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ACID AND ALKALINE PHOSPHATASE ACTIVITY IN THE TISSUES OF *LABEO ROHITA* FROM FRESHWATER LAKES OF BANGALORE

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L. rohita

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ABSTRACT

This study is aimed on the estimation of activity of acid and alkaline phosphatase (as biomarkers) in liver, gills and muscles tissues of *L.rohita* reared in the freshwater lakes of Bangalore. Acid and Alkaline Phosphatase are known to be involved in several cellular metabolic reactions. The study reveals a considerable reduction in the enzyme activity in the tissues obtained from the polluted lake. The study suggests that the effects of industrially pollutants from Yellamallapa Shetty lake (test site) when compared to those from Hebbal farm (control site) probably involve respiratory and metabolic acidosis and hypoglycemia as well as cellular damage.

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INTRODUCTION

Various organic and inorganic wastes in industrial and domestic effluents are responsible for water pollution. Water pollution is recognized globally as a potential threat to both human and other animal populations which interact with the aquatic environments (Svensson *et al.,* 1995). The conventional laboratory toxicity studies cannot be extrapolated to the natural environment because they lack ecological realism (Benson and Black, 1990). Variability and interaction of environmental factors in natural habitats complicate the responses of organisms to contaminants (Adams *et al.,* 1996). The water parameters such as, high BOD, pH, COD, TDS, nitrates, phosphates and free ammonia besides toxic metals cause deleterious effects on the aquatic biota which disrupt metabolic activities at the biochemical level causing changes in biologically important enzymes (Hirth, 1964).

Aquatic animals have been often used as bioassays to monitor water quality (Crains *et al.,* 1975 and Brugs *et al*., 1977). The development of biological monitoring techniques based on fish offers the possibility of checking water pollution with fast responses on low concentrations of direct acting toxicants (Poele and Strik, 1975). Fish are excellent subjects for the study of various effects of contaminants present in water samples since they can metabolize' concentrate and store water borne pollutants. Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans (Ali *et al*., 2007).

In recent years, pollution monitoring methods using enzyme inducement or enzyme dispersion in fish or other aquatic organisms have been proposed for studying polluted environment (Verma *et al.,* 1979). Changes in acid and alkaline phosphatase activities were shown in *Channa.punctatus* exposed to mercuric nitrate (Jeelani and Shaffi, 1989), in *C. punctatus* exposed to fenvalerate (Parthasarathi and Karuppasamy, 1998), *Heteropneustes fossilis* exposed to fenvalerate (Johal *et al*., 2002) and *Labeo rohita* exposed to domestic sewage (Rajan, 1990).

Phosphatases are good indicators of stress condition in the biological systems (Verma *et al.,* 1980). Subsequently, Balasubramanian *et al.,* (1984) and various other scientists reported the presence of phosphatases in various body tissues of invertebrates. Though number of literature is available regarding the effect of some pollutants and pesticides on enzyme systems in different fishes, studies on the effect of industrial effluents on the phophatases system in wild fishes reared in lakes are very scarce. The present investigation is an attempt to study the effect of industrial pollutants discharged in lake B on tissues like liver, muscle and gill of wild *Labeo rohita* reared in the lake for commercial purpose.

MATERIALS AND METHODS

Water samples were collected in water sampling bottles from Hebbal fish farm or control lake (A) wherein fish fingerlings are reared and Yellamallappa Shetty lake (B) or test site which receives effluents from a nearby pharmaceutical industry. The water was sampled from both the lakes in the morning at about 10.00 to 10.30 AM once in every week for a period of six months. The physico-chemical parameters e.g., temperature, pH, BOD, COD, DO, TDS, chloride, free ammonia, phosphates, sulphates, nitrates and alkalinity of the water samples were determined by following standard methods (APHA *et.al.*, 1995). The data showed that Yellamallappa Shetty lake was industrially polluted and Hebbal fish farm was non- polluted as per the parameters shown in Table 1.

Test fish, *Labeo rohita* were sampled at the same time as water sampling time period from the two water bodies (Hebbal fish farm (A) and Yellamallappa Shetty lake (B)). They were then anaesthetized using MS222 so as to retain the properties of enzymes. They were dissected and the tissues such as liver, muscles and gills were carefully removed and transferred to a suitable media for recording the activities of acid and alkaline phosphatase enzymes.

Table 1: Changes in physico-chemical parameters of Control fish farm (A) and Yellamallappa Shetty lake (B)

Analysis	Standard (Maximum Limits in mg/L)	Control A	Lake B
Temperature (°C)	22 - 28	22	22
pH	06.50 to 08.50	7.37	8.24
Turbidity (NTU)	05 to 25	12	11
Nitrates(ug/L)	10.00	11.0	40
Nitrites (ppm)	10.00	11.0	30.05
Chlorides	100.00	252.27	442.4
Salinity	200	400	800
COD	2.00	4.807	71.42
Phosphates	2.00	0.4	3.6
Total alkalinity	20.00	27	90
Total acidity	20.00	20	60
Iron	00.10	0.03	0.42
Zinc	00.15	0.06	0.19

Except Temperature, pH, conductivity, Turbidity, Nitrates and Nitrites all values are in mg/L

Acid phosphatase activity was determined following the method of Bergmeyer (1956) wherein Sodium p-nitrophenylphosphate was used as substrate. Buffer/substrate solution (0.5M citrate buffer, 0.0055 M p-nitrophenylphosphate, pH 4.8) was added to 10mL of homogenate. The reaction mixture was incubated for 30 min at 37°C. 4mL of 0.1M NaOH was then added to stop the enzymatic reaction. The absorbance was measured at 410nm. The activity of acid phosphatase was expressed as units per mg protein. Alkaline phosphatase activity was also determined with the similar method as of acid phosphatase excepting the Glycine buffer at a pH of 8.8 was used. The results were then statistically analyzed with the help of ANOVA.

RESULTS AND DISCUSSION

The physico-chemical parameters of water and biological diversity of the system determine the quality of an aquatic ecosystem. Discharge of pollutants into natural water resources results in, deterioration of physical, chemical and biological quality of water. The present investigation was undertaken to observe the effect of pollutants on the activities of acid and alkaline phosphatase. The availability of important abiotic factor that affects the survival of individuals in air and lake water is considered to be important for the existence of aquatic life. The level of various water parameters of lake A and B have been analysed and mentioned in detail in Table 1. The data showed that lake B has more amount of nutrients, high pH, chlorides, and also heavy metals as iron and zinc when compared to those of water from control A.

Enzymes are fragile substances with a tendency to undergo denaturation and inactivation under unsuitable conditions. The variations in the activities of acid and alkaline phosphatase enzymes in liver, muscles and gills of freshwater fish *L. rohita* sampled from the fish farm (A) and lake (B) was studied and is presented in Table 2 and Fig. 1 and Table 3 and Fig. 2. Acid and alkaline phosphatases are general enzymes present in almost all the tissues. They are hydrolytic enzymes concerned with the process of transphosphorylation and have an important role in the general energetics of an organism. They are associated with the transport of metabolites, with metabolism of phospholipids, phosphoproteins, nucleotides and carbohydrate, and with synthesis of proteins (Srivastava *et al.,* 1995).

In the present investigation a marked decrease in the activity of both the enzymes, particularly alkaline phosphatase was noticed in all the three tissues of the fish sampled from lake B when compared to those of fish farm (A). Acid phophatase showed a significant decrease in liver tissue when compared to those of muscle and gills in the fish sampled from lake B. This is in conformity with the report by Parthasarathi and Karuppasamy, (1998) in liver, intestine and muscle tissues of *C. punctatus* when exposed to fenvalerate. The decrease in acid phosphatase in liver suggested the uncoupling of phosphorylation by toxicity. Similar

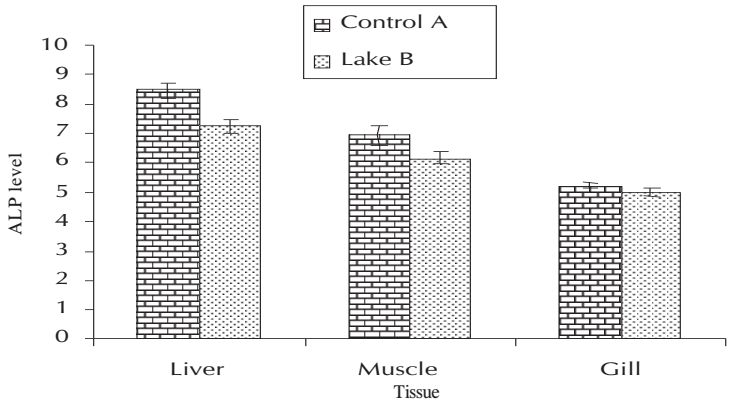

Figure 1: Levels of Acid Phosphatase in the different tissues of *Labeo rohita* from fish farm (control, A) and Yellamallappa Shetty lake (B)

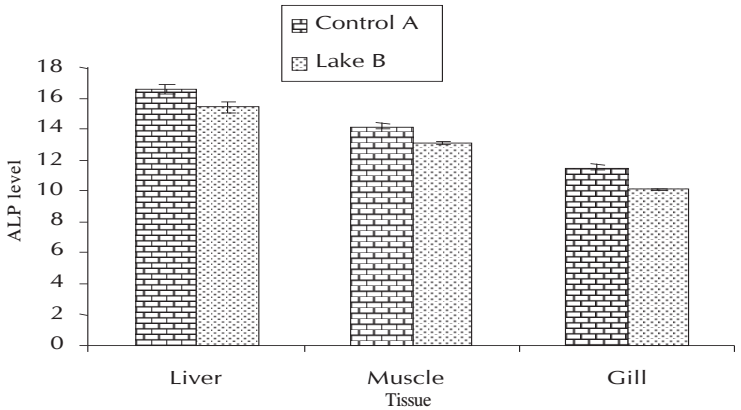

Figure 2: Levels of Alkaline Phosphatase in the different tissues of *Labeo rohita* from fish farm (control, A) and Yellamallappa Shetty lake (B)

decreasing trends were observed in *Ophiocephalus punctatus* exposed to copper and in *Notopterus notopterus* exposed to phenolic compounds (Dalela *et al*., 1982). The accumulation of toxicants beyond a tolerable level in the liver might cause such enzymatic changes. The acid phosphatase activity in the gills showed a

Table 2: Levels of Acid Phosphatase in the different tissues of *Labeo rohita* from fish farm (Control A) and Yellamallappa Shetty Lake (B)

Tissue	Control A	Lake B	
Liver	8.46 ± 0.26	7.26 ± 0.22	(-14.18%)
Kidney	5.85 ± 0.32	4.55 ± 0.19	(-22.22%)
Muscle	6.95 ± 0.10	6.16 ± 0.15	(-11.36%)
Gill	5.23 ± 0.21	5.00 ± 0.14	(-4.39%)

Values expressed as μ moles PNP/mg of protein/30min wet. weight of tissues; Values are expressed as mean ± S.E; sample size (n) = 6; Values given in parenthesis are % change over control; (-) indicates % decrease over control; *Statistically significant at 5% level

Table 3: Levels of Alkaline Phosphatase in the different tissues of *Labeo rohita* from fish farm (control, A) and Yellamallappa Shetty lake (B)

Tissue	Control A	Lake B	
Liver	16.61± 0.30	15.43 ± 0.31	(-7.1%)
Kidney	12.96 ± 0.11	12.65 ± 0.15	(-2.39%)
Muscle	14.2 ± 0.20	13.12 ± 0.10	(-7.6%)
Gill	11.55 ± 0.24	10.06 ± 0.10	(-12.9%)

Values expressed as μ moles PNP/mg of protein/30min wet.weight of tissues; Values are expressed as mean ± S.E; sample size (n) = 6; Values given in parenthesis are % change over control; (-) indicates % decrease over control; *Statistically significant at 5% level

minimum value of 5.23 ± 0.21 and liver showed the maximum value of 8.46±0.26 in the fish sampled from fish farm (A). A decrease in the trend was noticed in the tissues as liver > muscle > gill in the fish sampled from lake B (7.26±0.22, 6.16±0.15 and 5.0±0.14, respectively). The decreased acid phosphatase activity in the muscle was due to increased glycogenolysis or due to changes in the mitochondrial membrane function (Parthasarathi and Karuppasamy, 1998).

Alkaline phosphatase splits various phosphate esters at an alkaline pH and mediates membrane transport. In the present study a decrease in alkaline phosphatase was noticed in the all the tissues of the fish from lake B. Decrease in the activity of this enzyme may result in altered transport and an inhibitory effect on the cell growth and proliferation (Goldfischer *et al.,* 1964). In the fish sampled from fish farm the liver showed a maximum content of 16.61±0.30 and gill showed the minimum value of 11.55 ± 0.24. Whereas fish sampled from lake B showed comparatively less alkaline phosphatase content in liver (15.43 ± 0.31) and least was observed in gills (10.06 ± 0.10) when compared to those of fish sampled from fish farm (A). According to Parthasarathi and Karuppasamy (1998) alkaline phosphatase in liver is capable of inactivating phosphorylase enzymes, thus promoting glycogen synthesis. Therefore, inhibition in alkaline phosphatase activity may cause alterations in glycogen content. In the present research work, glycogen and protein content also is being estimated and the result is very much in correlation to the level of acid phosphastases and alkaline phosphastase. This may be due to the presence of trace metals present in the polluted lake B which was absorbed by the intestine and gills through blood into the body and inturn detoxified by the liver.

According to Shaikila *et al*., (1993) severe acidosis may be the cause for inhibition of alkaline phosphatase activity in intoxicated liver, which inturn could be adaptive for the fish to meet the energy demand by the anaerobic breakdown of glycogen. They further opined that inhibition of activity could also be due to interaction of toxicant with co-factors and regulators. Similar findings were reported in liver of *Oreochromis noloticus* when exposed to methyl parathion (Sarabadhikary and Sur, 1992) and in *Cyprinus carpio* exposed to vegetable oil factory effluent (Ramesh *et al.,* 1994).

The decreased activities of these enzymes indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system. (Nchumbeni *et al.,* 2007).

On the contrary, few scientist had a contradictory results such as an increase in the activity of both the enzymes reported by Sastry and Malik (1981) in *C.punctatus* exposed to diazinon, an increase in acid phosphatase and a decrease in alkaline phosphatase activity observed by Shrivastava and Shrivastava (1998) in *Mus musculus* treated with carbyl and also by Johal *et al*. (2002) in *Heteropneustes fossilis* exposed to fenvalerate. An increase in alkaline phosphatase and a decrease in acid phosphatase activity was also noticed by Ruparelia *et al*. (1992) in *Sarotherodon mossambica* exposed to cadmium.

In conclusion, decrease in acid and alkaline phosphastase activity could
result in enhanced toxicity of environmental chemicals or endogenous compounds and the possibility that some forms of neoplasms may be induced in fish. Fish live in an environment where they are exposed to various amounts of toxic chemicals. The answer to whether the inhibition of acid and alkaline phosphastase is good as an early monitoring for pollution depends on the chemical environment of the fish.

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