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RESEARCH ARTICLE

A Behavioral, Morphological and Growth Rate Alterations in Fresh Water Fish *Tilapia mossambica*under Fluoridation, Keenjhar Lake, Thatta, Sindh, Pakistan

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Fluoride is the most electronegative and active nonmetallic element. Fluoride has been known as a strong, persistent potent cumulative toxic agent, occurs naturally widely distributed in the rivers, lakes and seas of world. Fish and other aquatic animals tend to accumulate fluoride from the environment, chiefly in the skeleton (including the gills) and exoskeleton. Fluoride toxicity was studied to average weight 94.5gand standard length 24.62cmfresh water fish,*Tilapia mossambica* after their exposure to sub lethal concentration (1.5g/70 L and 3.0 g/70L NaF) for 7 days, 14 days, 21days and 28 days. A reduction in weight, length and growth were recorded in fresh water fish, *Tilapia mossambica*after their exposure to both sub lethal concentration of fluoride. The current investigations proposed that the long term administration of inorganic fluoride to laboratory fish have established that fluoride can elicit impairment of behavior, morphological and growth development.

Introduction

Fluoride is highly mobile and biological active element in aquatic systems, could potentially reach toxic levels in the water column during dredging to remove fluoride-contaminated sediment (Janiceet al., 2003). Fluoride has a tendency to accumulate in organisms, specifically deposits in bone or exoskeleton making adverse effects possible even at very low levels of exposure(Julshamn etal., 2004).Fish are extremely sensitive to many water-borne toxicants, due to their prolonged, constant and direct contact with the aquatic environment where chemical exposure occurs over the entire body surface and ecological significance in any natural systems (Little et al., 1993).

The toxicological impact of fluoride on a variety of aquatic species is well predictable with its harmful effects on humans, livestock, and plants (Gikunju, 1992).A number of studies on aquatic invertebrates like *Daphnia, Artema, Penaeus, and Hydropsyche* reveal that fluoride affects survival, growth, behavior, and reproduction. Fluoride also alters physiological, hematological parameters, morphological and behavioral parameters, and cellular structure in vertebrates (Dwivedi et al., 1997; Camargo, 2003; Mariappanet al., 2000).The effect of fluoride concentration on weight and length of *Johniusbelangerii* of Arabian Sea fish were studied by Azmat et al., (2007). A statistically significant relation was observed in between concentration of fluoride and condition.Since fluoride has been reported to affect nervous as well as endocrine systems in animals (Guan et al, 1999; Gao et al., 2009; Sharma et al., 2007 and Wang et al., 2009).It might also be having adverse effects on pigmentation in fishes after chronic exposure.

Hence the present study is designed to evaluate the toxic potentiality of sub lethal concentration of sodium fluoride on fresh water fish, *Tilapia mossambica*, collected from Keenjhar Lake, Thatta, Sindh, Pakistan.

Materialsand Methods

Collection of Fish

Healthy living *Tilapia mossambica* (average weight 94.5g and standard length 24.62cm) were collected from the Fish Farming Zone of Keenjhar Lake, Sindh in March 2010**(Fig.1)**. Temperature of lake was 30^{O}C. Humidity was 70%. Fish were caught with the help of professional local fisherman by using local fishing nets and motor boat.

Fig-1: Keenjhar lake: The largest freshwater lake in Pakistan

Fish Acclimation

Fish were transferred to laboratory under ordinary maintained laboratory conditions. Fish were grouped and placed in a fiber glass aquarium containing tap water, size: 36cm x 18cm x 15cm **(Fig: 2)**. Air pumps and filters were used to aerating the aquarium water by circulating it. During acclimation all control and treated fish were feed with commercial pellet once a day. Water in aquaria was changed after two days. Chemical analysis of water was done according to standard methods. The fish were divided into six groups with ten fish ineach group. Group 1 served as non-treated while Group II and IIIserved as experimental groupstreated with sub-lethal concentration of fluoride (1.5gm / 70 L and 3.0 g /70LNaF). A behavioral, morphological and growth rate measurements in fresh water fish *Tilapia mossambica*were made at 7 days, 14 days, 21 days and 28 days.

Fig-2: Fish were placed in fiber glass aquarium with proper aeration

Behavioral, Morphological and Growth rate measurements

Behavioral responses and morphological changes in fresh water fish, *Tilapia mossambica* after their exposure to sub lethal concentration of fluoride were visually recorded at7, 14, 21 and 28 days. Test and control fish were subjected to the determination of body weight (BW) and total length (TL) on 7, 14, 21 and 28 days at 1.5g and 3.0 g /70LNaF.Weight was taken on digital electric balance in gram while length was taken by measuring tape in centimeters. A complete and careful morphological, behavioral and growth observation on both treated and non-treated fish were made.

Statistical Analysis

The results are represented as Mean±S.E.M. The date was statistically analyzed by paired student t-test at 95% confidence interval of the difference to determine the level of significance. P values ≤ 0.05 were considered significant.

Results and Discussion

Fluoride is a widespread, non-biodegradable, highly oxidizing element of the periodic table with four electronegativity and relatively persistent pollutant of the environment (Lee, 1983). Due to its high biological activity and small ionic radius, it penetrates easily into the organisms and tissues. It has adverse chronic effects on different tissues (Rosenquist and Lemperg, 1974; Devi and Piska, 2006 a, b).

General behavioral responses, morphological changes and growth pattern upon fluoride toxicity in fresh water fish*Tilapia mossambica* at regular time intervals of 7, 14, 21 and 28 days at sub lethal concentration of sodium fluoride (1.5 g F/70L and 3.0 g/70LNaF) were carefully recorded.

Behavioral responses and Morphological changesof the control and exposed fish

In the present study, the control fish were healthy and active for locomotory activity, feeding, and common respiratory responses.Fish swims together in a synchronizedfashion. The behavior did not significantly vary in the control group; therefore, these results were taken as standards for the whole study. Changes in spontaneous locomotors activity and breathing responses are sensitive behavioral indicators of sub lethal exposure to toxicant in fish (Scherer, 1992).Fish exposed to sub lethal concentration of sodium fluoride (1.5 g /70L and 3.0 g/70L NaF) after 7 and 14 daysshowed marked apathy then loss of orientation and equilibrium, and increased mucus secretion and finally gasping for breath, reduction of swimming ability, darkening of color, and then death after long term of exposure (Camargo andTarazona, 1991).The accumulation and increased secretion of mucus in the fish exposed to fluoride may be an adoptive protective response to avoid the absorption of the applied toxicant by the overall body surface. (Das and Mukherjee, 2003; Yilmaz et al., 2004;Prashanth et al, 2005 and Subathra and Karuppasamy, 2003).

Other behavioral observationsrevealed fish suffering from anorexia lose weight, go through a period of violent movement which degrades into aimless wandering, and finally lose their equilibrium. Consequently, fish moved to the corners of the test chambers, which can be regarded as an adaptive/ avoidance behavior of the fish to toxicant (Dube andHosetti, 2010; Scherer, 1992). The response of fish to fluoride concentration (1.5 g/70L and 3.0 g /70L NaF) is related to environmental acclimatization and is species dependent because various types of fish were used to check the toxicity of fluoride. Several environmental pollutants e.g., heavy metals, acid rain, and organic xenobiotic have been identified to affect the architecture of the gill epithelium associated with these morphological pathologies, one finds changes in blood ionic levels, gill Na,K-activated ATPase activity and ionic fluxes (Evans, 1987).

A significant morphological change in gills structure, a major respiratory organin fish, associated with fluoride toxicity which may be due to the pronounced effects on gill solute and water transport by toxic substances and by alteration of energy metabolism during the period of acclimatization of under studied fish (Oleson et al., 2005).A change in respiration rate, which is commonly used to evaluate the changes in metabolism under environmental deterioration. A respiratory distress/ dysfunction as a consequence of the disturbance in oxidative metabolism were reported earlier under cypermethrin toxicity in *Tilapia mossambica* (David et al.,2003).The fish in the control

aquarium were observed to be healthy and normal and no mortality was recorded in it.Maximum mortality rate was observed after12 weeks.

Growth rate measurementsof the control and treated fish

The effect of fluoride concentration on weight and length of *Tilapia mossambica* of Keenjhar Lake at regular time intervals of at sub lethal concentration of sodium fluoride were studied.The weight and length of treated fish *Tilapia mossambica* were significantly decreased ($p<0.0010$) at 7, 14, 21 and 28 days at 1.5g and 3g/70L NaF. Results indicated that both sub lethal and lethal fluoride dose may have chronic effect on fish body size and fish weight/length ratio. A statistically significant relation was observed in between weight and length of control and experimental fish**(Table- 1)**.

Table-1: Length &weight of non-treated and treated fish *Tilapia mossambica* at 1.5g/70L and 3g/70L of NaF

Dose	Control Weight (g)	Control Length (cm)	Weight/ Length ratio	Exposure Time (d)	Test Weight (g)	Test length (cm)	Weight/ Length ratio
1.5g NaF/70L	**94.52±0.186** N=25;SD±0.930	**22.86±0.214** N=25;SD±1.072	4.134	7	**90.56±0.838*** ** N=25;SD±4.190	**19.75±0.232*** ** N=25;SD±1.634	4.585
				14	**88.85±0.171*** ** N=25;SD±0.857	**16.85±0.372** ** N=25;SD±1.861	5.272
				21	**86.76±0.341*** ** N=25;SD±1.709	**15.58±0.260*** ** N=25;SD±1.301	5.568
				28	**85.53±0.164*** ** N=25;SD±1.270	**14.75±0.181*** ** N=25;SD±1.537	5.798
3g NaF/70L	**95.87±0.171** N=25;SD±0.856	**26.44±0.208** N=25;SD±1.042	3.370	7	**89.00±0.206*** ** N=25;SD±1.034	**26.82±0.175*** ** N=25;SD±0.876	3.318
				14	**87.17±0.266*** ** N=25;SD±1.332	**23.926±0.149*** ** N=25;SD±0.745	3.685
				21	**83.39±0.316*** ** N=25;SD±583	**21.16±0.345*** ** N=25;SD±1.728	3.940
				28	**81.32±0.427*** ** N=25;SD±583	**19.26±0.425*** ** N=25;SD±1.728	4.222

N = no. of fish = 25; values expressed as Mean ± S.E.M; S.D = standard deviation; p = probability, ***represent highly significant ($p<0.001$), ** represent significant ($p<0.01$), * represent significant ($p<0.05$) compared to control

Fluoride causes alteration of size and growth in fish (Samal, 1994) and considered as a potent dangerous pollutant to fish (Bagaleetal., 2011).The reduced growth in the treated fish group may be due to disturbing major metabolic pathways, behavioral changes, histopathological changes in gill, kidney or intestine (Bhatnagar and Regar, 2007) and the accumulation of fluoride in bone tissues or exoskeletonmight affect growth rate offish (Shi etal., Yoshitomietal., 2006).

Conclusion

The exposure of living *Tilapia mossambica* of Keenjhar Lake to above-normal concentrations of fluoride, introduce fluoride accumulation by the fish, as fluoride is highly toxic and persistent bio accumulator in living organism, Its chronic effect may lead to a variation of the organism behavioural, respiration,morphological and growth measurements.

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