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Cypermethrin induced changes in inorganic composition in the freshwater fish, *Cirrhinus mrigala* (Hamilton)

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Abstract:

The variation in Na^+, K^+ and Ca^{2+} ions levels in gills, muscle and liver of freshwater fish *Cirrhinus mrigala* exposed to lethal and sub lethal concentrations of cypermethrin were studied. A decreased trend was observed in all the tissues. But in sub lethal concentration at 21 day there is an improvement tendency was observed in present study.

Key Words: Cypermethrin, *Cirrhinus mrigala*, Sodium, Calcium and Potassium

Introduction:

Pollution is now the important limiting factor for man. The growing population together with rapid industrialization has made the problem more serious. As the demands of the growing population increase, the waste thrown by the population grows up and the day will soon arrive when one person's trash basket become another's living space. Although pesticides produce good result in the control of pest, their harmful effect on the non-target animals are rarely investigated. Pesticides leave residues in the soil and water for several days their application. This process a constant threat to the non-target organisms especially to fishes.

In recent years, synthetic prethroid insecticides have been developed for major uses in agriculture and public health. The current commercial produces were evolved from the natural prethrins, which possess high insecticidal potency low mammalian toxicity and very short persistence. It is highly toxicity to fish and some aquatic invertebrates (Coats, 1979)[1]. The inorganic ions play an important role in osmotic phenomenon and in the requisition of cellular metabolism. These are required by all animals to provide suitable medium for protoplasmic activity. Any imbalance in the levels of these ions in animals will lead to impairment in various physiological activities [2-4].

Freshwater fishes are hyperosmotic to their medium. They gain water osmotically and tend to lose solutes by diffusion. In the regulation of osmolarity of system sodium, potassium, and calcium ions play a significant role to keep the hyperosmotic properties of these animals[5]. The inorganic ions play an important role in osmotic phenomena and in the regulation of cellular metabolism. These

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are required by all animals to provide suitable medium for protoplasmic activity. Any imbalance in the levels of these ions in animals will lead to impairment in various physiological activities [6]. Freshwater fishes are hyperosmotic to their medium. They gain water osmotically and tend to lose solutes by diffusion. In the regulation of osmolarity of system sodium, potassium, and calcium ions play a significant role to keep the hyperosmotic properties of these animals[7]. Considering this fact present study an attempt has been made to evaluate the inorganic ion (Na^+, K^+ and Ca^{2+}) activity in organs like gills, liver and muscle of freshwater fish, *Cirrhinus mrigala* exposed to cypermethrin.

Materials and Methods:

Procurement and maintenance of fish: Freshwater fish, *Cirrhinus mrigala* (length 15±1 cm; weight 10±1 g) were obtained from Karnataka State Fisheries Department Fish Farms, Dharwad, India and reared in large cement tank. During acclimatisation, the fish were fed with rice bran and oil cake in the ratio of 2:1 on alternate days. Water of the tank was changed daily to avoid any fungal and bacterial contamination.

Physico-chemical characterization of water: In order to know the quality of water used for fish rearing and bioassay experiments, physico-chemical characterisation was carried out according to the methods prescribed in Standard Methods (APHA-AWWA-WEF, 1998) [8]. The water quality parameters studied were as follows: pH 7.5±0.2, temperature 28±1°C, salinity 85 mg/L, dissolved oxygen 6.5 -7.0 mg/L, chlorides 46.3 mg/L, sodium 1.22 mg/L, potassium 30.5 mg/L, calcium 17 mg/L, magnesium 11 mg/L, carbon dioxide 9 mg/L, hardness 115 mg/L, oxygen per cent<R> saturation 57 mg/L (as $CaCO_3$) and specific gravity 1.00374.

Toxicant Selected and Preparation of stock solution: Technical grade of cypermethrin (95%) was obtained from United Phosphorus Ltd. Bombay. The stock solution was prepared by dissolving 10 mg of cypermethrin in 10 ml of analytical grade acetone. For further experiment, the pesticide was drawn from this stock solution.

Fixation of exposure periods: In order to understand the influence of time over toxicity the effect of lethal and sublethal concentration of cypermethrin on *Cirrhinus mrigala* was studied at different periods of exposure. Before experimentation, the healthy fish collected from the tank were acclimatized to laboratory conditions in glass troughs for fifteen days. Each trough contained 15 L of water with approximately uniform sized fish. They were fed with commercial fish food pallets during acclimatization. The fish were divided into two groups. One group without pesticide served as control and the other group was exposed to lethal and sublethal concentration of cypermethrin for 1, 2, 3 & 4 for short-term and 1,7,14 & 21 days for long term were chosen to observe the effects. The LC_{50} value for 96 h was determining by Porbit analysis method (Finney, 1971) [8] and was found to be 5 µg/L. For sublethal concentration the 1/5th of the LC_{50} value (1 µg/L) was selected for experiment.

Estimation of inorganic ions: The control and experimental fishes were scarified on each day of exposure. Tissues of gills, muscle and liver tissue were isolated and immediately transferred to deep freezer prior to analysis of ions (Sodium, Potassium and Calcium). The weighed organs were wet ashed in 50:50 (V/V) concentrated perchloric acid and nitric acid (Dall, 1967) [10]. After keeping the wet ash solutions for half an hour, until the organs were completely dissolved, they were evaporated at 100^{0}C to 200^{0}C temperature. The residues were dissolved in glass with distilled water and made up to 10 ml. It was filtered through whatman No.1 filter paper. Further, appropriate dilutions were made prior to estimations and the sodium, potassium and calcium ions were estimated with the help of Flamephotometer (Elico Pvt. Ltd., Model CL –22D). Standard solutions of sodium, potassium and calcium were prepared by using analar grade chemicals. The experiment was repeated for several times to get concurrent values. The values are expressed as mM/g wet wt of the organ. The data were subjected to analysis of variance and the means compared by Duncan's new multiple range tests at 0.05% level[11].

Result and Discussion:

Changes in Na^+, K^+ and Ca^{2+} levels in gills, liver and muscle tissues of fish, *Cirrhinus mrigala* exposed to lethal and sublethal concentration of cypermethrin present in table 1-3. There is a significant decrease in ionic composition when exposed to lethal concentration. But in sublethal concentration at 21 day there is an (enhancement) improvement of ions concentration were observed.

The osmotic and ionic characteristics of the body fluids and tissues of freshwater organisms are largely influenced by the ambient medium. The maintenance of homeostasis in such conditions is very much dependent on the osmoregulatory properties. It is a vital phenomenon to maintain the physiological balance between the external environment and internal milieu of an animal. Alteration in osmotic regulatory mechanism under toxic conditions may cause severe imbalance in biochemical composition of the tissue fluids followed by undesirable metabolic consequences. The principle components of osmoregulation are ions. The ions may be anionic or cationic in nature based on charge and these help in maintenance of perfect osmoregularity of the cell.

In fishes, gills form a major site for the ion transport and osmotic water movements, hence also of pesticide entry. They are the first organs to be exposed to pesticides as they are in constant touch with the polluted water. This affects the permeability characteristics and osmoregulatory function of the gills thereby resulting in the decrease of these ions in gill tissue upon exposure to cypermethrin. In the present study, the decrease in the levels of Na^+, K^+, Ca^{2+} ions in the gill, muscle and liver exposed to lethal and sub lethal concentrations of cypermethrin indicates changes in the permeable properties of the cell membrane of these organs and of deranged Na^+, K^+ and Ca^{2+} ionic pumps due to the probable consequences of tissue damage (Table 1-3).

Sodium (Na^+) is the principle cation of extra cellular fluids of most animals. It maintains electroneutrality and internal sodium ion concentrations (David, 1995) [12]. It also plays an important role in osmotic regulation of body fluids and also serves as an essential activating ion for specific enzyme system. The increased sodium ion content may cause a shift in ionic symmetry with a consequent change in membrane permeability and functional efficiency of Na^+, K^+ pumps. The results in the present study suggest that the sodium content decreased as a function of time of exposure to cypermethrin (Table 1). Sodium is the major component of the cations of the extracellular fluid. It is largely associated with chloride and bicarbonate in maintenance of acid base balance. It maintains the osmotic pressure of body fluid and thus protects the body against excessive fluid loss. It is known that sodium content in tissues mainly depends on the permeability functional efficiency of bio-membrane and efficient functional role of Na^+ pump, which regulates ionic content of tissues. The level of Na^+ signifies its importance in the mobilization of water transport, since sodium content in the membrane facilitates the water movement among the tissues [13, 14]. From the result, it is evident that the Na^+

loss is higher in the case of gill indicating the derangement in Na^+ transport. Also, the decreased sodium content in the tissues of exposed fish indicates changes in permeable properties of different bio-membrane systems to different extent by altering the Na^+ pump and rupture in the respiratory epithelium of gill tissue [15, 16].

Table: 1. SODIUM ion content (mM / g wet wt.) in the organs of fish, *Cirrhinus mrigala* on exposure to the lethal and sub lethal concentrations of cypermethrin.

Organ	Control	Exposure period in days							
		Lethal				Sub lethal			
		1	2	3	4	1	7	14	21
Gill	55.5498 B	46.1201 E	42.4342 G	34.9708 H	24.4891 I	49.2195 C	44.2552 F	47.3304 D	59.6982 A
SD ±	0.7848	0.5727	0.4907	0.9249	0.6952	0.5074	0.1459	0.0844	0.9318
% Change		- 16.9751	- 23.6104	- 37.0460	- 55.9150	- 11.3957	- 20.3323	- 14.7964	7.4679
Muscle	45.5892 B	39.8970 E	34.1373 F	26.8574 H	23.5097 I	32.7950 G	41.9143 D	44.7692 C	46.8526 A
SD ±	0.3138	0.1809	0.4890	0.2478	0.9848	0.1258	0.1197	0.0727	0.5411
% Change		- 12.4858	- 25.1197	- 41.0882	- 48.4316	- 28.0642	- 8.0611	- 1.7989	2.7712
Liver	53.8994 B	47.8566 D	44.3970 F	38.3090 H	31.9643 I	45.3198 E	41.7086 G	50.6403 C	57.4256 A
SD ±	0.1363	0.5378	0.9151	0.4509	0.6602	0.5638	0.6240	0.4206	1.4591
% Change		- 11.2112	- 17.6300	- 28.9250	- 40.6964	- 14.0625	- 22.6177	- 6.0468	6.5421

Means are ± SD (n=6) for a tissue in a column followed by the same letter are not significantly different (*P* £ 0.05) from each other according to Duncun's multiple range (DMR) test.

Table : 2. POTASSIUM ion content (mM / g wet wt.) in the organs of fish, *Cirrhinus mrigala* on exposure to the lethal and sub lethal concentrations of cypermethrin.

Organ	Control	Exposure period in days							
		Lethal				Sub lethal			
		1	2	3	4	1	7	14	21
Gill	60.9314 B	48.3094 E	46.1886 F	35.0102 H	29.0773 I	51.6427 D	43.6102 G	56.6189 C	63.3718 A
SD ±	0.4225	0.0075	0.4932	0.3405	0.5149	0.6246	0.1689	0.1549	1.5152
% Change		- 20.7151	- 24.1958	- 42.5416	- 52.2787	- 15.2446	- 28.4274	- 7.0777	4.0051
Muscle	64.6382 B	58.1295 D	48.6314 G	39.5956 H	32.1265 I	57.1285 E	51.6951 F	59.1349 C	66.5012 A
SD ±	0.2378	0.0083	0.7524	0.1942	0.5540	0.5660	0.1309	0.5926	1.4806
% Change		- 10.0694	- 24.7638	- 38.7427	- 50.2980	- 11.6182	- 20.0239	- 8.5141	2.8821
Liver	53.8395 B	46.2010 F	41.8753 G	38.0659 H	27.8994 I	49.4513 D	47.3287 E	51.0828 C	54.7226 A
SD ±	0.1013	0.5727	0.1363	0.5712	0.2980	0.4593	0.1047	0.5310	0.5386
% Change		- 14.1874	- 22.2219	- 29.2974	- 48.1805	- 8.1504	- 12.0930	- 5.1202	1.6403

Means are ± SD (n=6) for a tissue in a column followed by the same letter are not significantly different (*P* £ 0.05) from each other according to Duncun's multiple range (DMR) test.

Table: 3. CALCIUM ion content (mM / g wet wt.) in the organs of fish, *Cirrhinus mrigala* on exposure to the lethal and sub lethal concentrations of cypermethrin.

Organ	Control	Exposure period in days							
		Lethal				Sub lethal			
		1	2	3	4	1	7	14	21
Gill	82.2804 ^{B}	63.4942 ^{E}	51.1618 ^{G}	41.3094 ^{H}	33.2073 ^{I}	73.5871 ^{D}	53.4982 ^{F}	76.5644 ^{C}	86.4529 ^{A}
SD ±	0.5742	0.5723	0.6104	0.4456	0.4188	0.3784	0.4182	0.1867	0.6106
% Change		- 24.6632	- 38.1094	- 50.9858	- 60.5991	- 12.6878	- 36.5236	- 9.1551	2.5777
Muscle	64.2627 ^{B}	52.9672 ^{F}	42.2259 ^{G}	36.6721 ^{H}	24.9744 ^{I}	54.6691 ^{E}	58.6077 ^{D}	61.4310 ^{C}	62.5204 ^{A}
SD ±	0.5458	0.3687	0.4013	1.0593	0.4029	0.9453	0.2427	0.5071	0.6553
% Change		- 17.5770	- 34.2917	- 42.9340	- 61.1371	- 14.9286	- 8.7998	- 4.4064	1.9388
Liver	70.5805 ^{B}	64.8936 ^{E}	55.7022 ^{G}	47.6562 ^{H}	34.0375 ^{I}	66.1214 ^{D}	62.2018 ^{F}	68.5781 ^{C}	73.2372 ^{A}
SD ±	0.4157	0.2928	0.7711	0.5762	0.3241	0.6009	0.2117	0.2062	0.3813
% Change		- 6.7360	- 19.9457	- 31.5093	- 51.0818	- 3.2490	- 8.6055	- 1.4407	5.2553

Means are ± SD (n=6) for tissues in a column followed by the same letter are not significantly different (*P* £ 0.05) from each other according to Duncun's multiple range (DMR) test.

Potassium ion (K^{+}) is the prominent intracellular cation of animals and is an important co-factor in the osmotic pressure regulation and acid-base balance. Potassium ion activates certain enzymes (transferase) and is critical for the maintenance of normal membrane excitability. The consistency of intracellular potassium, even with varying total osmotic concentration of habit, may represent a very old cellular chamber [17]. It plays an important role as an osmotic inorganic effecter in animals (Lagesptez and Senius, 1979) [18]. A continuous decrease of K^{+} content in the tissue was observed in the present study (Table 2). In freshwater fishes, ions are actively taken up from water via the chloride cells in the gill epithelium. For the ionic movement, the membrane system in the chloride cells is important as this is the structure with which Na^{+} and K^{+} ATPase is associated [3]. It is known that any remarkable decrease in K^{+} level might be accompanied by serious disturbances in muscular irritability, myocardial function and respiration (Coles, 1967) [19]. The decrease in K^{+} content in the tissues of *Cirrhinus mrigala* exposed to cypermethrin might be attributed to the derangement in respiration at whole animal as observed in the present investigation.

Calcium ion (Ca^{+}) is another important osmotic effectors and is involved in conferring stability to the cell membrane. It is also a co-factor for several oxidoreductases, proteases and ATPases. Calcium couples the oxidation with contraction in muscle, for the maintenance of structural integrity of mitochondria, sarcoplasmic reticulum and rate of enzyme catalysis. Calcium content of tissues is an important factor (Harper, 1985) [20]. Calcium is a general regulator of permeability of cell membrane to water and other ions. High calcium level generally decreases permeability and low calcium increases it. Hence, calcium level can be taken as index of mitochondrial integrity and cellular metabolism [21]. Any change in calcium level can alter the mitochondrial function, protein synthesis and steady state of enzymatic reactions. All these ions exist in bound as well as in free forms. Bound ionic forms involve in metabolic functions and free ions involve in osmolarity in order contributing to homeostasis of the cell system.

From the decline of Ca^{2+} in the tissues (Table 3) on exposure to cypermethrin indicating increased decalcification, it is known that Ca^{2+} plays an important role in the regulation of cellular metabolism. It is required for regulation of muscle contraction, transmission of impulses neuromuscular excitability and regulation of protein binding capacity [5]. Mitochondria and endoplasmic reticulum are the two important sub cellular organelles involved in the maintenance of the calcium homeostasis (Borle, 1973) [22]. Mitochondrial Ca^{2+} ATPases and Ca^{2+} uptake are the two interlinked processes involved in the maintenance of calcium. It is generally accepted that many of the calcium's effect on the cellular processes are regulated by calmodulin. Calmodulin is responsible for Ca^{2+} dependent activation of a variety of enzymes involved in a number of fundamental cellular functions [23]. Lipophilic compounds have been found to bind with calmodulin with high affinity and reduce the stimulatory effect of this protein on several enzymes [24].

In the present study, the restlessness in *Cirrhinus mrigala* during cypermethrin stress might indicate alterations in the regulations of Ca^{2+} in the tissue (Table 3). Moreover, it has been reported that decreased calcium content during pesticide stress corresponds to structural changes in mitochondrial integrity [4]. Since mitochondria act as "sinks" for intra cellular Ca^{2+} (Bygrave, 1978) [25] and principle storehouses of Ca^{2+} deposition, it appears that the decreased Ca^{2+} in the present study might attribute to the disturbances in mitochondrial integrity and subsequent respiratory distress. Hoar suggested that the levels of amino acids and metabolites like pyruvate and lactate will be increased under stress conditions to compensate the loss of inorganic ions [26]. Amino acids and lactate were found increased in the tissue of *Cyprinus carpio* and *Labeo rohila* exposed to sub lethal concentration of fenvalerate [27], cypermethrin [12, 28, 29] causing metabolic diversion in fish to prolong its survivability under severe osmotic imbalance.

In sublethal concentration of cypermethrin the Na^+, K^+ and Ca^{2+} levels significantly decreased with ion competent in the organs of fish at day 1 and day 7 exposures (Table 1-3). The significant elevation in the ionic levels at days 14 and 21 indicate that on prolonged exposure the sub acute concentration of cypermethrin could not elicit inhibitory effect either on the uptake of ions instead it stimulated the uptake. Further recruitment of chloride cells has been proposed as a fundamental and physiologically significant response of freshwater fish to increase the capability to take up Na^+, K^+ and Ca^{2+} from water [6, 30]. Even the secretion might be increased to induce particularly hypercalcemia, by remobilization of Ca^{2+} from exchangeable Ca^{2+} stores. All these factors could be in active operation for the elevation in ion levels in the gills, muscle and liver of fish from 14 to 21 days. The increased ionic level may be helpful to the animal to prevent the entry of toxic cypermethrin by maintaining cat ion concentration gradient.

The present result clearly demonstrates the exposure to cypermethrin causes a decrease in ions. This is attributed to a direct action on the enzyme and the lipophilic nature of the pyrethroid in general. The synthesis of ATP by phosphorylation of ADP is mainly associated with glycolysis and biological oxidation involving the citric acid cycle and electron transport system [31]. Decreases and increases in the concentrations of inorganic ion follow changes in the membrane permeability of the tissues[5].

It may, therefore, be inferred that if the uptake of this compound by fishes in natural environment reaches tissue concentrations equal to that used in this study, the resulting disruption in ion concentration and inhibition in ion dependent ATPase may be sufficient to impair normal organ function.

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