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VARIATIONS IN ACID PHOSPHATASE (ACP) & ALKALINE PHOSPHATASE (ALP) ACTIVITIES IN LIVER & KIDNEY OF A FRESH WATER FISH *LABEO ROHITA* EXPOXED TO HEAVY METAL CONCENTRATIONS

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ABSTRACT

Fishes are aquatic and poikilothermic animals. Hence, their existence and performance is dominated by the quality of their environment. Pollution of water bodies forces them to acclimatize to various factors thus imposing a considerable amount of stress on their lives. Phosphatase is known to be sensitive to metal exposures and can be used to predict metal toxicity. The acid phosphatase (ACP) and alkaline phosphatase (ALP) enzyme activity brought an decrease in acid and alkaline phosphatase (ACP and ALP) in Liver and Kidney when a freshwater fish Labeo rohita exposed to Heavy Metal concentration as compared to the control group. The various concentrations of the heavy metals were found to alter the acidic and alkaline phosphatase activity in the studied organs. Hence, the results from present investigations may be useful in the assessment of environmental stress in the aquatic ecosystem.

KEYWORDS: Acid phosphatise, Alkaline phosphatise, Heavy metals, Labeo rohita.

I. INTRODUCTION

Nature, now a day, faces a serious problem of environmental pollution. The various chemicals entering the aquatic ecosystem through human activities, either accidentally or by design may cause adverse effects on the aquatic biota, including deleterious changes, which disrupt metabolic activities at the biochemical level. Heavy metals are common pollutants of the aquatic environment because of their persistence and tendency to concentrate in aquatic organisms (Ayas *et al. 2007*; Srivastava and Verma, 2009). Most heavy metals released into the environment find their way into the aquatic system as a result of direct input, atmospheric deposition and erosion due to rainwater. Therefore, aquatic animals are often exposed to elevated levels of heavy metals. Enzymes are biochemical macromolecules that control metabolic processes of the organisms, thus a slight variation in enzyme activities would affect the organism (Roy, 2002). Phosphatase is a hydrolytic enzyme, leading to the release of ortho-phosphate from phosphorus compound. The phosphatases, ACP and ALP are active at specific pH and are usually termed phosphomonoesterases. The ACP is a lysosomal enzyme and the raise in its activity is probably related to the cellular damage. It is difficult, however, to relate the decrease in ACP activity with necrosis. Increase in acid phosphatase and Alkaline phosphatase activities can be interpreted as a shift, which emphasise on energy break down pathway from normal ATPase system which includes phosphorylation. Any change in acid and alkaline phosphatase activities can affect the metabolism of the fish. In fisheries sciences, changes in phosphatase activities have been regarded as indices of growth, illness and spawning of fish (Goldemberg et al., 1987; Matusiewicz and Dabrowski, 1996). Thus, by estimating the enzyme activities in an organism, we can easily identify disturbances in its metabolism. Hence in the present study attempt is made to study the effect of the Heavy metals on the enzymes phosphates ACP and ALP in the fish *Labeo rohita*. (Hamilton).

II. MATERIAL AND METHODS

The fish *Labeo rohita* having mean weight 14-16 gm and length 12 – 14 cm were collected from Patrafish farm, at Bhopal and acclimatized to laboratory conditions. They were given the treatment of 0.1% KMNO4 solution and then kept in plastic pools for acclimatization for a period of seven days. They were fed on rice bran and oil cake daily. Fish samples were collected from the highly polluted belt and less polluted belt of the Betwa river. One fish sample was collected from the area of Mandideep (site 1). The second fish sample was taken at Bhojpor (site 2) while as other two fish samples were collected from Raisin (site 3) and Vidisha (site 4). The above samples collected from site 1, 2, 3 and 4th of river Betwa were considered fish samples from polluted water (test fish sample) and were compared with the fish samples collected from the Pathra fish form which is

contol fish sample. Acid and alkaline phosphatases activities were assayed spectrophotometrically as per Kind's and King's method (1954) using diagnostic reagent kit from SPAN was applied. The data were subjected to Analyses of Variance (ANOVA).

III. RESULT

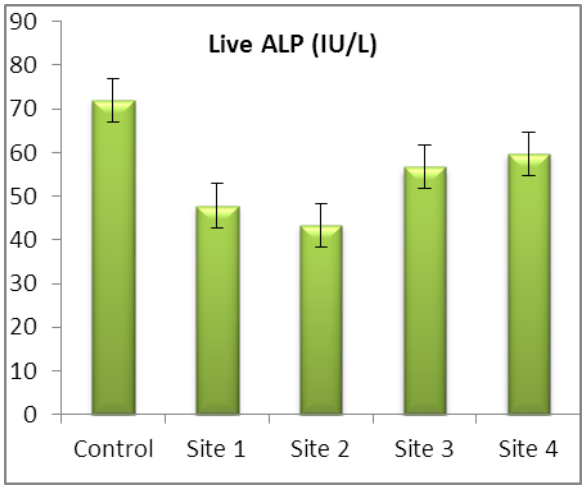
In *Labeo rohita*, the normal alkaline phosphatase activity expressed in IU/L was 71.98±0.18 for control group. At site I, the decrease (P<0.01) was observed in alkaline phosphatase activity in tissue of liver with mean±SE value of 47.84±2.04 IU/L. The alkaline phosphatase activity showed further decrease in tissue of liver at site II with mean± SE value of 43.39±2.80 IU/L. At site III & IV there was an increase in the activity of alkaline phosphatase which ranged from 56.62±1.76 IU/L to 59.71±1.19 IU/L. However in kidney, the normal alkaline phosphatase activity expressed in IU/L was 56.06±0.11 for control group. The lowest value was observed at site I with mean±SE value of 30.68±3.09 IU/L followed by site II with mean±SE value of 32.78±2.73 IU/L. At site III & IV there was an increase in the activity of alkaline phosphatase which ranged from 39.48±1.91 IU/L and 42.38±1.65 IU/L respectively.

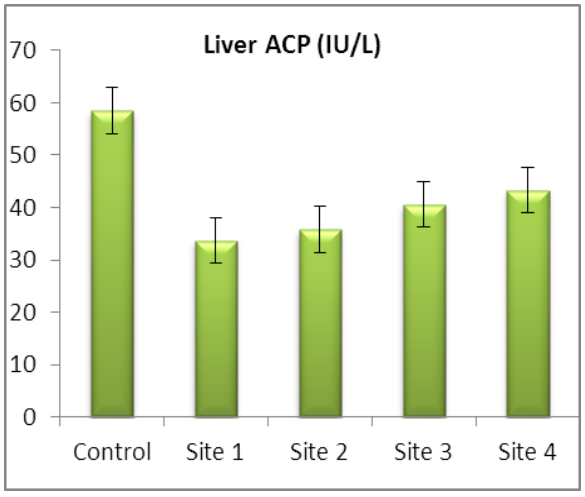
In liver of *Labeo rohita*, the normal acid phosphatase activity expressed in IU/L was 58.47±0.18 for control group. At various sites the acid phosphatase activity was found to be significantly decreased from 33.67±3.56 to 43.32±3.20 (IU/L) as compared to control. At site I, the decrease (P<0.05) was observed in acid phosphatase activity in tissue of liver with mean±SE value of 33.67±3.56 IU/L.However at site II, the increase was observed in tissue of liver with mean±SE value of 35.76±3.60 IU/L as compared to site I.The acid phosphatase activity showed further increase in tissue of liver at site III with mean±SE value 40.56±3.36 IU/L.The highest acid phosphatase activity in tissue of liver was at site IV with mean±SE value 43.32±3.20. However the activity of acid phosphatase in kidney of *Labeo rohita* was found significantly decreased (P<0.05) with mean±SE values from 25.63±1.95 to 32.58±1.56, when compared with the control group with mean ±SE value 42.57±0.19 expressed in IU/L. The lowest value was observed at site I with mean±SE value of 25.63±1.95 IU/L followed by site II with mean±SE value of 27.79±1.88 IU/L. At site III & IV there was an increase in the activity of acid phosphatase which ranged from 30.13±1.71 IU/L and 32.58±1.56 IU/L respectively.

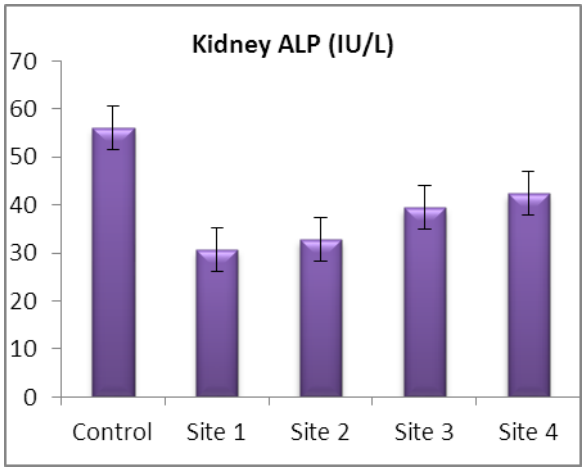
Table1: Showing change in enzymological activity in tissues of liver and kidney 0f *Labeo rohita* caught from different sites with relation to control.

Effect of heavy metals on enzyme activity of liver in *Labeo rohita*										
Parameters	**Control**		**Site I**		**Site II**		**Site III**		**Site IV**	
	Mean±SE	**SD**	**Mean±SE**	**SD**	**Mean±SE**	**SD**	**Mean±SE**	**SD**	**Mean±SE**	**SD**
ALP(IU/L)	**71.98±0.18**	**0.61**	**47.84±2.04****	**6.76**	**43.39±2.80****	**9.29**	**56.62±1.76****	**5.85**	**59.71±1.19****	**3.97**
ACP(IU/L)	**58.47±0.18**	**0.60**	**33.67±3.56***	**11.81**	**35.76±3.60***	**11.96**	**40.56±3.36***	**11.15**	**43.32±3.20^{NS}**	**10.62**
Effect of heavy metals on enzyme activity of kidney in *Labeo rohita*										
ALP(IU/L)	**56.06±0.11**	**0.39**	**30.68±3.09***	**10.25**	**32.78±2.73***	**9.05**	**39.48±1.91***	**6.35**	**42.38±1.65***	**5.48**
ACP(IU/L)	**42.57±0.19**	**0.64**	**25.63±1.95***	**6.47**	**27.79±1.88***	**6.25**	**30.13±1.71***	**5.67**	**32.58±1.56***	**5.17**

Note: Values are mean±SE with range and SD of three replications.* =significant at p<0.05 level, ** = extremely significant at p<0.01, ^{NS} = not statistically significant.







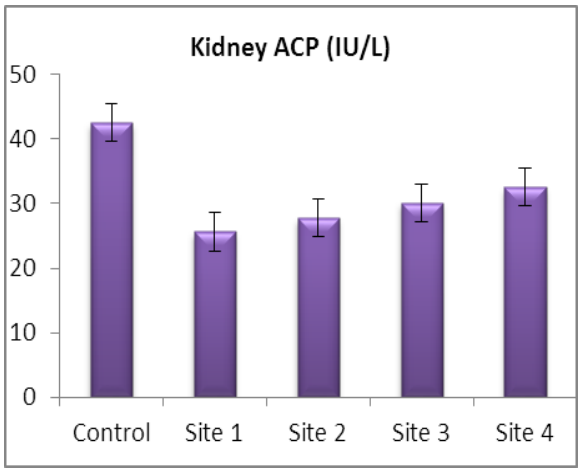


Fig. Enzymological responses in the liver & kidney of Labeo rohita showing increase (+) or decrease (-) in their contents, caught from different sites of Betwa river with relation to control:

IV. DISCUSSION

Occurrence of some heavy metals in all the environmental compartments including food chain of aquatic medium, despite their declining trend as the distance from the point of source increased and remained within the permissible limit, was responsible for the heavy metal toxicity that perhaps affected the succinate dehydrogenase enzyme activity of fish (Mukherjee and Jana, 2007). The use of biochemical approaches have been advocated to provide an early warning of potentially damaging changes in stressed fish. In toxicological studies of acute exposure, changes in concentrations and enzymes activities often directly reflect cell damage in specific organs (Casillas *et al.*, 1983). The Acid phosphatase and Alkaline phosphatase are plasma membrane derived enzymes which play a pivotal role in the cytolysis and differentiation process (Davidson 1949). They are present in almost all the tissues like liver, spleen, kidney, and reticulo endothelial cells and catalyze the libretion of inorganic phosphate from organic phosphate esters and help in maintaining buffer system in blood creating phosphate buffer system (Harper 1990). These enzymes may be important in the regulation of physical properties of membranes or in absorption of lipids (Sachdev 1999).

In the present study, it has been observed that the enzyme activity (ALP &ACP) in the tissue of liver and kidney decreased significantly as compared to control group. This inhibition in enzyme activities by heavy metals may be due to the direct binding of the metal with enzyme protein (Passow *et al.*, 1961) or the toxic effects produced by them on tissues leading to decreased synthesis of enzymes.The observed decrease in alkaline phosphatase and acid phosphatase activity in the liver and kidney is explained as due to the inhibition of the enzyme by the influx of metal ions, the destabilization of the plasma membrane and the resultant depletion of the enzyme away from the tissues. Onikienko (1963) noticed decrease in AL Pase activity may be taken as an index of hepatic parenchymal damage and hepatocytic necrosis. Hirth (1964) in his *in vitro* studies has shown the mechanism of enzyme inhibition by heavy metals revolving around the affinity of lead and mercury to the sulfhydryl group. Dalela *et al.*, (1978) noticed decreased ACP activity in liver, muscle and kidney of *Channa gachua* under sublethal concentration of rogor and endosulfan. Sastry and Gupta (1978) observed increased ACP and decreased ALP activity in liver of *H.fossilis* after a chronic treatment with 2.8 mg/l of lead nitrate. Reduced ALP activity in different tissue of *Channa punctatus* were reported by Sastry and Sharma (1979) due to endrin exposure.

Gautam and Parihar (1995) noticed enzymological alterations in the liver and kidney of fresh water teleost *Heteropneustes fossilis* after intoxication with lead and mercury. Inhibited enzyme activity of ALP and ACP and lipase were seen which might be due to the damage of the plasma membrane, lysosome and endoplasmic reticulum. Kumar *et al.*, (1997) observed the alkaline phosphatase activity in the liver and kidney of *Notopterus notopterus* and found a significantly decreased in alkaline phosphatase activity. They suggest that the inhibition of phosphatase activities due to malathion exposure, affected the physiological function of tissue. The decrease could be attributed to the destruction of cell membrane and lysosomes which in turn leads to hepatic damage. The significant fall in the activity of alkaline phosphatase might be due to decrease in the rate of transphosphorylation.

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