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Research Article

Effect of Cadmium Chloride on the Activity of ATPases in Functionally Different Tissues of Freshwater Fish, *Cyprinus carpio* (Linnaeus)

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ABSTRACT

In the present study, an endeavour has been made to study Na^+-K^+, Mg^{2+} and Ca^{2+} adenosine triphosphatase (ATPase) activities and Oxygen Consumption in the freshwater fish, *Cyprinus carpio* on short-term and long-term exposure to the median lethal and sub lethal concentrations of Cadmium Chloride. ATPase activity and Oxygen Consumption were found affected in fish exposed to Cadmium Chloride. Whereas the toxicity effect changes is not highly pronounced at sub lethal level indicating low Concentration of Cadmium Chloride and also it shows non-toxic effect at chronic exposure. Na^+–K^+, Mg^{2+} and Ca^{2+} ATPases activity were also found decreased in correspondence to decrement in the Oxygen Consumption under median lethal and sub lethal concentrations in target tissues. With this effect this might have lead to behavioural changes and create wide-spread disturbance in the normal physiology, ultimately causing the death of the fish. The results suggest that in biomonitoring programmes, inhibition of associated ATPases can be a good diagnostic tool for Cadmium Chloride toxicity.

Key words: Na+–K+, Mg^2+ and Ca^2+ ATPases, Oxygen Consumption, *Cyprinus carpio,* Cadmium Chloride

INTRODUCTION

Heavy metals include transition metals like arsenic, antimony, bismuth, Cadmium, cobalt, copper, lead, mercury, nickel, zinc etc., having the atomic number 22 to 92 in all the groups from periods III to VII in the periodic table. The common feature of these metals is that they are relatively toxic to aquatic organisms even at fairly low concentrations and readily accumulate in their tissues. The presence of heavy metals in aquatic media is further compounded by the fact that they are water soluble, non-degradable and having the ability of binding to many biochemical's, especially polypeptides and proteins. All heavy metals are toxic to aquatic organisms, but some are highly toxic even at lower concentrations. Metals like mercury, copper, Cadmium, lead and zinc are very toxic, of which, except copper and zinc, and the others are nonessential metals.

The Cadmium is the second member of the Group IIB triad (Zn, Cd and Hg) in the periodic table and the classification of elements. The stable state of cadmium in the natural environment is Cd (+2). It has a medium class B character compared to zinc and mercury. This character imparts moderate covalency in bonds and high affinity for sulfhydryl groups, leading to increased lipid solubility, bioaccumulation and toxicity. There has been rapid and continuous increase in the world-wide production and use of Cadmium since 1925. Cadmium is primarily used in zinc smelting and electroplating. In addition Cadmium is widely used in the metal industry as protective coverings for iron, copper and steel. This element is used to make Cadmium-electroplated parts in television and radio sets. Cadmium oxide is used as the negative electrode in Cadmium-Nickel and Cadmium-Silver rechargeable batteries and is an important component in low melting alloys in bearings, solder, Nuclear reactor control rods and in Cadmium-Copper telephone wires etc. Cadmium is of pathophysiological interest not only because of its toxic properties but because there is

some evidence that it may serve as a physiologic function. Cadmium interacts with several other essential elements such as iron, copper, zinc, calcium and selenium and could play an important regulatory role with these elements. Some enzymes, containing zinc have been reported to be activated by Cadmium *in vitro*.

In recent times, enormous quantities of Cadmium are being discharged into the freshwater environment due to the activities of highly industrialized societies. Studies on the toxicity of this nonessential element reported its harmful nature for the productivity of freshwater fishes, which serve as staple food for human beings. In the invertebrates heavy metal ions may also enter through the cuticle [1].The role of any way of uptake depends on feeding behaviour, life cycle, animal body size, and duration of exposure [2]. However, most of them are confined to adult fishes but comparative studies on the effect of cadmium on different physiological, biochemical and histological aspects in fry and fingerlings exposed to lethal and sub lethal concentrations are very limited. This wide lacuna in the field of cadmium toxicity on freshwater fishes prompted to take up this investigation to access the toxic effect of Cadmium Chloride on ATPses in freshwater fish, *Cyprinus carpio*. Since, *Cyprinus carpio* is sufficiently available in the freshwater tanks and ponds in and around tumakuru. Therefore the current investigation is carried on this species.

MATERIALS AND METHODS

Cyprinus carpio weighing 5 ± 2 g and measuring an average length of 4-5 cm were collected from the State Fisheries Department, Tumkur, Karnataka, India and kept in large aquarium previously treated with potassium permanganate to clean it from microbial infection if any. All the experimental fish were acclimated to laboratory conditions for 15 days with tap water whose physico-chemical characters were analyzed by following the APHA [3]. Cadmium Chloride about 95% purity was procured from local market of Bangalore, Karnataka, India, under the trade name Thermo Fisher Scientific India Pvt Ltd., Supplied by Vasa Scientific Co., Bangalore, Karnataka, India. Required quantity of Cadmium Chloride at a Concentration of 9 mg/l, 11mg/l, 14mg/l, 18 mg/l and 22 mg/l was prepared and exposed to ten fish per concentration along with control. The LC_{50} value at 96hr was determined by following the method of Finney [4].

Based on the results of LC_{50}, the fish were exposed to lethal concentrations for 1, 2, 3 and 4 days and sub lethal concentrations for 1, 5, 10 and 15 days.

Whole Animal Oxygen Consumption (Lethal and Sub lethal Concentration)

The rate of Oxygen Consumption by the whole animal was estimated adopting Winkler's iodometric method as described by [5] and the apparatus setup was the same as described by [6]. The difference in the oxygen content of the initial and final samples were taken as the amount of oxygen consumed by the fish during the period of experiment. The Oxygen Consumed by the normal and Cadmium Chloride exposed fish was determined. After experiment, the fish were individually weighed and their unit metabolism was calculated and expressed as ml oxygen consumed/g wet weight/h.

Na^+- K^+, Mg^{2+} and Ca^{2+} ATPase activities (ATPase phosphorylase, EC. 3.6.1.3.):

Na^+- K^+, Mg^{2+} and Ca^{2+} ATPase activities were estimated separately in the organs by the method described by [7] with slight modification. 1% tissue homogenate (W/V) were prepared in ice-cold 0.25 M sucrose solution containing 5 mM EDTA (Prepared in 40 mM tris-HCl buffer at pH 7.5) and 0.01 M imidazole. The homogenates were centrifuged at 2500 rpm for 10 minutes and the supernatants were taken as crude enzyme extract for the assay of the ATPase enzyme activities.

After due standardization of enzyme kinetic parameters, three sets of incubation mixtures were prepared. In a total volume of 2 ml, the first set consisted of 100 mM disodium adenosine triphosphate (Prepared in 20 mM tris-HCl buffer at pH 7.5), 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$ and 0.3 ml of enzyme extract. The second set consisted of 100 mM disodium ATP (Prepared 2 mM tris-HCl buffer at pH 7.5), 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$ 1 mM ouabain (potent inhibitor of Na^+-K^+ ATPase) and 0.3 ml of enzyme extract and the third set consisted of 100 mM disodium ATP (Prepared in 20 mM tris-HCl buffer at pH 7.8), 5 mM $CaCl_2$ and 0.3 ml of enzyme extract. All the three incubation sets were incubated at 37^0C for exactly 15 minutes and then the reaction was arrested by adding 2 ml of cold 10% TCA. All these three ATPase activities are expressed as µM of Pi liberated/mg protein/h. Protein contents were measured according to the method of [8] using Bovine Serum Albumin as standard.

The data were subjected to analysis of variance and the means were compared by Duncan's new multiple range to test at 0.05% level [9] to draw the mean comparison among the results.

RESULTS

LC_{50} Value of Cadmium Chloride for the Indian carp, *C. carpio* was found to be 14 mg/l. The present observation deals with the rate of whole animal oxygen consumption of control and Cadmium Chloride treated

fish, which is presented in the (**Table 1**). The data indicates that the fish exposed to lethal and sub lethal concentrations of cadmium chloride, oxygen consumption was reduced. The whole animal oxygen consumption is reduced by about 28.36% on day one and reached maximum reduction of 72.28% on 4th day. Even during day 2nd and 3rd there was high rate of decrement (**Table 1**). The decrement was a sudden reduction from day 1 to day 2, from then it was gradual reduction. However in sub lethal concentrations, the steady decline in the rate of oxygen uptake observed and it was maximum on 5th day. Day one showed the decrease of about 29.67%, which reached 47.25% by day 5th. Subsequently, there was an improvement in Oxygen Consumption as by 10th and 15th day (25.74 and 14.22%) respectively (**Table 1**).

ATPases:

The data on the activities of Na^+-K^+, Mg^{2+} and Ca^{2+} ATPase (µM of Pi formed/mg protein/h) in gill, muscle and liver of freshwater fish, *C. carpio* at 1, 2, 3 and 4 days on exposure to lethal and 1, 5, 10 and 15 days on exposure to the sub lethal concentrations of Cadmium Chloride are presented in the (**Table 2, 3 & 4**). Na^+- K^+ exhibited a decrease in the lethal level and fluctuations and finally showing an elevation on day 15 of sublethal level in all the two target organs (gill, kidney and liver). Mg^{2+} ATPase did not differ in exhibiting the trend of decrease in both lethal and sub lethal levels, simultaneously showing an increase on day 15 of the sub lethal level, which was also seen as in other two ATPases. Ca^{2+} ATPase activity showed a gradual decrease at lethal level and variations at sublethal level up to day 10 and enhancement on day 15 in gill, kidney and liver (**Table 2, 3 & 4**).

DISCUSSION

In this study the toxicity effect were observed decrease in Oxygen Consumption by the whole animal it might be due to the respiratory distress as well as a consequence of the impairment of oxidative metabolism [10]. In consonance to this, he also reported that the depletion in Oxygen Consumption was due to the disorganization of the respiratory function caused by rupture in the respiratory epithelium of the gill [11]. And this study reported that the Cadmium is efficiently absorbed across the gill and diffuse into the blood stream resulting toxic to the fish. Decrease can also attribute to the induction of hypoxic conditions within the animal due to the intimate contact of the respiratory surface with the polluted water resulting in the alteration of normal respiratory area of the animal. The secretion of mucus layer over the gill lamellae has been observed during Cadmium stress. Oxygen deficiency is a wide spread phenomenon encountered in tropical lakes and ponds. Fish living in these water bodies often exhibit numerous physiological adjustments [12]. And the toxicant mechanism of fish uptake through gills probably occurs through pores and by simple diffusion and is then absorbed through cell membranes [13]. In this Studies the course of Oxygen Consumption in lethal and sub lethal concentration indicates the series and the type of compensatory mechanism, if any, which operates within the animal to overcome the load of toxic stress.

Adenosine triphosphatases have the central role in physiological function of cells as energy transducers by coupling the chemical reactions of ATP hydrolysis [14]. Membrane bound Na^+- K^+ ATPase is the enzymatic machinery for the active transport of sodium and potassium across the cell membrane. Magnesium translocation is dependent on membrane bound Mg^{2+} ATPase [15]. Living system needs a continuous input of energy for the build-up and maintenance of their organization. And the energy imitative during the cellular oxidation, primarily with adenosine in the form of ATP (Adenosine triphosphate) of organic fuels stored in the phosphate bond and sometime with creatine in the form of CP (Creatine phosphate). With this the ATPases have been described as prominent energy linked enzymes in fishes [16].

The present results suggested the activities of Mg^{2+}, Na^+- K^+ and Ca^{2+} ATPases are decreased in kidney and liver of the fish on exposure to Cadmium Chloride. The first set gave the total ATPase activities of Na^+- K^+ and Mg^{2+}, whereas the second set gave only the Mg^{2+} ATPase activity as ouabain inhibits Na^+- K^+ stimulated ATPase. Hence, the Na^+- K^+ activity was derived by subtracting the Mg^{2+} ATPase from total of Na^+- K^+ and Mg^{2+}, ATPase activities. The third set directly gave the Ca^{2+} ATPase activity.

Decrease in these activities indicates the destruction of cellular ionic regulations in the organs of the fresh water fish as reported [17]. In this connection, it is of interest to note that Oxygen Consumption has decreased in the fish, *Cyprinus carpio* under fenvalerate and cypermethrin stress [18]. And possible metabolic diversions adopted by the cockroach, *Periplaneta americana* to counteract the toxicity of fenvaleate [19].The decrease in activities may also be due to interaction of heavy metal with Mg^{2+} and Na^+- K^+ ATPases thereby inducting inhibition [20]. According to [21] the inhibition is due to phosohorylation of active site of the enzyme as in the case of acetylcholinesterase inhibition. It is reported that the heavy metals are lipophilic in nature. Hence it is reasonable to assume that they should interact with tissue having more lipid content or involved in the lipid metabolism. The Mg^{2+} ATPase is a phospholipid dependent enzyme [22] and alterations in the chemical and physical characteristics of phospholipids would therefore alter the enzyme activity.

Table 1
Whole Animal Oxygen Consumption of the fish (ml/gm wet wt/h) *Cyprnius carpio* on exposure to the lethal and sub lethal concentrations of Cadmium Chloride.

Estimations	Control	Exposure period in days							
		Lethal				Sub lethal			
		1	2	3	4	1	5	10	15
Mean	0.4520^{A}	0.3228^{D}	0.3023^{F}	0.1716^{H}	0.1243^{I}	0.3178^{E}	0.2374^{G}	0.3356^{C}	0.3868^{B}
SD ±	0.0568	0.0873	0.0886	0.011	0.067	0.462	0.872	0.0873	0.740
% Change	-----	-28.36	- 33.11	- 59.82	- 72.28	- 29.67	- 47.25	-25.74	- 14.22

Values are Means ± SD (n=6) for oxygen consumption in a column followed by the same letters are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.

Table 2
Na^{+}-K ATPase activity («M of Pi formed / mg protein / h) in the organs of fish, *Cyprnius carpio* on exposure to the lethal and sub lethal concentrations of Cadmium Chloride.

Organ	Control	Exposure period in days							
		Lethal				Sub lethal			
		1	2	3	4	1	5	10	15
Gill	4.7590 ^{A}	4.3175 ^{C}	4.2125 ^{D}	3.9010 ^{G}	2.0678 ^{I}	4.4112 ^{B}	4.2046 ^{E}	3.7801 ^{H}	4.0511 ^{F}
SD ±	0.0002	0.0002	0.0004	0.0003	0.0003	0.0002	0.0004	0.0005	0.0003
% Change	------	-9.26	-11.82	-18.02	-56.53	-7.30	-11.63	-20.55	-14.86
kidney	4.2062 ^{A}	3.8003 ^{C}	3.0995 ^{G}	2.1653 ^{H}	1.4805 ^{I}	3.9907 ^{B}	3.6285 ^{E}	3.1926 ^{F}	3.6775 ^{D}
SD ±	0.0003	0.0002	0.0003	0.0003	0.0004	0.0002	0.0003	0.0004	0.0002
% Change	-----	-9.63	-26.31	-48.51	-64.83	-5.12	-13.73	-24.07	-12.55
Liver	3.5495 ^{A}	3.0213 ^{C}	2.5923 ^{F}	2.2675 ^{H}	1.5872 ^{I}	2.9652 ^{B}	2.6413 ^{E}	2.1531 ^{G}	2.7755 ^{D}
SD ±	0.0003	0.0002	0.0003	0.0004	0.0004	0.0003	0.0005	0.0002	0.0004
% Change	------	-14.86	-26.95	-36.11	-55.26	-16.45	-25.56	-38.88	-21.80

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

Table 3
Mg^{2+} ATPase activity («M of Pi formed / mg protein / h) in the organs of fish, *Cyprnius carpio* on exposure to the lethal and sub lethal concentrations of Cadmium Chloride.

Organ	Control	Exposure period in days							
		Lethal				Sub lethal			
		1	2	3	4	1	5	10	15
Gill	4.2395 ^{A}	3.6447 ^{D}	3.0940 ^{G}	2.7150 ^{H}	1.9445 ^{I}	3.8847^{B}	3.3028 ^{F}	3.4972 ^{E}	3.7341 ^{C}
SD ±	0.0004	0.0005	0.0003	0.0003	0.0006	0.0003	0.0004	0.0003	0.0002
% Change	-------	-14.02	-27.01	-35.95	-54.11	-8.35	-22.05	-17.50	-11.91
kidney	4.5180 ^{A}	4.1490 ^{D}	3.5982 ^{F}	2.4132 ^{H}	2.2880 ^{I}	4.3237 ^{B}	3.6601 ^{E}	3.2085 ^{G}	4.3091 ^{C}
SD ±	0.0003	0.0002	0.0003	0.0002	0.0004	0.0654	0.0987	0.8965	0.0541
% Change	------	-8.15	-20.36	-46.56	-49.36	-4.30	-18.97	-28.96	-4.62
Liver	5.8952 ^{A}	4.6453 ^{D}	4.4351 ^{E}	3.4623 ^{H}	2.1476 ^{I}	5.6521 ^{B}	5.2456 ^{C}	3.8661 ^{G}	4.3941 ^{F}
SD ±	0.0224	0.0332	0.0532	0.0621	0.0321	0.0423	0.0412	0.0321	0.0521
% Change	------	-21.20	-24.75	-41.26	-63.56	-4.11	-11.01	-34.42	-25.45

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

Table 4
Ca^{2+} ATPase activity («M of Pi formed / mg protein / h) in the organs of fish, *Cyprnius carpio* on exposure to the lethal and sub lethal concentrations of Cadmium Chloride.

Organ	Control	Exposure period in days							
		Lethal				Sub lethal			
		1	2	3	4	1	5	10	15
Gill	5.9767 ^{A}	5.5406 ^{D}	5.3772 ^{E}	4.0825 ^{G}	3.2653 ^{I}	5.6875 ^{B}	5.2986 ^{F}	4.0335 ^{H}	5.6152 ^{C}
SD ±	0.0986	0.0873	0.3697	0.0546	0.0545	0.8765	0.9861	0.1125	0.3577
% Change	------	-7.28	-10.04	-31.67	-45.35	-4.83	-11.34	-32.49	-6.04
kidney	4.2590 ^{A}	3.2521 ^{F}	2.9431 ^{G}	2.7667 ^{H}	2.1431 ^{I}	3.8508 ^{C}	3.4971 ^{D}	3.2966 ^{E}	3.9690 ^{B}
SD ±	0.0115	0.0556	0.9855	0.2587	0.01453	0.6985	0.3697	0.0986	0.0561
% Change	------	-23.63	-30.87	-35.03	-49.67	-9.59	-17.87	-22.58	-6.81
Liver	2.4296 ^{A}	2.2262 ^{C}	2.1880 ^{E}	1.7507 ^{H}	1.5592 ^{I}	2.2035 ^{D}	1.9251 ^{F}	1.8233 ^{G}	2.3491 ^{B}
SD ±	0.366	0.0563	0.5201	0.0895	0.0063	0.0351	0.095	0.0651	0.320
% Change	-----	-8.36	-9.94	-27.94	-35.83	-9.30	-20.78	-24.95	-3.32

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

Thus Mg^{2+} ATPase seems to be an enzyme that is readily susceptible to the action of heavy metal. Greater imbalance caused to the gill structures also is one of the probable reasons for observed perturbations of ATPase activities in the fish. At cellular level the availability of heavy metal to interact with the ATPase might depend on the cell surface area.

In the sub lethal concentrations of Cadmium Chloride, significant elevations in the ATPase activities in the organs of fish, from day 10 to 15 days of exposure, indicate the greater efficiency to resist the sub lethal concentrations of Cadmium. The ability for recovery from the state of imbalance was seen at 10 days of exposure but maintained at 15 days with an initial struggle for survival. Most ion exchange in freshwater telosts occurs across the gills. 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 inhibition in ion dependent ATPase may be sufficient to impair normal organ function. Adenosine triphosphatase (ATPase) is a membrane bound enzyme group vital for regulating oxidative phosphorylation, ionic transport, muscle function and several other membrane transport dependent phenomena. Na^{+}- K^{+} ATPases has a central role in branchial transepithelial ion transport in fish [23]. This enzyme is present in the cell membranes of virtually all vertebrates and is particularly abundant in tissues associated with ionic and osmotic regulation. It represents a complex enzyme system, which requires Mg^{2+}, Ca^{2+}, Na^{+}- K^{+} ions for their activity. Na^{+}- K^{+} ATPase is a biochemical expression of active transport of Na^{+}- K^{+} of the cells and Mg^{2+} ATPase is involved in the biosynthesis of ATP in the mitochondrial system [24].

CONCLUSION

The analysis of data from the present investigation evidenced that Cadmium Chloride primarily induce high physiological stress on the freshwater fish, *Cyprinus carpio* at both lethal and sub lethal concentrations resulting decrease in the whole animal oxygen consumption. This has lead to the imbalance in cellular homeostasis. The decreased oxygen consumption in the toxicant exposed fish is probably due to incorporation of Cadmium Chloride into the fish biosystem. With this ATPases, the membrane bound enzymes play and an outstanding role in maintaining inter-cellular ionic gradient, osmoregulatory and other physiological processes which shows the Inhibition in the ions specific ATPases, could be attributed to membrane damage and cellular leakage.

CONFLICT OF INTEREST

We declare that we don't have any sort of conflict of Interest.

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REFERENCES

1. Robinson KA, Baird DJ, Wrona FG. Surface Metal Adsorption on Zooplankton Carapaces: Implication for Exposure and Effects in Consumer Organisms, Environ. Pollut, 2003; 122(2):159–167.
2. Gerhard A. Review of Impact of Heavy Metals on Stream Invertebrates with Special Emphasis on Acid Conditions. Water, Air, and Soil Pollut, 1993; 66(3–4): 289–314.
3. APHA. American Standard methods for the examination of water wastewater. 20th Edn. Amer. Publ. Health. Assoc., Washington, DC, 2005.
4. Finney DJ. Probit Analysis, 3rd Edition, Cambridge University, Press, London, 1971; 333.
5. Welsh JH, Smith RI. Laboratory exercises in invertebrate's physiology. Burgess publish Co., Minnepolis, 1953; 126.
6. Saroja K. Oxygen consumption in relation to body size and temperature in the earthworm, Megascholex mauritii when submerged under water. Proc. Indian. Acad. Sod. (Animal Sci), 1959; 49: 183-193.
7. Watson TA, Beamish WH. The effects of Zinc on bronchial ATPase activity in vivo in rainbow trout, Salmo gairdneri. Camp. Biochem. Physiol, 1981; 66: 77-82.
8. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin-phenol reagent. J. Biol. Chem, 1951; 193: 265-275.
9. Duncan DM. Multiple range and multiple tests. Biometrics, 1955; 42: 1-42.
10. David M, Shivakumar HB, Shivakumar R, Mushigeri SB, Ganti BH. Toxicity of cypermethrin and its impact on oxygen consumption in Tilapia mossambica. Indian. J. Environ. Toxicol, 2003; 13(2): 99-102.
11. Kalavathy K, Sivakumari A, Rashmi Chandran. Toxic effect of the pesticide cadmium on the fish, Saratherodon mossambicus. J. Eco. Res. Bicon, 2001; 152(2): 27-32.
12. Esther Joice P, Mallikaraj D, Parthi N, Natarajan GM, Sasikala G, Tamilselavi G, Kasthuri M. The effect of hypoxic stress on the bimodal respiration of Macropodus cupanus. J. Eco. Res. Biocon, 2001; 2(1&2): 76-80.
13. Opperhuizen A, Van Der Velde EW, Gobas FAPC, Liem DAK, Van Der Steen JMD. Relationship between bio-concentration in fish and steric factor of hydrophobic chemicals. Chemosphere, 1985; 14(11/12): 1871-1896.
14. Laugher P. Physiol. Rev, 1987; 116-124.
15. Beeler TT. Characterization of the membrane bound Mg2+-ATPase of rat skeletal muscle. Biochem. Biophys. Acta, 1983; 734(2): 221.
16. Desaiah RC, Bhatnager MC, Tyagi AK, Varma SR. Adenosine triphosphatase activity in few tissues of freshwater teleost, Channa gachua following in vivo exposure to endosulfan. Toxicol, 1975; 11(4): 361-368.
17. Schemidt, Nielson B. Osmoregulation: Effect of salinity and heavy metal Fed. Proc, 1975; 33(10): 2137-2146.
18. Malla Reddy P. Effects of fenvalerate and cypermethrin on the oxygen consumption of a fish, Cyprinus carpio. Mendel, 1987; 4: 209-211.
19. Reddy ATV, Yellamma K. The possible metabolic diversions adopted by the cockroach, Periplaneta americana to counteract the toxicity of fenvaleate. Biochem. International, 1991; 23(2): 359-365.
20. Dikshith TSS, Tandon SK, Datta SK, Gupta PK, Jay Raj B. Comparative response of male rats to parathion and linden: Histopathological and biochemical studies. Environ. Res, 1978; 17(1): 1-19.
21. Price NR. Disruption of excitation contraction coupling by organic insecticides. Mode of action on the muscle of the flounder, Platichthys flesus. Comp. Biochem. Physiol, 1978; 59(2): 127-133.
22. Cutkomp LK, Koch RB. Inhibition of ATPase by Chlorinated hydrocarbons. Presented in the Symposium on Mode of Chlorinated hydrocarbons. 1981.
23. Fenwick JC, Lam TJ. Studies on effect of air exposure on gill Na^+-K^+ ATPase of the marble goby, Oxyeleotris marmorata, a facultative air-breathing fish. Fish. Phy and Biochem, 1988; 5(3): 121-130.
24. Mary Chandravathy V, Reddy SLN. In vivo alterations in the activities of ion-specific ATPase in tissue of Anabas scandens associated with sublethal lead nitrate toxicity and recovery responses during post-exposure period. J. Environ. Biol,1995; 16 (4): 301-304.