EFFECT OF ALUMINIUM TOXICITY ON THE ACID PHOSPHATASE AND ALKALINE PHOSPHATASE OF FISH *CYPRINUS CARPIO* VAR *COMMUNIS*

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Abstract:

The present investigation is aimed to assess the impact of aluminium toxicity on the changes in the aquatic fauna in general and fish in particular. To study in the aquatic ecosystems affect growth and enzymes in aquatic organisms. Aluminium metal in acidic waters has also been reported. Na^+ K^+ ATPase activity in gills, plasma, acid and alkaline phosphatase activity in plasma of control and experimental freshwater *Cyprinus carpio var. communis* were studied. The blood was collected from live fish (*Cyprinus carpio* var. *communis)* of control and experimental groups for enzyme assay. The sample was centrifuged at 9000 rpm for 5 min. This left a clear yellow fluid, the plasma, which was used for the determination of Na^+ K^+ ATPase, acid phosphatase and alkaline phosphatase. The gills were separated from the control and experimental fish and 200 mg tissue from each was weighted.

Keywords: Aluminium, Enzyme, freshwater fish *Cyprinus carpio* var. *Communis* Na^+ K^+ ATPase activity in gills and plasma and acid and alkaline phosphatase.

1. Introduction:

Pollutants in the aquatic ecosystems affects the growth and enzymes in aquatic organisms. The occurrence of metal toxicity in animals exposed to pollutants is corresponds to an overwhelming capacity of accumulated metals which can easily binding to macro molecules especially with many enzymes. Recent trend is that, fish an aquatic fauna can be studied as one of the bioindicator organisms which can be predominantly used to assess the extent of heavy metal toxicity. Na^+, K^+ ATPase plays an imminent role in whole body ion regulation by providing energy for the movement of Na^+ K^+ across the cellular membranes against an electrochemical gradient which is required for the uptake of metabolites such as glucose and amino acids by cells (Epstein *et al.*, 1967; Trachtenberg, 1981; de Renzis and Bornancin, 1984; Evans, 1987). The xenobiotics can alter Na^+ K^+ ATPase activity due to disruption of energy producing metabolic pathways or direct interact ion with the enzyme (Watson and Beamish, 1980).

Among various enzymes studied, much attention has been focused on alkaline phosphatase (ALP). Alkaline phosphatase is a polyfunctional enzyme which hydrolyses a broad class of phosphomonoester substrates and acts as a transphosphorylase at alkaline p^H.

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The enzyme may also play an important role in the mineralization of the skeleton of aquatic animals. Determination of alkaline serum phosphatase is great importance in the differential diagnosis of many diseases in man. According to in ecotoxicology, alkaline phosphatase could serve as a good indicator of intoxication because of its sensitivity to metabolic salts. Hence, in the present study the nature and degree of enzyme response in *Cyprinus carpio* var. *communis* to aluminium toxicity in polluted waters was investigated.

2. Material and Methods:

Na^+ K^+ ATPase activity in gills and plasma, acid, alkaline phosphatase activity in plasma of control and experimental fishes were studied.

2.1 Preparation of Sample:

The blood was collected from live fish of control and experimental groups for enzyme assay. The blood was drawn from the heart region by cardiac puncture using a syringe pre-rinsed with the anticoagulant heparin and it was transferred into small vials. The sample was centrifuged at 9000 rpm for 5 min. This left a clear yellow fluid, the plasma, which was used for the determination of Na^+ K^+ ATPase, Acid and alkaline phosphatases.

2.2 Gills:

The gills were separated from the control and experimental fish, and 200 mg tissue from each was weighed. They were homogenized with 1 ml of 0.1 M. Tris-HCl buffer (pH 7.5) using a Teflon homogenizer, and then centrifuged at 10,000 rpm at 4^0C for 15 min. The supernatant was used for Na^+ K^+ ATPase activity studies in gills.

2.3 Estimation of Adenosine Triphosphatase (ATPase):

Sodium and Potassium ATPase:

The specific activities of Na^+ K^+ ATPase were assayed following the method of Shiosaka*et al.*,(1971).

2.4 Standard Preparation:

0.315 g of pure monopotassium phosphate was dissolved in distilled water and transferred quantitatively to 1 L volumetric flask. Then 10.00 ml of 10 N sulphuric acid was added to this and diluted to the mark with distilled water and mixed well. This solution contains 0.40 mg of phosphorus in 5.00 ml.

2.5 Estimation of Acid Phosphatase and alkaline phosphatase:

Acid phosphatase activity was estimated by the method of Moctified King's method of King and Jagatheesan (1959) using Diagnostic Reagent Kit supplied by Stangenlmmuno diagnostics, Hyderabad. Alkaline phosphatase activity was estimated by Kind and King's (1954) Method using Diagnostic Reagent Kit supplied by Stangen immunodiagnostics, Hyderabad, India.

3.0 Results:

Table 1 and Fig.1 depict the changes in the enzyme activities of Na^+ K^+ ATPase in gills and plasma and acid and alkaline phosphatase in plasma of *Cyprinus carpio* var. *communis* exposed to lethal concentration of aluminium sulphate. In gills, the Na^+ K^+ ATPase activity decreased significantly showing -35.49% whereas in plasma it increased by +6.37% relative to the controls. Both acid and alkaline phosphatase activity increased in experimental fish, showing a +144.66% and +387.69% respectively. Table 2 and Fig. 2 gives the data on the changes in Na^+ K^+ ATPase activity in the gills of fish, *Cyprinus carpio* var. *communis* exposed to sub lethal concentration of aluminium. The enzyme activity decreased to -3.54% after 7th day in treated fish. The declining trend continued up to middle of the experiment recording -18.66% and -46.92% decreases on 14th and 21st day, respectively. After 21st day, the enzyme activity gradually recovered recording a percent decline of -16.02% and -8.69% at the end of 25th and 35th day respectively.

Table 3 and Fig.3 show data on Na^+ K^+ ATPase activity in plasma of fish, *Cyprinus carpio* var. *communis* exposed to sublethal aluminium stress. Na^+ K^+ ATPase activity decreased by -15.40% and -27.09% after 7th and 14th day, respectively. However, after 21st day enzyme activity in plasma of treated fish suddenly increased by +83.12% than that of the control. Subsequently Na^+ K^+ ATPase activity reached to near normal value of control recording -1.30% decreases and +6.29% increases after 28th and 35th day, respectively. Changes in the plasma acid phosphatase activity of *Cyprinus carpio* var. *communis* exposed to sublethal aluminium sulphate were given in Table 4 and Fig 4. The enzyme activity increased to +96.01% in

the experimental fish after 7th day treatment. However, after 14th day, it decreased to -19.81%. In the following weeks, again the enzyme activity substantially increased showing +79.12%, +222.11% and +285.53 % after 21st, 28th and 35th day, respectively. Table 5 and Fig. 5 illustrate the changes occurred in the alkaline phosphatase level in the plasma of fish by the exposure of sublethal concentration of aluminium sulphate. Alkaline phosphatase activity decreased to -54.35% at the end of 7th day, after when the activity gradually recovered showing -27.72% at the end of 14th day then the value reached to near nonnal by registering +4.63% increase after 21st day. Then the enzyme activity decreased showing -51.79% and -29.91% after 28th and 35th day, respectively.

Table 1. Na^+ K^+ ATPase activity in the gills and plasma and plasma phosphatases activity of *Cyprinus carpio* var. *communis* exposed to acute aluminium sulphate toxicity.

S.no	Parameters	Control	Experimental	Percent change	Calculated 't'
01.	Na^+ K^+ ATPase.				
	a. Gills (µg/h/gm.)	42.402 ± 1.045	27.362 ± 0.890	-35.49	10.9817
	b. Plasma (µl/h/L)	0.0241 ± 0.009	0.0256 ± 0.0009	+ 6.37	1.4769
02.	Acid phosphatase in KA units	22.87 ± 0.529	55.902 ± 1.176	+144.66	25.6750
03.	Alkaline phosphatase in KA units	14.602 ± 0.178	71.202 ± 1.478	+ 387.69	38.0901

Values are mean ± S. E. of five individual observations. - Denotes per cent decrease over control. + Denotes per cent increase over control. Values are significant at 5% level. Degrees of freedom at 8 t 0.05 = 2.306.

Table 2. Na^+ K^+ ATPase activity in the gills of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

S.no	Exposure period (in days)	Gill Na^+ K^+ ATPase activity µg/h/g tissue		Percent change
		Control	Treatment	
01.	07	42.002 ± 1.047	40.525 ± 1.260	-3.54
02.	14	36.322 ± 1.230	29.552 ± 0.709	-18.66
03.	21	36.162 ± 1.149	19.202 ± 0.874	-46.92
04.	28	40.002 ± 1.822	33.602 ± 1.056	-16.02
05.	35	42.402 ± 0.714	38.722 ± 1.506	-8.69

Values are mean ± S.E of five individual observations. - Denotes per cent decrease over control

Table 3. Na^+ K^+ ATPase activity in the plasma of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

S.no	Exposure period (in days)	Plasma Na^+ K^+ ATPase µl/h/L		Percent change
		Control	Treatment	
01.	07	0.234 ± 0.0006	0.0198 ± 0.0009	-15.40%
02.	14	0.0229 ± 0.0010	0.0167 ± 0.0006	-27.09%
03.	21	0.0227 ± 0.0010	0.0415 ± 0.0007	+83.12%
04.	28	0.0234 ± 0.007	0.0231 ± 0.0006	-1.30%
05.	35	0.0239 ± 0.0010	0.0254 ± 0.0010	+6.29%

Values are mean ± S.E. of five individual observations. - Denotes per cent decrease over control. + Denotes per cent increase over control.

Table 4. Acid phosphatase activity in the plasma of *Cyprinus carpio* var. *communis* exposed to sub lethal concentration of aluminium sulphate for varying periods.

S.no	Exposure period (in days)	Plasma and phosphatase in KA units		Percent change
		Control	Treatment	
01.	07	24.950 ± 0.590	48.900 ± 1.064	+95.99
02.	14	27.265 ± 0.375	21.860 ± 0.825	-19.79
03.	21	28.590 ± 1.205	51.210 ± 0.839	+79.10
04.	28	21.400 ± 0.744	69.110 ± 0.637	+222.9
05.	35	21.400 ± 0.450	82.500 ± 0.951	+285.51

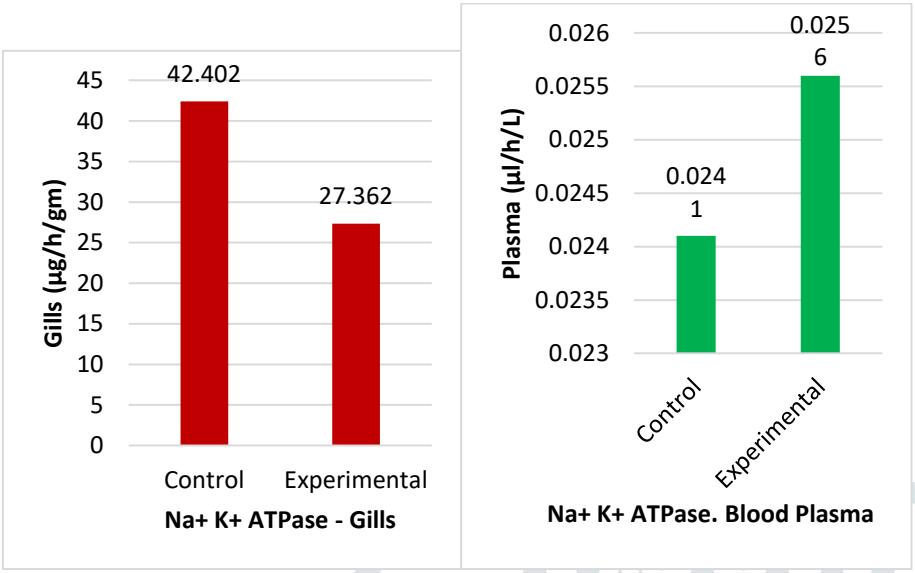
Values are means ± S E. of five individual observations. - Denotes per cent decrease over control. + Denotes per cent increase over control

Table 5. Alkaline phosphatase activity in the plasma of *Cyprinus carpio* var. *communis* exposed to sub lethal concentration of aluminium sulphate toxicity for varying periods.

S.no	Exposure period (in days)	Plasma alkaline phosphatase activity in KA units		Percent change
		Control	Treatment	
01.	07	16.600 ± 0.698	7.580 ± 0.308	-54.33
02.	14	14.800 ± 0.853	10.700 ± 0.491	-27.70
03.	21	15.200 ± 0.455	15.900 ± 0.420	+4.61
04.	28	14.100 ± 0.487	13.670 ± 0.830	-51.77
05.	35	14.700 ± 0.302	10.306 ± 0.804	-29.89

Values are means ± S.E. of five individual observations. - Denotes per cent decrease over control. + Denotes per cent increase over control.

Fig.1. Na^+ K^+ ATPase activity in gills and plasma phosphatase activity of *Cyprinus carpio. Var. communis* exposed to 24 h LC_{50} concentration of aluminium sulphate toxicity.



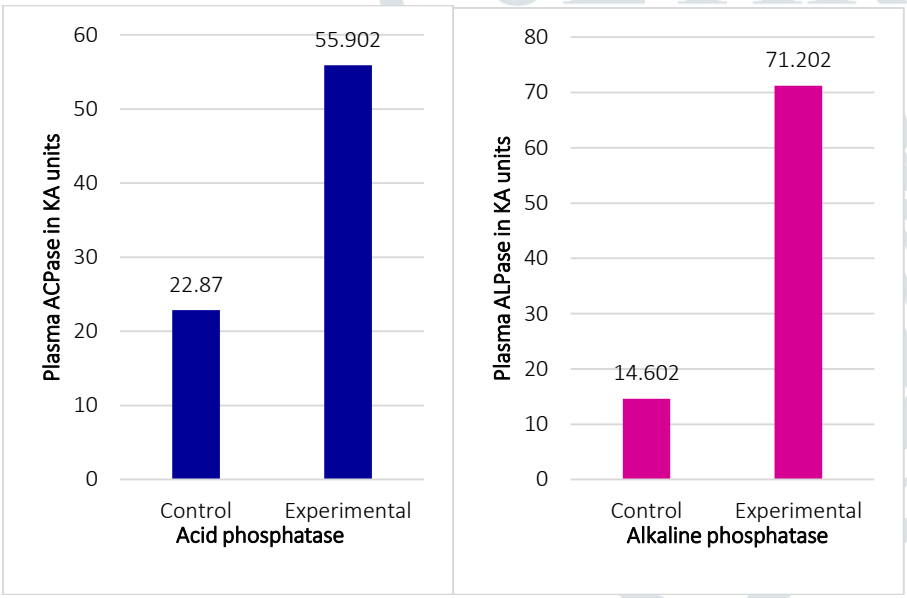
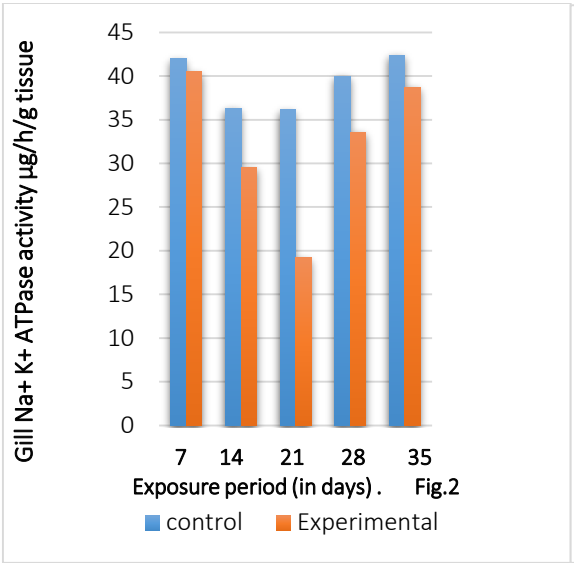


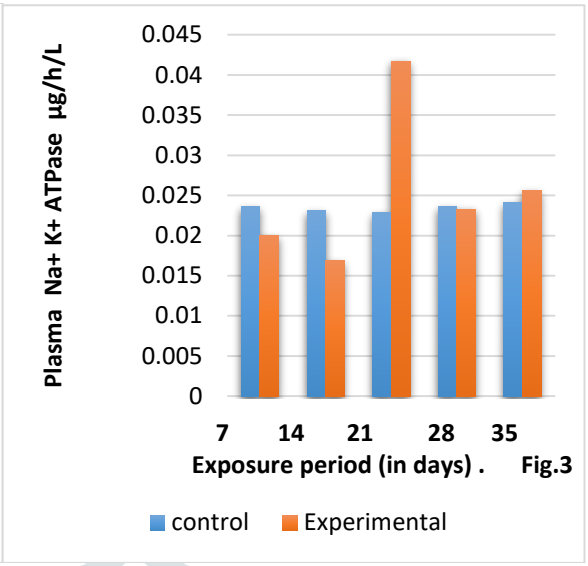
Fig.2. Na^+ K^+ ATPase activity in gills of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

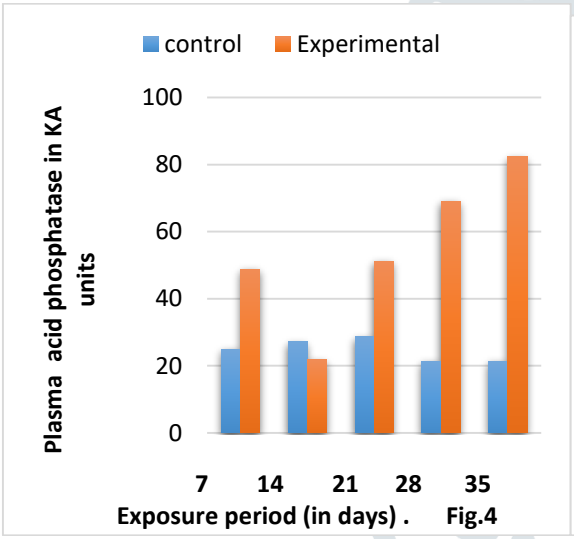
Fig.3. Na^+ K^+ ATPase activity in plasma of *Cyprinus carpio* var. *communis*exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

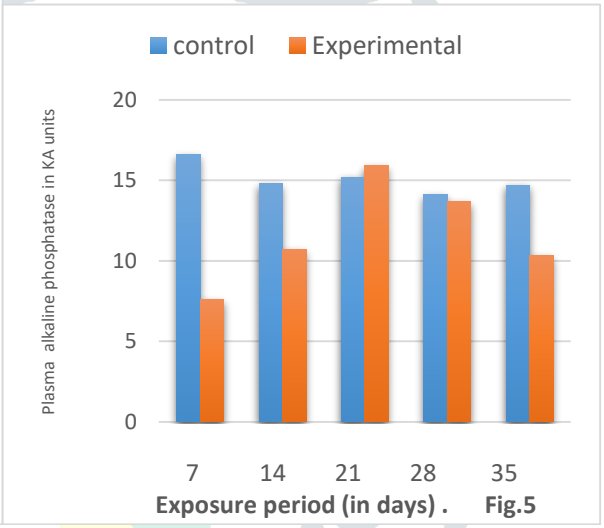
Fig.4. Acid phosphatase activity in the plasma of *Cyprinus carpio* var. *communis*exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

Fig.5. Alkaline phosphatase activity in the plasma of *Cyprinus carpio* var. *communis*exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.









4. Discussion:

In the present investigation, the selected experimental fish when exposed to the estimated LC_{50} concentration of the toxicants were found prone to changes in the acid and alkaline phosphatase enzyme titer. These present findings are in agreeing with the earlier findings of several authors. Metals may alter enzyme activity in animals in several ways (Kench, 1972; Verma *et al.*, 1983). They may bind to macromolecules inside the cells particularly with enzymes (Vallee and Ulmer 1972; Britten and Blank, 1973) or change the concentrations of co-factors or reactants by altering membrane permeability and indirectly affecting enzyme activity (Tucker and Matte, 1980). Na^+ K^+ ATPase has been reviewed and assessed as a potentially useful indicator of pollution stress in aquatic animals (Haya and Waiwood, 1983; Torreblanca*et al.*, 1989). Inhibition or stimulation of Na^+ K^+ ATPase activity could be expected to have metabolic or ionic effect in fishes in relation to osmoregulation as stated by Verma *et al.* (1983).

Aluminium toxicity is expressed by its ability to bind phosphate, ATP, or another functionally related biological compound containing phosphorus. Hence, ATP forms stronger complexes with AL^{3+} than with Mg^{2+} and the Al^{3+} ATP complex acts as a strong inhibitor to Mg^{2+} ATP as indicated by Ganrot (1986).The possibility of direct inhibition of enzyme by aluminium also exists as *Al* has been shown to accumulate in the chloride cells of both brown trout, *Salmo trutta*and rainbow trout, *Oncorhynchus mykiss*, when kept in *Al* rich acid water (Karlsson-Norrgren*etal.*, 1986a, 1986b; Youson and Neville, 1987).(In brook trout, *Salvelinus* fontinalis aluminium has been found to accelerate the turnover and degeneration of the chloride cells (Chevalier *et al.*, 1985).

In the present study the inhibition of Na^+ K^+ ATPase activity in the gills of fish *Cyprinus carpio* var. *communis* may be due to direct inhibition of enzyme or replacement of normal co-factor Mg^{2+} ions or

'chloride cell' damage or by binding of the metal ion with the phosphate groups of enzymes recalling the observations of the above authors. In the present study the increase in the plasma Na^+ K^+ ATPase activity after the 21st day could be described as a form of 'hormesis' as reported by the above authors. The xenobiotic can interact directly with the enzyme or alter Na^+ K^+ ATPase activity due to disruption of energy producing metabolic pathways (Watson and Beamish, 1980; Yadwad*et al.,(1990).* According to Mayer *et al.*,(1992) serum enzyme concentration may increase from the following: 1. Enzyme leakage from a cell with a damaged cell membrane 2. Increased enzyme production and leakage from the cell and 3. Decreased enzyme clearance from the blood. Increased serum lysosomes enzymes are an indication of toxicity which may be due to increased enzyme production, decreased lysosomes stability or tissue damage as opined by Mayer *et al.* (1992). According to Ganrot, (1986) within cells, Al^{3+} accumulates first in the Lysosomes and then is slowly transferred to the nucleus of the cell.

The author further reported that the toxicity effect could be even greater at a more neutral pH if Al^{3+}has adequate time to bind in sufficient quantities to the membranes. Ganrot (1986) stated that inhibition of phosphodiesterase (ALP) is probably the result of a high affinity interaction between Al^{3+} and calmodulin. Inhibition of alkaline phosphatase activity in *Cyprinus carpio* var. *communis* during sublethal exposure with aluminium may have been due to direct inhibition of enzymes which may find support from the observations of above workers.Selamoglu Talas *et al.,* (2012) also found during their investigation that granulocyte, erythrocyte, haemoglobin, haematocrit values were decreased in Cyprinus carpio by the use of as in comparison with control group. Fish exposed to as exhibited anxiety in breathing due to the clogging of gills by coagulated mucous and suffered direct damage of as ions to blood vessels, resulting in vascular collapse in the gills and anoxia (Mondal and Samantha (2015)). Lam *et al.*,(2006) and Li *et al.*,(2016) found metabolic and histopathological liver when the zebra fish exposed with arsenic. In particular Wolf *et al.*, (2015), which was co-authored by a team of fish path logistics, provides many illustrated examples of morphologic misdiagnosis and non-lesions in fish used for toxicological bio assay. Toward that end several recent articles further describe issues that lead to and are caused by, poor quality fish histopathology data (Wolf (2011); Wolf *et al.,* (2015); Wolf and maack (2017); Wolf (2018).

Acknowledgement:

The authors express their gratitude to the Principal and Dr. A. Yousuf Khan, Professor and Head, Department of Zoology, Govt. Arts College for Men (Autonomous), Nandanam, Chennai600 035, for providing me an opportunity to study in their prestigious institution.

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