

Impact of Triazophos on Ionic Regulation in the Blood of Freshwater Fish, *Channa punctatus* (Bloch)

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Abstract: The blood of fresh water, edible fish, *Channa punctatus* (=murrel) was exposed to sub lethal concentration of triazophos for 24, 48, 72 and 96 h to evaluate alterations in the levels of minerals i.e., sodium, potassium, chloride, calcium, inorganic phosphate, magnesium and iron. The increased chloride mineral along with sodium and potassium play an important role in neuromuscular excitability, acid-base balance and increased osmotic pressure of the fish. The decreased calcium is of great importance in blood coagulation, muscle contraction and nerve transmission while decreased iron is an essential constituent of heame in hemoglobin of the fish. The increased magnesium participates in principal metabolic activities, in formation of bones and teeth. Inorganic phosphate acts as a major buffer and is the basis of energy exchange. Triazophos altered the activities of the macro minerals in *C. punctatus* during stress periods.

Key words: *Channa punctatus*, calcium, chloride, iron, magnesium, inorganic phosphate, potassium, sodium, triazophos

INTRODUCTION

Wide spread application of various pesticides has aggravated the problem of pollution in aquatic environment. In a residual form or as a whole, they enter into the aquatic ecosystem and cause serious threat to aquatic organisms, especially for the fishes (Mukhopadhyay and Dehadrai, 1980a; Sastry and Siddiqui, 1984). Aquatic ecosystems that run through agricultural areas have high probability of being contaminated by run off and ground water leaching by a variety of Chemicals, and on entering the aquatic environment brings multiple changes in organism by altering the growth rate, nutritional value, behavioral pattern, etc. A major part of the world's food is being supplied from fish source, so it is essential to secure the health of fishes (Tripathi *et al.*, 2002). The most important mineral salts are calcium, sodium, potassium, phosphorus, iron, magnesium and chlorine. The deficiency of these macro mineral elements induced a lot of malfunctioning, reduces productivity, irritability of blood to clot, osteophoresis, anaemia (Shulman, 1974; Mills, 1980). In freshwater fishes, blood and electrolyte concentration are regulated by interacting processes, such as absorption of electrolytes from surrounding medium through active mechanisms predominantly at the gill control of water permeability and selective reabsorption of electrolytes

from urine. Any alteration in one or more of these processes results in a change in the plasma electrolyte composition. These ions play a vital role in several body functions, viz. the monovalent ions sodium, potassium and chloride are involved in neuromuscular excitability, acid base balance and osmotic pressure (Verma *et al.*, 1981), whereas divalent cations calcium and magnesium facilitate neuromuscular excitability, enzymatic reactions and retention of membrane permeability. Further, inorganic phosphate acts as a major cytoplasmic buffer and is the basis of energy exchange (Aurbach *et al.*, 1985). In the present study an attempt has been made to study the alterations in the blood minerals of fresh water fish *C. punctatus*.

MATERIALS AND METHODS

Healthy fresh water edible fish *Channa punctatus* (commonly called murrel), with an average weight 80-120 g and 15-25 cm in length, were collected from lake of Nirmal, Andhra Pradesh. It is carnivore's fish and voracious eater. The fish were stored in cement tank (6×3×3 feet) containing 60L-dechlorinated tap water for 3 week for acclimatization under continuous water flow. The average temperature of water was 22±1.0°C. They were fed *ad libitum* with groundnut cake along with commercial feed pellets (1-1.5% body weight). Prior to

the experiment the fish were starved for one day (Butterworth, 1972). Acclimatized fish were treated with 90% pure technical grade triazophos (OP) and 10% other ingredients. The IUPAC name of triazophos pesticide is O, O - diethyl, 0.1 phenyl, 1H, 1, 2, 4, Triazol 3yl - phosphorothio. It is commonly known as triazophos. Triazophos was introduced into the water tubs by dissolving it in acetone (0.5% w/v). The LC₅₀ (0.019 ppm) for 48 h was determined by the method of (Bayne *et al.*, 1977). Batches of six fish were exposed to sub acute to acute periods in sublethal concentration 0.006 ppm of triazophos in tap water for 24, 48, 72 and 96 h. After removing the fish at stipulated time interval blood was quickly isolated and kept in ice-jacketed petri-dishes for biochemical estimations. The physico-chemical parameters of tap water in which fish acclimatized are as follows. Temperature 30-35°C, hydrogen ion concentration PH 7.2, electrical conductivity 0.052 milliohms, calcium 5 mg/L sodium 2.1 mg/L, bicarbonates 142 mg/L, total alkalinity 69 mg/L, sulphates 7.1 mg/L; biological oxygen demand 1.6 mg/L; chemical oxygen demand 0.008 mg/L; fluoride 0.03 ppm.

Calcium levels were estimated by the method of Clark and Collip (1925). Measure 2 mL of serum 2 mL of water and 1 mL of 4% ammonia oxalate in a centrifuge tube. Mix thoroughly and allow standing for at least 30 min. Mix again and centrifuge at 1500 rpm for 15 min. Pour off the supernatant fluid and drain the tube by keeping in inverted on a filter paper for a few minutes. To this add 3 mL of 2% ammonium solution shake, centrifuge again pour out the supernatant, drain the test tube and add 2 mL of 1N sulfuric acid and shake vigorously. Keep the test tube on boiling water bath, shaking intermittently until the precipitate.

Sodium and Potassium levels were estimated by the method of Jacobs and Haffman (1931). Prepare 1 in 100

dilution of the sample by diluting 0.1 mL of serum in 10 mL of deionized water. Take 10 mL of deionized water removed 0.1 mL from an added 0.1 mL of sample. Blank the instrument with deionized water, adjust the instrument with sodium and potassium standards and then aspirate the test sample. Values are expressed in micrograms/100mL.

Chloride levels were estimated by the method of Schales and Schales, (1941). Add 0.2 mL serum or plasma to 1.8 mL water followed by micro burette calibrated to 0.01 mL. Repeat the titration on 2 mL of the standard chloride solution the expected titration volume which gives an intense violet-blue colour on adding the first drop of excess mercuric nitrate. On adding more titrant this becomes pale yellow or colourless until a sharp change to pale violet denotes the end point. Values are expressed in µmol/L.

Inorganic phosphorus levels were estimated by the method of Fiske and Subbarow (1965). Measure 9 mL of 10% trichloroacetic acid in test tube. Add 1ml of serum drop by drop with constant shaking. Transfer 5 mL of filtrate to tube labeled unknown. Pipette 5 mL of the working standard phosphorus solution into a tube labeled Standard and 5 mL of 10% TCA into a tube labeled blank. Values are expressed in mg/100 mL.

Iron levels were estimated by the method of Peters *et al.* (1956). Measure 2 mL serum into a test tube, add 3 mL water, one drop concentrated hydrochloric acid and one drop thioglycollic acid. Mix and stand for about 30 min. Put up a blank and standard at the same time with 2 mL water and 2 mL working standard respectively instead of serum. To each them add 1 mL trichloroacetic acid, mix, stand for about 10 min, then centrifuge. To 3 mL of the supernatant add 0.4 mL sodium acetate and 2 mL bathophenanthroline. Mix and read after at least 5 min, using a green filter or transmission at 540 nm. The colour remains unaltered for several hours. Values are expressed in µg/100 mL.

Table 1: Alterations in the concentration of mineral elements in the blood of *Channa punctatus* exposed to sublethal concentrations of triazophos

Parameters	Exposed Triazophos				
	Control	24 h	48 h	72 h	96 h
Sodium	11.51±0.13	12.01±0.04	12.46±0.14*	13.46±0.02	13.96±0.27
micromoles/litre of blood		PC = 4.34	PC = 8.25	PC = 16.94	PC = 21.28
Potassium	5.36±0.11	6.14±0.05*	6.97±0.12	5.65±0.09	6.25±0.09
microgram/100ml of blood		PC = 14.5. 2	PC = 30.03	PC = 66.04	PC = 127.61
Chloride	65±3.83	82.16±1.29	101.71±2.71	5.01±0.12	1.37±0.21
Eq/litre of blood		PC = 26.4	PC = 56.47	PC = -27.39	PC = 44.21
Calcium	6.90±0.05	5.8±0.13*	0.547±0.042	1.62±0.30	3.700.20
mg/100 ml of blood		PC = -15.94	PC = 69.34	PC = 70.52	PC = -46.37
Inorganic Phosphate	0.95±0.34	1.15±0.13	163.66±5.96	0.59±0.10	1.95±0.10
mg/100ml of blood		PC = 21.05	PC = -21.31	PC = 84.37	PC = 105.26
Magnesium	0.323±0.059	0.42±0.10	119.21±3.08	147.50±2.50	0.6290.026
(Micromoles liter of blood)		PC = 31.25	PC = 83.40	PC = -29.08	PC = 94.73
Iron	208±7.23	186.33±4.49*	4.06±0.10	138.21±2.08	131.001.50
(Eq/liter of blood)		PC = -10.41	PC = -41.15	PC = 112.63	PC = -37.01

Each value is mean SD of 6(Six) observations. All values are statistically significant from control at 5% level (p<0.05); PC: percent change over control. *: Not significant; Values in parenthesis show exposure period in days, C = Control, T = Treated

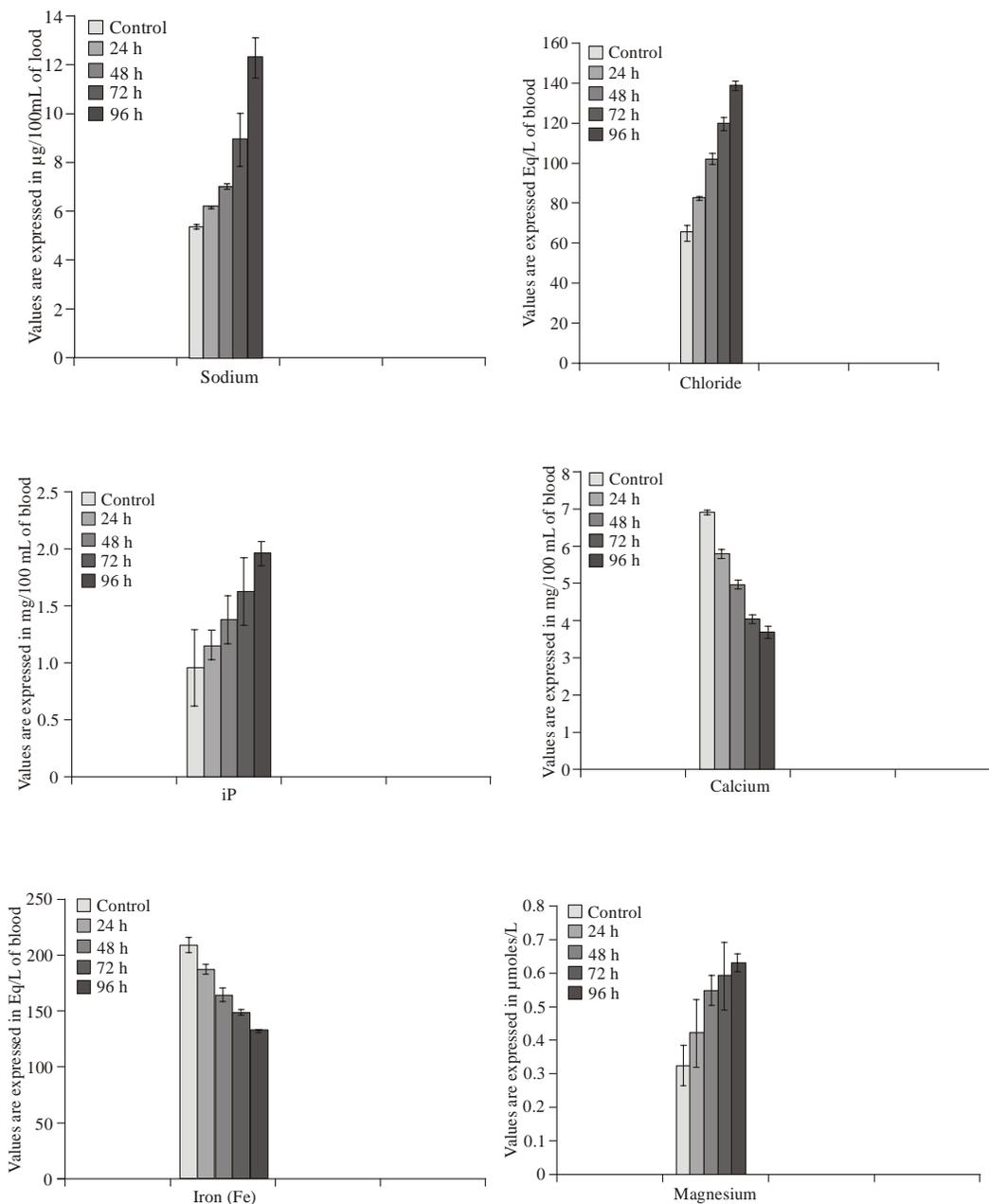


Fig. 1: Changes in the concentration of mineral elements in the blood of *Channa punctatus* exposed to sublethal concentrations of triazophos during 24, 48, 72 and 96 h exposure periods

Magnesium levels were estimated by the method of Cohen and Daza (1980). Label the rest tubes i.e., blank, standard and unknown to all the tubes add 5 mL of working reagent to standard tube add only 0.05 mL of standard solution to the unknown tube and add 0.05 mL serum. Mix well and wait for 20 min take the absorbance of standard and test against blank at 532 nm. Values are expressed in µmole/L of blood.

RESULTS AND DISCUSSION

Mineral elements have a great diversity of uses within the animal body these are recognized as essential for body functions in the fish. The quantitative changes of blood parameters like, calcium, sodium, potassium, chloride, inorganic phosphate and magnesium and iron the changes of the blood cells in the fish *C. punctatus* both in

control and sublethal concentration of triazophos exposed after 24, 48, 72 and 96 h are given in (Table 1, Fig. 1).

The levels of chloride significantly increased during prolonged toxic exposure periods of triazophos. The percentage change in chloride content in triazophos exposed fish is altered during exposure periods. Chloride ions along with sodium and potassium play an important role in neuromuscular excitability, acid base balance and osmotic pressure of the body. A biphasic response in blood chloride concentration has been reported in fishes subject to various concentrations of insecticides. Eisler and Edmunds (1966) observed hyper and hypochloremia in the northern puffer exposed to different doses of endrin. In the same species, (Misra and Srivastava, 1983, 1984) recorded both hyperchloremia and hypochloremia at different time intervals of acute exposure of the fish to triazophos respectively. Acute stress of insecticides causes diuresis in fishes and a severe loss of water and electrolytes due to increased GFR and deficient tubular reabsorption (Hickman and Trump, 1969). The decreased levels of calcium and iron observed in control triazophos exposed fish during 24, 48, 72 and 96 h exposure periods. Calcium is of great importance in blood coagulation and as regulator of permeability of cell membrane to water and inorganic ions. It also contributes to the maintenance of the membrane potential as well as the development of action potential in muscles and nerves. The organophosphates and carbamates are AchE inhibitors due to which skeletal muscle paralysis and death from asphyxiation occurs in the animals exposed to these insecticides. Increased serum calcium levels have been reported in *L. rohita*, *H (=s) fossilis* and *M. vittatus*, exposed to insecticides and their combinations (Bansal *et al.*, 1979; Verma *et al.*, 1979, 1981) and mineral composition in selected fresh water fish in Nigeria (Fawole *et al.*, 2007). However, (Abdul Naveed and Venkateshwarlu, 2005) reported raised level of serum calcium along with its decreased concentration in liver, muscle, brain and kidney of *Channa punctatus* exposed to sub lethal concentration of triazophos. Bansal *et al.* (1979) suggested that an increase in serum calcium may be due to release of calcium ions into the blood from vital organs due to toxic effect of the insecticides.

The levels of sodium gradually increased during prolonged toxic exposure time periods of triazophos. Sodium is the chief regulator of osmotic pressure of the body fluid. It initiates and maintains the contraction of heart and involuntary muscles and excites the nerves. Eisler and Edmunds (1966) observed an increase concentration of sodium in blood and decreased levels in the liver of the marine fish, northern puffers *Sphaeroides maculatus*, on exposure to lethal concentration of endrin. Grant and Mehrle (1970) also reported that a high dose of endrin and chloride ion loss due to complete failure of

osmoregulatory processes. Shastry and Dasgupta, (1991) recorded a significant decline in sodium ion concentration in liver and muscle of *Channa punctatus* exposed to 1 ppm of nuvacron for 60 days.

The percentage increased in potassium levels when triazophos exposed to fish potassium is the main intracellular cation involved in several physiological functions viz., nerve and muscle function, acid base balance and osmotic pressure. Elevated levels of sodium and potassium have been reported in the blood of *Heteropneustes (=Saccobranchus) fossilis*, *Mystus vittatus* and *Clarias batrachus*, exposed to various insecticides and their combination (Verma *et al.*, 1979; Dalela *et al.*, 1981; Bano, 1982). However, Larsson *et al.* (1981) reported a pronounced decrease in potassium level during long term exposure of flounders to acute cadmium stress. They observed that disturbed potassium regulation might be due to an impaired active reabsorption of potassium in renal tubules (Gill *et al.*, 1989). The levels of inorganic phosphate increased during prolonged toxic exposure periods of triazophos. The inorganic phosphate acts as a major buffer and is the basis of energy exchange and component of protein (Taylor *et al.*, 2002) Both hypo- and hyperphosphatemia have been recorded in teleosts exposed to various insecticides (Colvin and Phillips, 1968; Srivastava *et al.*, 1997a, b). Singh and Srivastava (1998) suggested that insecticides being lipophilic in nature can gain an easy access to the mitochondrial membrane and inhibit the enzymes involved in electron transport chain. Mineral elements magnesium is important in the metabolism of fats, carbohydrates and proteins and iron is essential constituents of heame in hemoglobin, Cytochromes, Peroxidases etc., (National Research Council, 1977).

CONCLUSION

The increased sodium and potassium ions function as extra cellular and intracellular fluids in regulating acid based balance, osmotic pressure and nerve and muscle function. Chlorine regulates acid-base balance. The decreased calcium plays major role in muscle contraction in transmission of nerve impulses. Inorganic phosphates act as a major buffer is the basis for energy exchange and component of proteins. The increased magnesium involved in metabolism of fats, carbohydrates and proteins; whereas iron is essential constituents of heame in hemoglobin.

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