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BIO-CHEMICAL ALTERATIONS IN CHANNA PUNCTATUS DUE TO FLUORIDE TOXICITY

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ABSTRACT

The high productive areas are encountering sustainability problems after enjoying the benefits of the 'Green revolution programme'. There are reports of declining factor in the productivity especially the 'Fish', hence declining graph in the 'Blue Crop' production. This is because of deteriorating biosphere and ecosystem caused by environmental pollution as a result of urbanization, rapid industrialization and deforestation. All these have become an alarming menace to human being besides being a threat to all other fauna and flora. The global population about 5.8 billion in 1997 has immensely increased to about 8 billion. Therefore, the pressure on land in the world is increasing due to rapid demand of food to increasing population at alarming rate to cope up with the food shortage. Much attention has been given towards aquaculture, especially to 'Fishes'. Since the commencement of 'blue revolution' programmes in the world has enhanced the production of fish on the line of agriculture. In our country milk and meat consumption is very low, fish has special importance as a supplement to ill balanced cereal diets. Now a days 'Protein' deficiency is the world's most serious malnutrition problem as about 30-35 per cent of the world population is suffering from protein deficiency diseases. Fishes are the only good and cheap source of protein and other useful oils. Since fishes occupy a very privileged position in relation to human civilization from time immemorial. Today, India stands sixth largest producer of the fish in the world and second in' the inland fisheries. But now a days physico-chemical characteristic of aquatic ecosystem has experienced a tremendous deterioration due to several anthropogenic activities, viz.over siltation, intensification of aquaculture as well as various industrial, agricultural and sewage discharge.

KEYWORDS: *blue revolution, drinking water, Fishes, inland fisheries*

INTRODUCTION

Fluoride is found mainly in earth crust as ores of fluorspar (CaF2), cryolite (Na3AlF6), fluorapatite [Ca10F2(PO4)6], topaz [Al2SiO4 (OH7F)2] sellaite (MgF2), and villiamite (NaF) which finds way into water bodies either by natural run off or several anthropogenic activities. Other sources of fluoride are steel and aluminium smelters, elemental phosphorus, phosphate fertilizer, wet process phosphoric acid, plants, brick and ceramics work and combustion of coal. Several industrial and agricultural activities are also involved in contaminating the water. Fluoride containing effluents released from the industries is generally discharged into rivers or the sea. Fluoride contaminated water related health hazards are being wide spread in human beings as well as in animals directly through drinking, bathing and indirectly through food chain. The highest natural fluoride concentration (2800 mg/L) ever found in water was recorded in lake Nakuru in the Rift Valley in Kenya, (Murray, 1986). Nearly 100 million people suffer from mottled teeth and 1.70 million people suffer from skeletal fluorosis (Vasavada, 1998). The concentration of fluoride in natural water depends on the solubility of fluoride bearing rocks and period of contact with water. Absorbed fluoride is distributed between two compartments. One with a short half-life of a few hours probably comprising blood and soft organs and one with a much longer half-life mostly about eight years. Corresponding to the skeletal studies have shown that about 50% of absorbed fluoride is retained for a long time in the skeleton while the other half of the fluoride absorbed is excreted in the early hours.

In aquatic habitat fishes are the most sensitive organisms to get affected even upon a mild change in their surroundings. There are several reports about the accumulation of fluoride in skin, gills, scales, muscles and bone tissue of fish (Gikunju, J.K., 1992) Wright, D.A. and Davison, A.W., 1975 and Christenson B. 1987). In several countries fish and marine products have been identified as major source of dietery fluoride (Minoguchi, G., 1970, Shertock, J.C., 1984 Siber, G. and Trautner, G., 1985).

Fluoride problem is much severe in aquatic ecosystem mainly in fresh water as in low concentration calcium ions in water which precipitate fluoride as calcium fluoride. Excessive ingestion of fluoride gets accumulated and damages various vital organs and systems viz.liver, kidney, gills and reproductive system (Kaur& Singh, 1980, Chinoy, et al., 1994 and Gupta, et al., 2001) which effects the population and growth of animals in terms of quantity as in case of fish production which is being gradually reduced. The problem is much severe at certain places where the people are getting exposed to fluoride from both the sources from drinking water and food chain through fish consumption. That is why it is necessary to protect the human population exposed through the fish contaminated with fluoride. This can only be done by protecting fish from toxic level of fluoride which has to be evaluated so fish population can be saved and improved in terms of both quality and quantity to some extent by utilizing the data obtained by different studies.

Earlier reports suggested that daily ingestion of over 28 mg of fluoride would be harmful. Subsequent studies cited 20 mg as the maximum safe limit, but in endemic areas where the presence of certain local factors and prolonged exposures tend to aggravate fluoride toxicity the safe level of fluoride intake may be even lower. Studies of the cases of endemic skeletal fluorosis in India revealed an average fluoride intake of 9.88 mg and it is held that a daily fluoride intake could be deemed safe.

To overcome all these problems related to fluoride toxicity many workers in India and abroad have carried out research on various experimental animalsto reduce the effect of fluoride on different organs & organ systems but there is lack of data on bio-chemical parameters of the fish. Thus if seems there is a paucity of data of fluoride toxicity on fresh water fish.

The above studies certainly add something new to our existing knowledge of the environmental toxicity of fluoride to the fish fauna as a whole. The parameters selected for toxicological evaluation of fluoride may be used for rapid screening test or early signals to Fluoride intoxication added to natural aquatic system.

METHODOLOGY

1. Test Animal:-

Channa (=Ophiocephalus) punctatus

Phylum	-	Chordata
Sub-Phylum	-	Vertebrata
Class	-	Pisces
Sub-Class	-	Teleostomi
Order	-	Ophiocephaliformes
Family	-	Ophiocephalidae
Genus	-	Channa (=Ophiocephalus)
Species	-	punctatus

Channapunctatus is found throughout plains of India and Pakistan. commonly known as “Snake-headed Fish” and locally called as “Girai”, one of the air breathing fish and prefer to live in stagnant water. The major food of the animal is aquatic insects, microorganisms, small fishes, molluscs and shrimps they are prolific breeders and the development is much rapid.

2. Collection and acclimatization of fish:-

Healthy living specimens of the fresh water teleost,Channapunctatus of weight (25+3 gms) and size (12+2 cms) were brought from Central Institute of Fisheries Education (CIFE), Chinhat Centre, Lucknow, Gomti Hatchery, Lucknow and local fresh water resources or purchased from the fish market at Quasar Bagh in Lucknow. Fishes were disinfected with 1% Potassium permanganate (KMnO4) solution and acclimatized in well aerated and DE chlorinated (Few drops of “Clear ‘O’ Liquid”, For DE chlorination, Bengal Chemicals Kolkata were added)

tap water in glass aquaria under standard laboratory conditions.

Other physico - Chemical characteristics viz. temperature, pH, dissolved oxygen, hardness, electrical conductivity and fluoride of the water used were determined by standard methods (APHA, 1998, 20th edition).

3. Toxicant used:-

Toxicant - Fluoride
Compound used -Sodium Fluoride (Excel-R grade)
Molecular Formula - NaF
Molecular weight -41.99
Manufacturer - QualigensFineChemicals,Mumbai.
Solubility -Easily solublein water to form Na+ ascation and F- as anion (Na+ is Non-toxic to animals).

A stock solution of concentration 10 mg / ml (as fluoride) was prepared by dissolving 11.0550 gm. of sodium fluoride (NaF) in 500 ml of distilled water in volumetric flask. This stock solution was further diluted according to the desired concentrations needed in the aquaria. This stock solution could be kept at room temperature for one month for safe bioassay.

4. Toxicity Test:-

Preliminary toxicity test was conducted under laboratory conditions to determine the LC50 values of fluoride for 96 hours. Stock solution of the toxicant fluoride prepared in distilled water by simply disolving sodium fluoride (APHA, 1998, 20th ed.)

Prior to experimentation the best fishes were examined carefully for pathological symptoms and fish were disinfected with 1% KMnO4 solution. Then these test animals were transferred from the acclimatization glass aquaria to experimental glass aquaria. From the stock solutions measured quantities of fluoride solution were added separately to each aquarium. Requisite quantity of water was added to each aquarium for further dilution ensuring the availability of at least one litre of the test solution from each gram biomass of fish.

After 24 hours dissolved oxygen and pH of the experimental solution were recorded. The fish were considered dead when they did notrespond on being prodded with glass rod and respiration ceased.

The mortality data was statistically analysed by Trimmed Spearman Karber Method (Hamilton el al., 1977). The LC50 value for 96 hours was 306 mg/L for fluoride. In order to observe the chronic effects of the fluoride fish were exposed to sub lethal concentration 30mg/L and 60mg/L of fluoride for 90days (Fig. 3). Water in the experimental aquaria was renewed every 48 hours and fresh solution of the fluoride was added to bring the concentration to the desired level. During the entire tenure of experiments, fish were regularly checked for infection, diseaseand other unhealthy conditions. At the expiry of each experimental period control and fluoride exposed fish were processed simultaneously.

5. Food and Feeding:-

The Fishes were conditioned to pelleted diet with Prawn powder, fish powder and minced goat liver in 2:2:1 at the rate of 3% of body weight. Feeding was discontinued during the acute exposure period. For chronic exposure period, the fishes were fed twice daily.

6. Blood Glucose Estimation:-

Blood from fish was taken by severing the caudal peduncle and gluocose in blood was estimated by Kit, Hemo, GlukoTesReflollux S Type, 1172115 (Germany).

Protein Estimation (Liver and Muscle)

The quantitative estimation of total protein in tissue ofChanapunctatus was made according to the method of Lowry et al., (1951) using BSA as standard. A one percent homogenate of liver and muscles was prepared in 0.15 M KCl solution. After centrifugation for 10 minutes the supernatant was discarded. The precipitate was dissolved in 1ml of 1.0 N NaOH. Equal volume (0.5 ml) of dissolved precipitates, distilled water and alkaline copper reagents were placed in test tubes. The mixture was kept for 10 minuites for complex formation. The 0.5 ml of Folin's reagent was added to each tube and was left about 30 minutes at room temperature. Blank and standards were also prepared by the same procedure and optical density was read at 660 nm with spectrophotometer.

7. Estimation of Metal ions:-

The level of calcium (Ca++) and Magnessium (Mg++) were estimated in blood plasma of Channapunctatus. Plasma was deproteinized with perchloric acid and after centrifugation the supernatant was used for the estimation of different metal ions after suitable dilution in 1% HCl on Perkin Elmen Model, 5000, Atomic absorption Spectrophotometer (SP-500).

BIO-CHEMICAL STUDIES

(i)Gluocose

There was significant decrease ($P < 0.01$ and $P<0.001$) in blood glucose in both the exposed group, 30 mg/L and 60 mg/L fluoride concentration is Channapunctatusafter 90 days as compared to control (Table-7).

(ii) Protein

There was no significant change in the protein content in the liver and muscle of the exposed fish at both the concentrations (30 mg/L and 60 mg/L) of fluoride after 90 days as compared to control

(iii) Ionic Composition

(a)Calcium (Ca++)

Calcium ion concentration in blood of exposed fish Channapunctatus has been found 15.12 mg/100ml blood at 60 mg/L fluoride concentration and the value is significant ($P < 0.001$) and at 30 mg/L fluoride concentration the value was found 23.10 mg/100 ml blood and the value is very near to control 25 mg/100ml blood (Table-8).

(b)Magnesium (Mg++)

Magnesium ion concentration was also found decreased in blood of exposed fish at 60 mg/L the value 7.53 mg/100ml blood and at 30 mg/L the value10.21 mg/100 ml. blood as compared to control (Table-8). The value obtained at 60 mg/L of fluoride concentration is significant (P < 0.001).Both Ca++ and Mg++ ion concentration decreased at higher concentration of fluoride in the fishChannapunctatus exposed for 90 days and the value obtained was significant (P< 0.001).

Table 1 : Results of bio-chemical Parameters in fish *Channapunctatus* exposed to fluoride.

Parameters	Concentration of Fluoride		
	Control	**30 mg/l**	**60 mg/l**
Glucose in blood (mg/dl)	56.00 ± 3.70	48.00 ± 4.50**	42.00±2.31***
Protein in liver (mg/100 mg)	25.00 ± 3.52	26.51 ± 1.50	25.73 ± 2.12
Protein in muscle (mg/100 mg)	32.5 ± 4.72	30.11 ± 1.83	28.17 ± 1.81

Values are mean ± SE, **P<0.01, ***P<0.001,

Table 2 :Ionic composition in blood of fish *Channapunctatus*exposed to fluoride

Ions	Concentration of Fluoride		
	Control	**30 mg/l**	**60 mg/l**
Ca^{++} (mg/100 ml blood)	25.00 ± 3.51	23.10 ± 2.67	15.12± 2.89 ***
Mg^{++} (mg/100ml blood)	11.00 ± 1.37	10.21 ± 2.38	7.53 ± 1.53 **

Values are mean ± SE, **P<0.01, ***P<0.001

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