelines of the Institutional Animal Ethical Committee, Department of Zoology, University of Burdwan, Burdwan, West Bengal, India.

Test media

Dechlorinated tap water was used as the test media in this study. The physico-chemical character of water was estimated as per the standard method[26] and the essential water quality parameters were temperature 29.4°C, pH 7.8, dissolved oxygen 7.46 mg/L, free CO_2 10.12 mg/L , total alkalinity 90 mg/L, hardness 125 mg/L, fluoride 1.48 mg/L and aluminium less than 0.01 mg/L.

Test chemicals

Analytical grade aluminium sulphate [$Al_2(SO_4)_3$ mw 630.38 g/mol] and sodium fluoride (NaF mw 41.98) were used as the test chemicals. The stock solution of each toxicant was prepared by dissolving appropriate quantity of test chemical in distilled water in a volumetric flask (1000 mL). On the other hand, working solution of each toxicant was prepared from the stock solution at the time of the experiment.

Experimental design

Acute static renewal toxicity assays were conducted as per the method of Sprague[27] and USEPA[28] in which test media were replaced daily with freshly prepared ones as some of the toxicants degraded in due course of time, in order to provide a constant effect of the toxicants on test animals. To carry out preliminary toxicity bioassays for each toxicant, 10 well acclimated fingerlings were introduced in each small glass aquarium containing 10 L of tap water. The preliminary rough range finding tests for 48 h was conducted as per the method of Ramakritinan *et al*.[29] with some modification, in which the nominal test concentration of each toxicant was prepared in a linear scale (50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 and 1000 mg/L) using three replicate for each concentration with sufficient control. The preliminary rough range finding tests were repeated several times until mortality occurs. After preliminary rough range finding tests, a narrow range of concentrations of each toxicant was taken for the definitive toxicity tests in order to estimate the LC_{50} value[29]. Selected nominal concentrations of fluoride (F) were 680, 682, 684, 686, 688, 690, 692, 694, 696 and 698 mg/L whereas the aluminium (Al) were 224, 225, 226, 227,228, 229, 230, 231, 232 and 233 mg/L. The measured mean (±SD) concentrations of fluoride[26] at each treatment were 460.33±0.57, 462.00±1.00, 463.33±0.57, 464.66±0.57, 467.66±1.52, 468.66±1.15, 470.33±0.57, 472.66±1.15, 474.33±1.15 and 476.00±1.00 mg F /L, respectively. On the other hand, mean (±SD) aluminium[26] concentrations at each treatment were 0.21±0.02, 0.22±0.02, 0.22±0.01, 0.25±0.01, 0.26±0.01, 0.28±0.02, 0.30±0.03, 0.33±0.02, 0.37±0.02 and 0.40±0.05 mg Al/L, respectively. The fishes were not provided any food during the period of experiments and the water was not aerated. The tests were rejected when the mortality was more the 10% in the control. A fish was considered to be dead if it gave no response by gentle touch of a net[24] and when there was no opercular movement[30]. Dead fishes were counted and removed immediately in every 24 h to prevent deterioration of water[24].

Stastistical analysis

The cumulative mortality of the test animals after 96 h was used to estimate LC_{50} values with 95% confidence limit by Probit analysis[31] using Statistical Package for Social Science (SPSS) version 20. The correlation between different concentrations of toxicants (fluoride and aluminium) with mortality rate of test animals as well as with exposure times were analyzed by SPSS. The safe level of concentration of fluoride and aluminium were determined as per the methods of Kamewara (1974)[32] and Miller & Miller (1986)[33].

Results & Discussion

The lethal and safe concentrations of fluoride and aluminium to *C. carpio* fingerlings are presented in

Table 1. There was no mortality in the control group of *C. carpio*, which is in agreement with the standard EPA/COE procedure wherein mean survival should be 90%[29,34]. The 96 h LC_{50} value of fluoride (675.615 mg/L) was greater than aluminium (224.214 mg/L) indicating that aluminium was highly toxic to *C. carpio* than fluoride. The calculated LC_{50} values of fluoride and aluminium for *C. carpio* were found to be reduced with increasing of exposure time. In addition, there was a significant correlation (P <0.01) between mortality rate of *C. carpio* with all concentrations of fluoride as well as aluminium. The exposure time (24, 48, 72 and 96 h) and different concentration of fluoride (680, 684, 686 and 692 mg/L) and aluminium (228, 229, 231 and 232 mg/L was also found to be correlated significantly (Table 2 & 3). Acute toxicity test conducted in the present study demonstrate that *C. carpio* is sensitive to both fluoride and aluminium.

In the present study, 96 h LC_{50} value of fluoride to *C. carpio* (675.615 mg/L) is much higher than the 96 h LC_{50} value of fluoride for *Oncorhynchus mykiss* (200 mg/L) and *Puntius sophore* (126.12 mg/L) as estimated by Smith *et al.*[35] and Narwaria & Saksena[30], respectively. The wide variation of the LC_{50} value observed in the present study from that of the *O. Mykiss* and *P. sophore* was probably due to higher hardness of water. The hardness of water has a protective effect against fluoride toxicity due to precipitation of insoluble calcium fluoride from hard water[35,36]. The higher hardness (125 mg/L $CaCO_3$) of the water in the present study probably reduces the toxicity of fluoride. Apart from fishes, the 96 h LC_{50} value of sodium fluoride for Tadpole Skittering Frog, *Euphyctis cyanoplyctis* was reported to be 647 mg/L at water hardness ($CaCO_3$) of 89 to 98 mg/L[37]. Whereas, 96 h LC_{50} value of fluoride to adult fresh water snail *Physella acuta* was 120.3 mg/L at water hardness ($CaCO_3$) of 147 mg/L[38].

On the other hand, the 96 h LC_{50} value of aluminium for *C. carpio* (224.214 mg/L) is resembling to the 96 h LC_{50} value for *Clarias batrachus* (220 mg/L) estimated by Naskar *et al.* 2006[11]. However, Azmat *et al.*[39] determined the mean 96h LC_{50} value of aluminium to the *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* and the values were 81.68, 105.46 and 118.36 mg/L respectively at pH 7.5 and water hardness of 300 mg/L. In an earlier study, Rani[40] reported the 96 h LC_{50} of aluminium to *C. carpio* as 26.42 mg/L in a water hardness of 18 mg/L.

The fluoride and aluminum exposed *C. carpio* fingerlings showed various abnormal behaviors like erratic swimming movement, rapid opercular activity and excessive secretion of mucous. The intensity of the abnormal behaviors was more prominent in aluminium exposed groups compared to the fluoride exposed groups. However, the control group of fishes showed normal behaviour. Different erratic swimming movements shown by the *C. carpio* exposed to fluoride was probably due to the result of acute toxicity of fluoride. Narwaria & Saksena[30] also

Table 1 — Parameters of acute lethal toxicity bioassays with fluoride and aluminium for *C. carpio* exposed under static renewal bioassay tests

Toxicant	Exposure time (h)	LC_{50} (mg/L)	95% confidence interval	Safe conc. (µg/L)
Fluoride	24	689.948	687.165-693.230	
	48	683.394	678.037-686.052	
	72	678.254	663.142-682.099	
	96	675.615	636.978-680.674	6.75
Aluminium	24	229.599	228.071-231.906	
	48	228.322	226.755-229.814	
	72	227.141	225.207-228.461	
	96	224.214	221.365-225.108	2.24

Table 2 — Correlation (r± SE) of fluoride and aluminium concentration with mortality rate for *C. carpio* significant at 1% level

Test chemical	Exposure time	r	SE	P value
Fluoride	24	0.936	2.254	P <0.01
	48	0.988	1.054	P <0.01
	72	0.924	2.462	P <0.01
	96	0.934	2.353	P <0.01
Aluminium	24	0.863	1.620	P <0.01
	48	0.909	1.338	P <0.01
	72	0.969	0.798	P <0.01
	96	0.826	1.811	P <0.01

Table 3 — Correlation (r ±SE) of exposure times (24, 48, 72 and 96 h) with mortality rate for common carp to each fluoride conc.

Test chemical	Conc. (mg/L)	r	SE	P value
Fluoride	680	0.992	0.196	P <0.05
	682	0.939	0.542	P >0.05
	684	0.990	0.225	P <0.05
	686	0.990	0.224	P <0.05
	688	0.944	0.522	P >0.05
	690	0.913	0.645	P >0.05
	692	0.983	0.293	P <0.05
	694	0.887	0.731	P >0.05
	696	0.775	10.00	P >0.05
Aluminium	224	0.894	0.707	P >0.05
	225	0.913	0.645	P >0.05
	226	0.923	0.607	P >0.05
	227	0.894	0.707	P >0.05
	228	0.956	0.463	P <0.05
	229	0.956	0.463	P <0.05
	230	0.887	0.731	P >0.05
	231	0.983	0.293	P <0.05
	232	0.983	0.293	P <0.05

reported the similar abnormal movement in *P. sophore* exposed to fluoride. The fluoride damages the gill epithelia resulting in the higher opercular activity in the fluoride exposed fishes than the control[17]. On the other hand, different abnormal behaviour displayed by aluminium exposed fishes was probably due to acute aluminium toxicity[19,41]. Behavioural changes like erratic swimming movement as well as secretion of mucous from the body were more prominent in aluminium exposed fishes compared to the fluoride exposed fishes, demonstrating the higher toxic effect of aluminium than fluoride in fish.

Conclusion

The acute toxic effect of fluoride and aluminium in *C. carpio* was determined in the present study. The LC_{50} values of the fluoride and aluminium were 675.615 mg/L and 224.214 mg/L, respectively. Whereas, the calculated safe concentrations of fluoride and aluminium were 6.75 and 2.24 µg/L, respectively. Concentrations of fluoride and aluminium above the safe concentration levels can have adverse effects on the *C. carpio*. Owing to the high toxicity of fluoride and aluminium in fish, it is strongly recommended to prevent the indiscriminate use of different fluoride and aluminium containing compounds or substances like phosphate fertilizer, pesticides, dyes, baking powder, antacids, anticoagulant, etc. hence the harmful effects of fluoride and aluminium to aquatic life can be minimized.

Conflict of Interest

Authors declare no competing interests.

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