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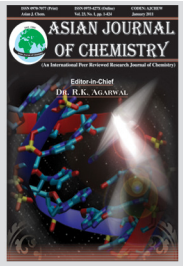

Accumulation of Metals in Gill Tissues of *Notopeterus notopeterus* Under Fluoride Stress

RAFIA AZMAT[1,*], FARHA AZIZ[2] and SYEDA SALUHA BIBI[1]

[1]Department of Chemistry, Jinnah University for Women, 5C Nazimabad, Karachi 74600, Pakistan
[2]Department of Biochemistry, Jinnah University for Women, 5C Nazimabad, Karachi 74600, Pakistan

*Corresponding author: E-mail: rafiasaeed200@yahoo.com

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Sub lethal physiological effects of fluoride on macro and micro metals residue accumulation in gills tissues were measured in *Notopeterus notopeterus* from fresh water reservoir. Fishes were bare to two concentrations (1.5 and 3 g/70 L) of fluoride for 24, 48 and 72 h and 45 days beside the control group in separate aquarium. High concentration of essential and non essential metals showed that fluoride induced permeability in gills tissues results in mineral nutrients unbalancing due to free movement of non essential metals (Pb, Hg, As and Cd) in gills of fish under studied. This may be related with permeability of gills epithelium which significantly affects the scale loss due to fluoride mobility with increase in metal accumulation in gill tissues of fish. It was observed that in gill tissues the bioaccumulation factors of Mn, Zn, Fe and Co were affected as compared to control fish, which ultimately associated with the physiological processes of fish with dissolved gills tissues structure.

Key Words: Fluoride, Essential and Non-essential metals, Gills, Permeability.

INTRODUCTION

Like lead and arsenic, fluoride is a cumulative poison and the most damaging environmental pollutant may be labeled as mutagenic which can induced or increase the frequency of mutation of an organism[1,2]. Fluoride ions are directly toxic to aquatic life and accumulate in the tissues, at concentrations where absorption rates exceed excretion rates[3,4]. Fluoride may be xenobiotics to the biological system at elevated level disturb the normal metabolic pathway. Xenobiotics are usually very lipophilic and need to undergo biotransformation to be converted into more water-soluble compounds that allow them to be excreted from the body with significant increases in fluoride concentrations were observed[5] in bone, cartilage, skin and gill of Siberian sturgeon exposed to ≥ 4 mg fluoride L^{-1}. Gills tissue of fish is directly exposed to toxicant present in the aquatic resources. Any contaminants of marine resources first directly affect the tissue of gills which is the main absorption sites of respiratory of fish for oxygen absorption. Fluoride is the most electronegative element of the periodic table, with very high electro negativity and reactivity[6]. The rate of absorption of oxygen depends on the availability or fluoride concentration. Higher the fluoride concentration lowers the absorption of oxygen which will be later on produced alteration in all biochemical processes occurring in fish.

Research on the micronutrients like Fe, Na, Mg and Zn by toxicants caused minerals misbalancing which may result in the alteration of physiological processes of organs. Micronutrients interact with toxic substances at several points in the body increase adaptation and excretion of ionic substances, transport non-essential substances in the body, binding to target proteins, metabolism and seguestation of toxic metals and finally in secondary mechanism of toxicity such as oxidative stress[7]. Some toxic substances increases the permeability of cell membrane, in such a way that it provide the help in accumulation of essential and nonessential metals in the organ directly exposed to toxicants and increase and decrease the micronutrients metals adversely affect the function of the organs. Fluoride is highly mobile inorganic pollutant and presence of fluoride support the accumulation of essential and non-essential metals in living organism. Discharges of fluoridated municipal waters also cause significant increase (about five times greater than natural background level) in the fluoride concentration of recipient rivers[8,9].

The aim of present research is to focus on availability of essential macronutrients and micronutrients in presence of fluoride in gill tissues of fresh water fish *Notpetrius notopeterus* in relation with the permeability of gills tissue.

EXPERIMENTAL

Kanjher lake is one of the most important fresh water reservoir in Sindh Pakistan, which feed by river Indus and water is used as a drinking water for Karachi city people. Now a

days it is a critical environment situation pollution by industrial discharge, non-treated urban waste and pesticides which had accumulatively negative effects. Fishes were collected with the help of fisherman and placed in glass aquarium containing tap water, size: 36 cm × 18 cm × 15 cm for 2 weeks before analysis. The aquarium water was changed on alternate days and a fresh dose of fluoride was supplemented after feeding. Both control and treated fishes were sacrified after 24, 48, 72 h and finally after 45 days.

Equal weights of all fishes were put into 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 ºC. After that the samples were mineralized at 400 ºC in the chemical oven for 24 h then 2 mL of nitric acid was added and sampled were dehydrated at 450 ºC. To each sample 10 mL of hypochlorous acid was added and then make up to 50 mL with double distilled water. The detection of macronutrients, micronutrients and heavy metals were carried out by atomic absorption spectrophotometry with graphite furnace and acetylene flame. The data was subjected to statistical analysis.

RESULTS AND DISCUSSION

A 45 days assessment of essential and non-essential metals in gills tissues of *N. nonparties* collected from the above lack was conducted to determine the accumulation trend of these metals in presence of fluoride ions. Fluoride is a strong, hard anion and cumulative toxic agent[9], occurs naturally widely distributed in the rivers, lake and seas of world[10]. The analysis of essential and non-essential metals was carried out after regular time interval of 24 h for 45 days. *N. notopeterus* was exposed to two different sub lethal concentration of sodium fluoride for 45 days. Trace metal accumulation patterns in aquatic biota; along with bio-concentration and bio-magnification processes in gills have been studied in relation with fluoride concentration. The research analyze the effect of fluoride, as a most important biologically active and mobile toxicant with a micronutrients approaches by using invasive fish (*N. notopeterus* as a sustainable specie,) on minerals contents of gills. As minerals metal elements play an important role in the growth, maintenance and recovery of tissues. Minerals are also needed for the contraction of muscles, the functioning of the nerves and in the energy household. Fluoride uptake occurs both in soft and bone tissues. Gills tissue were examined after 24, 48 and 72 h for essential macronutrients(Na, K, Ca and Mg), micronutrients (Mg, Cu, Zn and Fe) and non essential metals (Hg, Cd, Pb and As) as first site of interaction of any toxicants of aquatic resources. The results are summarized in the Tables 1-6. It was observed that the gills tissue after exposure of 24 h show much variation on day one. No mortality was observed during the trial of 45 days except the weight and movement loss but after 45 days all fishes were died with deformalities of neck and body. It was observed that significant effect of fluoride toxicity was recorded in the early days of exposure which was persistent in which the hyperexitability, darken black backs, weakness with decrease in respiration before their death were the visual symptoms during the study of 45 days in fluoride aquaria and gills were dissolved and rapture with no tissues in the fish gill which may be attributed with the total decline in protein and lipids contents of gills.

TABLE-1
EFFECT OF FLUORIDE (1.5 g/70 L) ON MACRO ELEMENTS (ppm) IN FRESH WATER *N. notopeterus* AT 24, 48 AND 72 h; TEMPERATURE: 30 ºC, pH = 7.8

Macro elements	Control	24 h	48 h	72 h
Na	44.0±0.210†	30.5±0.268‡	39.0±0.488‡	33.3±0.221‡
K	35.0±0.210†	22.0±0.210‡	21.3±0.488‡	19.2±0.674‡
Ca	45.0±0.210†	22.3±0.213‡	19.2±0.201‡	38.5±0.632‡
Mg	32.5±0.223†	10.4±0.213‡	10.6±0.221‡	45.3±0.500‡

N = 10, †SD = 0.666, †V = 0.444, ‡SD = 0.849, ‡V = 0.6749, P‡ < 0.0001

TABLE-2
EFFECT OF FLUORIDE (3.0 g/70 L) ON MACRO ELEMENTS (ppm) IN FRESH WATER *N. notopeterus* AT 24, 48 AND 72 h; TEMPERATURE: 30 ºC, pH = 7.8

Macro elements	Control	24 h	48 h	72 h
Na	44.0±0.210†	26.6±0.221‡	25.3±0.300‡	29.1±0.233‡
K	35.0±0.210†	16.6±0.221‡	16.6±0.221‡	17.7±0.213‡
Ca	45.0±0.210†	20.1±0.233‡	31.7±0.213‡	38.0±0.210‡
Mg	32.5±0.223†	9.2±0.200‡	17.6±0.221‡	40.6±0.221‡

N = 10, †SD = 0.666, †V = 0.444, ‡SD= 0.699, ‡V = 0.4889, P‡ < 0.0001

TABLE-3
EFFECT OF FLUORIDE (1.5 g/70 L) ON MICRO ELEMENTS (ppb) IN FRESH WATER *N. notopeterus* AT 24, 48 AND 72 h; TEMPERATURE: 30 ºC, pH = 7.8

Micro elements	Control	24 h	48 h	72 h
Zn	13.3±0.213†	08.7±0.260‡	07.8±0.249‡	07.7±0.213‡
Fe	31.4±0.163†	22.4±0.266‡	30.8±0.249‡	23.6±0.305‡
Mn	12.7±0.260†	12.7±0.260‡	14.5±0.166‡	18.0±0.210‡
Co	14.1±0.233†	16.6±0.221‡	20.8±0.249‡	19.5±0.307‡

N = 10, †SD = 0.666-0.737, †V = 0.677-0.544, ‡SD = 0.0.842, ‡V = 0.622, P‡ < 0.001

TABLE-4
EFFECT OF FLUORIDE (3.0 g/70 L) ON MICRO ELEMENTS (ppb) IN FRESH WATER *N. notopeterus* AT 24, 48 AND 72 h; TEMPERATURE: 30 ºC, pH = 7.8

Micro elements	Control	24 h	48 h	72 h
Zn	13.3±0.213437	9.2±0.200000	10.2±0.290593	14.6±0.2211080
Fe	31.4±0.163299	16.7±0.260342	27.4±0.266667	24.2±0.2494440
Mn	12.7±0.260342	13.6±0.221108	15.7±0.260342	16.9±0.2276887
Co	14.1±0.233333	16.7±0.260342	18.3±0.300000	20.7±0.2603420

N = 10, †SD = 0.455-0.737, †V = 0.677-0.544, ‡SD = 0.0.943-0.632, ‡V = 0.622, P‡ < 0.001

TABLE-5
EFFECT OF FLUORIDE (1.5 g/70 L) ON TRACE ELEMENTS (ppb) IN FRESH WATER *N. notopeterus* AT 24, 48 AND 72 h; TEMPERATURE: 30 ºC, pH = 7.8

Elements	Control	24 h	48 h	72 h
Pb	3.7±0.152†	5.7±0.152‡	19.6±0.163‡	15.7±0.152‡
Hg	–	1.31±0.150†	1.3±0.152‡	3.7±0.152‡
Cd	–	–	–	1.7±0.152‡
As	9.6±0.163†	10.6±0.163†	12.5±0.166‡	10.0±0.210‡

N = 10, †SD = 0.0-0.152; †V = 0.0-0.266, ‡SD = 0.0153-0.516, ‡V = 0.266-0.444, P‡ < 0.0001

TABLE-6
EFFECT OF FLUORIDE (3.0 g/70 L) ON TRACE ELEMENTS (ppb) IN FRESH WATER *N. notopeterus* AT 24, 48 AND 72 h; TEMPERATURE: 30 ºC, pH = 7.8

Elements	Control	24 h	48 h	72 h
Pb	3.7±0.152†	12.7±0.213‡	7.2±0.249‡	17.1±0.233‡
Hg	–	6.8±0.249‡	8.5±0.223‡	13.6±0.221‡
Cd	–	3.7±0.213‡	3.7±0.213‡	5.7±0.260‡
As	9.6±0.163†	11.7±0.213‡	12.7±0.213‡	20.0±0.210‡

N = 10, †SD = 0.0-0.516; †V = 0.0-0.266; ‡SD = 0.213-0.823, ‡V = 0.266-0.444, P‡ < 0.0001

Effect of fluoride on macro minerals of gills: In the present experiment the toxicological affects of sodium fluoride on accumulation of essential and non essential metals in gills tissues was observed as gills are directly exposed to any pollutant present in the water. Results are reported in the Tables 1-6 which showed the mineral imbalancing and accumulation of heavy toxic metals with the elevated concentration of essential micronutrients and reduced macronutrients content.

The sodium contents at two sublethal concentration of fluoride (1.5 g/70 L and 3.0 g/L) was found to be decreased with in 24 h of exposure and reduced in concentration. The decrease in concentration of Na depends upon the time of exposure and the concentration of fluoride as reported in the Table-1. The potassium content in gills was adversely effected by the fluoride when compared with the control (35 mg kg^{-1}) where the decrease in concentration at 24 h was 22 and was 17 at 72 h. Sodium and potassium are essential macronutrients which regulates together the water household in a cell (osmotic pressure). Potassium performs these tasks with in the cell and sodium outside the cell. It is involved in the optimal functioning of the nerves and muscles. Decrease in concentration of these essential nutrients disturb the functioning of gills tissues and these effects were observed visually as a slow movements of gills of treated fish as compared to control one. Fluoride inhibits oxygen consumption and blood clotting and diminishes erythrocyte glycolysis. It also induces efflux of potassium from red blood cells. The resulting hyperkalaemia and hypocalcaemia have been implicated as contributing factors in fluoride-induced. The depilation in level of Na and K may be the risk for the gill tissues Na cell which is the main respiratory organ and regulate the all body functions. Ca and Mg contents of fish under fluoridation showed different behaviour as compared to other macronutrients metals. It was observed that Ca and Mg contents first decreases in early days of exposure but at 72 h it increases at both the concentration of sodium fluoride which may be due to the adaptation for protection of tissues of gills as calcium is stored in the muscle cells in particular cell organelles (called, the sarcoplasmatic reticulum) because the bioavailability of fluoride is reduced by increasing level of calcium contents. Increase in calcium contents may affect fish response or tends to enhance the resistance of fish to fluoride or it may be due to the reaction between fluoride and calcium. Magnesium is anti-stress mineral, raises the resistance against stress, depressions and tensions. It also involved in releasing energy from the diet and is involved in a good functioning nervous system and good functioning muscles. Increase in contents of Mg may be related with resistance produced under fluoride stress or to decrease the permeability of gills[2,7]. A positive correlation ($p < 0.001$) in between macro elements and fluoride may lead to show the macro-minerals misbalancing which results in the distortion of gills cell respiration.

Effect of fluoride on micro minerals of gills: Effect of fluoride was studied on micronutrients of gills of *N. notopeterus*. Results were reported in the Tables 3 and 4 and showed that the mineral elements reflect the adverse effect of fluoride. All micronutrients (Zn, Fe, Mn and Co) showed the increase in concentration after 72 h at high dose of fluoride which indicate that the increase in concentration of micronutrients element with trace elements results in distortion in the cell organelles which may bring about elevation or initiation in activity of various enzymes and mitochondial damage leads to decreased respiration and or partially uncouple oxidative phosphorylation, which may be correlated to increase in Cd and Co concentration in gills tissue which induced damage gills epithelium, that can reduce oxygen uptake capacity and bring about hypoxia in organs of vital importance[11]. The above facts were also supported by lower oxygen consumption in the experimental fish. This may be related with Zn unbalancing as Zn plays a role in hundreds of biological processes in the body. It is among others involved in the synthesis of DNA, RNA and hormones (testosterone)[11]. The mineral is also involved in the vitamin A function and in the metabolism of carbohydrates, alcohol and fatty acids. Zinc works as co-factor of the enzyme superoxide dismutase (SOD) that protects the body against radicals. Zinc is also of interest for the immune system. The decrease in Zn contents may be related with the weak immune system of fish under fluoride stress whereas increase in Zn level at high dose at 72 h with high concentration of fluoride may be due to the damage gill structure. Iron has an important contribution to the oxygen transport and the formation of red blood cells[12]. Results showed that iron contents after exposure of two doses and with the passage of time was found to be decreased as iron is a part of the enzymes haemoglobin (in the blood) and myoglobulin (in the muscles) and also of other enzymes therefore any decreased in the concentration of iron in gills tissue will tend to decreased the absorption of oxygen in the gills. Iron is also an important in the energy production and it raises the immune system. Iron deficiency leads to decrease the immune system of fish which results in the decrease movement of fish. Cobalt contents was found to be increased with exposure time and at both concentration of fluoride, indicating that this trace mineral element may be helpful in the production of DNA in the tissues and red blood cells to increase the absorption of oxygen under fluoride stress or to send the signal in a nervous system or to maintain the blood pressure under toxicity of fluoride.

Manganese is involved in the synthesis of protein like substances, bones and cartilage. Slight increase in concentration at two sublethal concentrations in gill tissues related with fluoride toxicity which may be increase for protection from fluoride ion as manganese is also part of cartilage. An enzyme superoxide dismutase (SOD) contains manganese. This enzyme protects the body against free radicals. The correlation matrix for fluoride and the studied heavy metal contents like zinc,

iron, cobalt and manganese shows a positive significant relation between micro elements and fluoride concentration of treated and non treated fish ($p < 0.001$).

Effect of fluoride on trace metal accumulation of gill: Results reported in the Tables 5 and 6 showed that Hg and Cd were detected in the control fish whereas treatment with fluoride especially at high dose heavy toxic metals accumulation recorded with time and concentration. These results suggest that fluoride facilitate the trace metals concentration in gill tissue which is the vital part of absorption of oxygen for the body of fish. Although fluoride unique role in mineralization provide the bases for its recognition as a beneficial trace element but the permeability of gills structure also allow the accumulation of the heavy toxic metals which are non-degradable and regarded as hazardous to aquatic ecosystem because of their environmental persistence and their tendency for bioaccumulation[13,14]. Results showed that toxic metal (Hg, Cd, Pb and As) accumulation increases with an increase in the concentration of fluoride.

The response of fish to moderate fluoride concentration (1.5 to 3.0 ppm) is related to environmental acclimatization and is species dependent. It is difficult to assign specific values as toxic levels because so many factors in the environment, including the physiological state of the fish, species, even race or grains, governs the response of fish to intoxication[11,15,16]. The development of gills tissue disorder in *Notpetrius notopeterus* fish is linked to a poorly understood relation ship between nutrition, environment and fluoride concentration which results in neck deformities with gills tissue, unfortunately limited research efforts has been made to characterize the pathological changes associated with disorder link to gills deformalities[17], link to mineral nutrient disorder and accumulation in trace metals in fish.

Conclusion

It is concluded that fluoride ions are directly toxic to aquatic life and accumulate in the tissues, at concentrations where absorption rates exceed excretion rates whereas the concentration of fluoride directly effect the aquatic organism by accumulating many trace metals in vital part of fish. These results clearly demonstrate that permeability in gills tissue due to fluoride exposure result in entering various toxic metals in fish under study, which may cause detritus changes with disrupt tissue structure at respiratory level that ultimately decrease the absorption of oxygen in gills filament results in death of fish at long exposure.

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