



Effect of Fluoride on Levels of Selected Toxic Heavy Metals in Gills of Edible Fresh Water Fish *Tilapia mossambica* from Keenjhar Lake, Sindh, Pakistan

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Abstract

The present research work was conducted to examine the lethal effects of inorganic fluoride (F⁻) on accumulation of toxic heavy metals in gills of edible fresh water fish *Tilapia mossambica* collected from Keenjhar Lake, Sindh, Pakistan. Levels of selected toxic metals like Lead (Pb), Mercury (Hg), Cadmium (Cd), Cobalt (Co) and Arsenic (As) at different time intervals with fluoride concentration 1.5 g/70L NaF and 3 g/70L NaF in the gills of *Tilapia mossambica* were determined by using Perkin – Elmer Atomic Absorption Spectrophotometer at parts per million (ppb) level. High concentration of toxic heavy metals (Pb, Hg, Cd, Co and As) showed that fluoride increase gills membrane permeability results in minerals unbalancing in fresh water fish *Tilapia mossambica* from Keenjhar Lake, Sindh, Pakistan. The order of metals accumulation in gills, vital organ for respiration was cobalt > arsenic > lead > mercury > cadmium at both doses and all exposure time intervals.

Keywords: Toxic heavy metals, fluoride, gills, atomic absorption spectrophotometer, metals accumulation.

Introduction

Fluoride has been known as strong, hard anion and cumulative toxic agent¹ occurs naturally mostly distributed in the rivers, lake and seas around the world². Fluoride is a cumulative toxin and the most damaging environmental pollutant, has affinity to accumulate in the tissues of organisms, making adverse effects to aquatic life at very low levels of exposure^{3, 4, 5}. Fluoride may be considered as a xenobiotic to the biological ecosystem at elevated level disturb the normal metabolic pathways of an organism.

Fish are the main aquatic food chain organisms may often accumulate large amounts of certain metals⁶. Fish are commonly used to assess the quality and health condition of aquatic ecosystems and as such can serve as bio indicators of environmental pollution⁷⁻¹⁰. A number of pollutants including toxic heavy metals like cadmium, copper, mercury, lead and zinc are found to be universally present in rivers, lakes, reservoirs and are destructive for aquatic life and can exhibit toxic effects and death in the aquatic systems at elevated concentrations^{11,12}. In general, toxic heavy metals are non-degradable and regarded as hazardous to aquatic ecosystem because of their environmental persistence and their tendency for bioaccumulation¹³⁻¹⁵. Gills are the major sites of gas exchange, acid – base regulation and ions transport. It is the organ directly exposed to toxicants and an increase and decrease

in aquatic metals concentration results in a disruption to gill osmoregulatory function of fish gills.

In the present study, we estimated the levels of selected heavy toxic metals in the presence of fluoride in gill tissues of fresh water fish *Tilapia mossambica*, collected from Keenjhar Lake, Thatta, Sindh, Pakistan (figure-1).

Material and Methods

Fish collection: Healthy living and active *Tilapia mossambica* (average weight 89.5g and standard length 8.9cm) were collected from the Fish Farming Area of Keenjhar Lake, Sindh in February 2012 (figure-2). Temperature of lake was 29°C. Humidity was 70%. Fish were caught with the help of skilled local fisherman by using local fishing nets and motor boats.

Fish Acclimation: Fish were transported to laboratory under ordinary conditions. Fish were grouped and placed in a fiber glass aquarium containing tap water, size: 36cm x 18cm x 15cm (figure- 3). Air pumps and filters were used to aerating the aquarium water by circulating it. 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 two groups with ten fishes per group. Group 1 serves as non-treated while group II and group III served as experimental group. Group II was treated with sub-lethal concentration of

fluoride (1.5g / 70 L of NaF) while group III was treated with lethal concentration of fluoride (3 g / 70 L of NaF). Both control and treated fish were scarified after 7, 14, 21 and 28 days and gills were removed, washed with distilled water and stored at 4 °C for further studies.

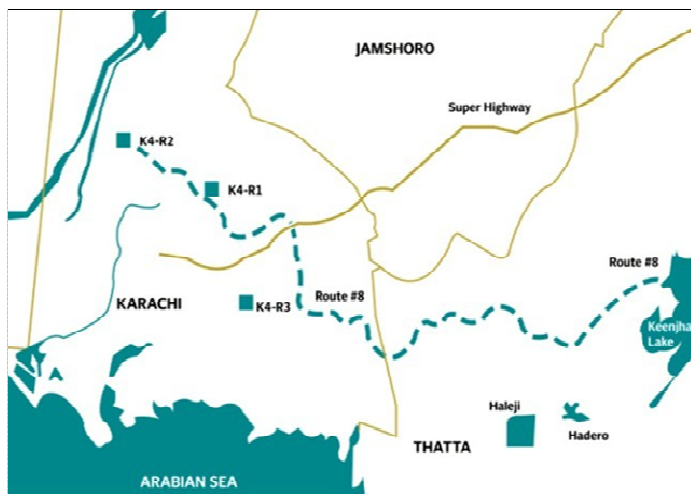


Figure-1

Study Area map of Keenjhar Lake, Thatta, Sindh, Pakistan



Figure-2

Fish collection region at Keenjhar Lake, Thatta, Sindh

Digestion Method for Preparation of Sample for Metals

Analysis: Equal weights of all control and treated fish gills were put into separate crucibles (using 0.2 g dry mass and a dilution factor of 50). The crucibles were placed in the oven for 2 h at 135 °C. After that the samples were mineralized at 400 °C in the chemical oven for 24 h then 2 mL of nitric acid was added and samples were dehydrated at 450°C. To each sample 10 mL of hypochlorous acid was added and then make up to 50 mL with double distilled water. The detection of heavy toxic metals was carried out by atomic absorption spectrophotometry with graphite furnace and acetylene flame.



Figure-3

Fish were placed in fiber glass aquarium with proper aeration

Statistical Procedure: The data was subjected to statistical analysis. Student's *t*-test was used to determine the level of significance of the heavy metal concentration in control and treated fish. Differences were regarded as significant at $p \leq 0.05$.

Results and Discussion

Fluoride is a well-known, non-decomposable and relatively persistent contaminant in the environment¹⁶. Due to its high biological activity and small ionic radius, it penetrates easily into the organisms and tissues. It has adverse effects of high and chronic effects on different tissues¹⁷⁻¹⁹.

The present study was carried out in gills of fresh water fish *Tilapia mossambica* at regular time intervals of 7, 14, 21 and 28 days at 1.5 g/70L NaF and 3 g/70L NaF. This research work analyzes the effect of fluoride, as a most important biologically active and mobile toxicant on accumulation of toxic metals on gills. The results are reported in the tables 1-2 showed that the adverse effect of fluoride on gills structure and function.

The level of cadmium and cobalt were not detected in control fish at both dose. All other toxic metals like (Pb, Co and As) showed the increase in concentration after 7, 14, 21 and 28 days at 1.5 g/70L and 3 g/70L of fluoride (figure-4 and 5) which indicate that the increase in concentration of toxic metals adversely affect gills and other tissues like liver, muscle, kidney of fish, disturb metabolism, development and growth of fish²⁰⁻²² in alteration in the cell organelles structure and function. Increase Cd and Co concentration in gills tissue are highly toxic which brought gills epithelium dysfunction that can decrease oxygen uptake capacity and bring about condition called hypoxia in organs of vital importance²³.

Results showed that toxic metal (Hg, Cd, Pb and As) accumulation increases with an increase in the concentration of fluoride and time of exposure.

Table- 1
Effect of fluoride (1.5g/70LNaF.) on toxic metals (ppb) in gills of fresh water *Tilapia mossambica* at 7, 14, 21 and 28 day temperature 29°C, pH= 7.2

Toxic metals	Control	7day	14day	21day	28day
Pb	2.90±0.152	5.70±0.152**	12.70±0.152***	15.6±0.163***	19.46±0.167***
Hg	ND	1.51±0.150**	2.30±0.152***	4.30±0.152***	5.90±0.233***
Cd	ND	1.20±0.260**	1.70±0.160***	1.94±0.124***	2.25±0.130***
Co	14.10±0.223	16.60±0.221**	17.80±0.249***	18.50±0.137***	19.50±0.187***
As	7.26±0.123	8.96±0.163**	10.14±0.201***	10.98±0.134***	12.27±0.210***

N = no. of fish = 10; values expressed as Mean ± S.E.M; SD ± standard deviation; p = probability, *** represent highly significant (p<0.001), ** represent significant (p<0.01), * represent significant (p<0.05) compared with non-treated values, ND = not detected.

Table- 2
Effect of fluoride (3g/70LNaF.) on toxic metals (ppb) in gills of fresh water *Tilapia mossambica* at 7, 14, 21 and 28 day temperature 29°C, pH= 7.2

Toxic metals	Control	7day	14day	21day	28day
Pb	2.90±0.152	8.71±0.192**	10.70±0.156***	12.61±0.185***	15.36±0.217***
Hg	ND	2.41±0.156**	2.94±0.143***	4.90±0.184***	6.33±0.151***
Cd	ND	1.89±0.160**	2.12±0.167***	2.74±0.114***	2.95±0.120***
Co	14.12±0.223	17.60±0.201**	18.30±0.219***	19.52±0.157***	20.50±0.117***
As	7.26±0.123	7.96±0.123**	8.64±0.501***	11.23±0.193***	12.98±0.154***

N = no. of fish = 10; values expressed as Mean ± S.E.M; SD ± standard deviation; p = probability, *** represent highly significant (p<0.001), ** represent significant (p<0.01), * represent significant (p<0.05) compared with non-treated values, ND = not detected

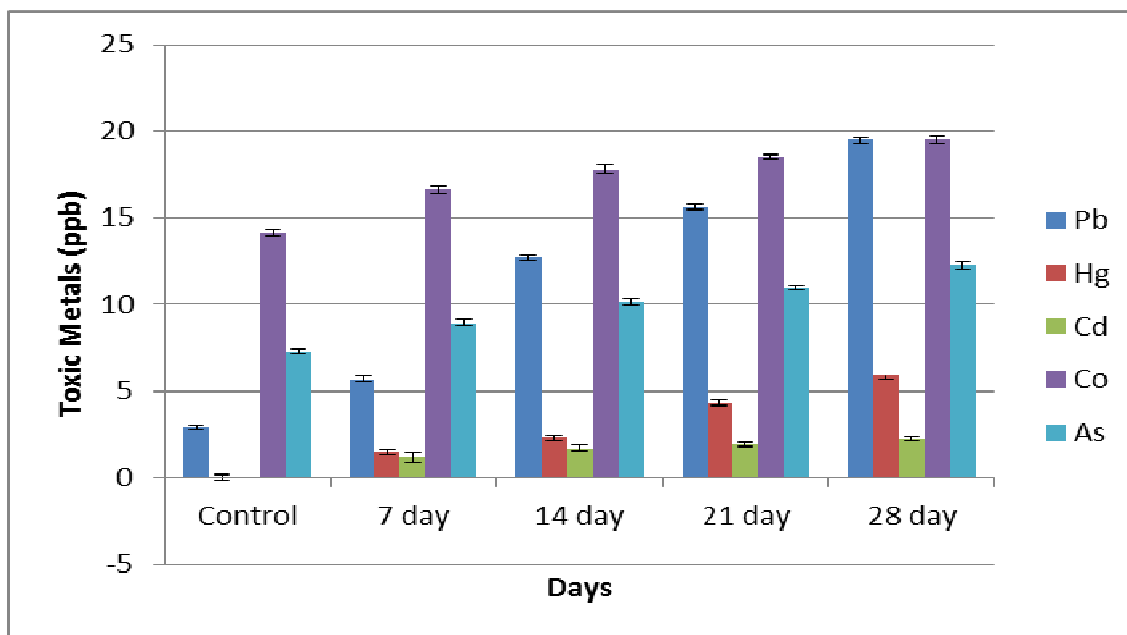


Figure-4
Effect of fluoride (1.5g/70L NaF.) on toxic metals (ppb) in gills of fresh water *Tilapia mossambica* at 7, 14, 21 and 28 day, temp = 29°C, pH= 7.2

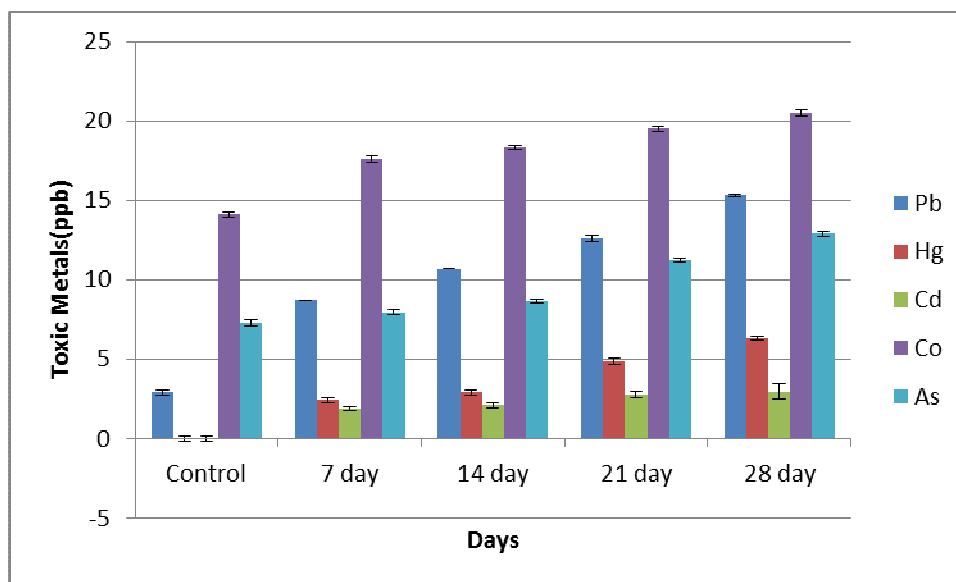


Figure-5

Effect of fluoride (3g/70L NaF.) on toxic metals (ppb) in gills of fresh water *Tilapia mossambica* at 7, 14, 21 and 28 day, temp = 29°C, pH= 7.2

The altered biochemical and physiological responses of fish to sub lethal concentration 1.5 g/70L NaF and 3 g/70L NaF is associated to environmental adaptation and species dependent. Several factors in the environment, including the physiological state of the fish, species, even race or grains, govern the general response of fish to heavy metals intoxication²³. Therefore it is challenging to assign specific toxic exposure levels to experimental fish.

Conclusion

Fluoride is known to have a profound toxic effect on a wide range of animals including freshwater aquatic life and it's readily permanently accumulates, in the long bones of vertebrates, causing fluorosis, when present in excessive amounts. The permeability of gills induces the accumulation of the heavy toxic metals which are highly toxic even in minor quantities and considered as dangerous to aquatic life especially fish. These results propose that fluoride support the accumulation of trace metals in gills tissue which is the vital part of absorption of oxygen for the fish.

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