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ORIGINAL ARTICLE

EFFECTS OF INDUSTRIAL EFFLUENTS ON THE ACTIVITIES OF ACID AND ALKALINE PHOSPHATASES OF FISH *Arius maculatus* FROM UPPANAR ESTUARY, CUDDALORE DISTRICT, TAMILNADU

T. Kumaresan

Department of Zoology,Annamalai University,Chidambaram,Cuddalore Dist-608002,Tamilnadu

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ABSTRACT

The present study is aimed to analyse the acid and alkaline phosphatases inmuslce and kidney tissues of *Arius maculatus* exposed to sub lethal concentration industrial effluents collected from Uppanar estuary, Cuddalore, District,Tamilnadu. The activities of acid and alkaline phophatases were decreseaed in muslce and kidney tissues of *Arius* maculatus exposed to sub lethal concentration industrial effluents. The present study concludes that the effect of industrial effluents changes the activities of acid and alkaline phosphatases in *Arius* maculatus.

Keywords: Industrial effluents, ACP,ALP, *Arius maculatus*

1.INTRODUCTION

Man uses estuaries as a source of food, for transport, recreation and disposal of wastes. These activities have in many cases, altered their physical, chemical and biological characteristics and often conflicted with one another. As more of the human population lives near the coastal, pressures on the limited resources of estuaries are increased. Toxic contamination has led to closure of several estuarine fisheries, including the striped bass fishery in New York. The Southeast-coast estuary in India, has also been subjected to serious industrial pollution problems for more than a decade resulting in a reduction in diversity and abundance of living marine organisms.

Estuaries receive inputs of contaminants from a variety of sources. The most direct inputs are from point sources, that is, pipe discharges from power plants which use ambient water for cooling, can stress estuarine organisms through temperature changes, entrainment and impingement the toxicity of the biocide chlorine. The waste water also contains a wide variety of inorganic and organic pollutants.

Corresponding author: *Dr.* ***T. Kumaresan*** *Assistant Professor, Department of Zoology,Annamalai University, Annamalainagar-608002, Tamilnadu*

Some of the most heavily industrialized areas of the world are situated near the estuaries which are particularly at risk from metallic infusion. Industrial discharges containing toxic and hazardous substances, including heavy metals (Woodling *et al.,* 2001) contribute tremendous to the pollution of aquatic ecosystem. Hence, the problem of toxicity in marine organisms from natural causes or manmade pollutants is coming into critical focus.

There are five types of pollutants that affect the estuarine and coastal resources as under: (i) bacterial infection due to the discharge of untreated domestic sewage along with storm water runoff from the cities which can cause epidemics of water borne diseases such as dysentery, typhoid, cholera, poliomyelitis and hepatitis (Custodio, 2010); (ii) industrial wastes that deplete dissolved oxygen (Mondal and Singh, 2011); (iii) toxic chemicals of industrial wastes and land runoff comprising, pesticides and herbicides (Sankaran *et al.,* 2009) interfering with the metabolism; (iv) fertilizer's runoff that tend to stimulate growth of some life forms and cause eutrophication (Mondal *et al.,* 2011) and (v) inert chemical sediments and counteracting with the delicate benthos of the estuary (Sankar *et al.,* 2010).

The priority list of pollutants compiled by the Environmental Protection Agency of United States contains the eight more widespread heavy metals such as arsenic, chromium,

cadmium, copper, lead, mercury, nickel and zinc (Moore and Ramamoorthy, 1984), of which the last six are generally known as "toxic heavy metals" (Dara, 1997), while cobalt, manganese, molybdenum and selenium as "essential heavy metals." According to George (1987) aluminium, arsenic, antimony, cadmium, chromium, copper, iron, manganese, mercury, lead, uranium and zinc are more toxic to the aquatic system.

Fish is an important as well as cheap source of animal protein and hence farming of fish has become imperative to meet the growing demand for protein worldwide. In recent years in India too, intensive aquaculture practices are on the increase, and aquaculture programmes at present largely depend on riverine and estuarine seed resources (Meehan, 2002). Wide variable effects of different toxicants on a given species (Dalela, 1977; Savinov *et al.,* 2003) and the variable effects of the given toxicants on different species (Ruangsomboon and Wongret, 2006) warrants, no generalization be made on the toxic effects of disposed industrial wastes surrounding aquatic environment.

Fishes are sensitive to contaminants of the water and pollutants may damage certain physiological and biochemical processes when they enter the organs of the fish (Tulasi *et al.,* 1992; Kumaresan and Karuppasamy, 2011b). The fishes which are largely being used for the assessment of the quality of the aquatic environment and can cause bioindicator of environmental pollution (Dautrempuits *et al.,* 2004; Lopes *et al.,* 2001).

2.MATERIALS AND METHODS

Fish *Arius maculatus* was collected from three different stationsat Uppanar estuary and they were immediately transferred to ice box.From the experimental station of Uppanar estuary, fish were caught either using cast net or hand lines. Cast net are made of nylon. The mesh varies from 10 to 15 mm. Hand lines with mono filament and hooks were used with prawn or mussel as bait for the capture of *Arius maculatus*. The fish were transported to the laboratory and maintained in the same way as the control fish collected from Perumal Lake. The water was renewed once in two days.

rocurement of fish

Live specimens of *Arius maculatus* with an average length of 8.5 ± 0.50 cm and weight of 15.0 ± 0.5 g were collected from Uppanar brackish water by operating cast net. The fish were acclimatised in the aquaria of 120 litres capacity containing well aerated sea water (salinity 28 ‰; pH 7.69; oxygen content 4.32 mg/l) and water temperature (32.6°C) for a period of one week prior to experiment. During acclimatization, the fish were fed withchopped prawn and clams. Food was withheld one day before the' commencement of the experiment. The water was changed along with waste feed and faecal matter every 24 hours. Fish collected from Perumal lake (Plate 1A) were used as control and Uppanar brackishwater area was selected as experimental site (Plate 1B).

Acute Toxicity Test

The raw and partially treated effluent was collected from the discharging point of industries surrounding the Uppanar eaturry for acute toxicity test. In the acute toxicity bioassay, mortality could be observed within a short period. LC_{50} was calculated by the following method of Finney (1978) to observe mortality and behavioural response of the test fish, *Arius maculatus* on exposure to effluents of different concentrations.

Static acute toxicity was employed to evaluate the adverse effects of industrial effluents surrounding the Uppanar estuary on the fish, *Arius maculatus* under standardised laboratory conditions.

Food was withheld one day before the toxicity test with a view to avoid the possible change in the toxicity of the pollutants after addition of the effluent into the test tank with 100 litres of sea water having 10 fishes. Mortality was recorded after 24, 48, 72 and 96 hr and five replicates were maintained simultaneously for the purpose. Fishes showing respiratory and lack of response to tactile stimuli were considered, nearing dead and removed immediately. Percentage mortality was calculated and the values were subjected to Probit analysis (Reddy *et al.,* 1992). Confidential limits (upper and lower) of the Regression coefficient with Chi - square test were calculated.

Design of sublethal toxic study

Sublethal studies are helpful to assess the response of the test organism to stress caused by the effluents. Based on acute toxicity test two sublethal concentrations (2% and 6%) on *Arius maculatus* were derived and used as the experimental concentrations. Sublethal of safe level concentration were derived from 96 hr LC_{50} value.

In the present study 2% (1/50) and 6% (1/30) dilution (in *Arius maculatus*) of the 96 hr LC_{50} were selected as sublethal concentrations. The experimental fish were exposed in each concentration for a period of 7, 15 and 30 days. A control batch corresponding to each test group was maintained simultaneously.

Estimation of Acid and Alkaline Phosphatase

Acid and alkaline phosphatase were analysed following the procedure of Tenniswood *et al.* (1976).

Acid Phosphatase

100 mg of wet tissues (gill, muscle, liver and intestine) were homogenized in glass homogenizer using 10 ml of ice cold double distilled water. Six test tubes each containing 0.5 ml of the substrate solution (P- nitrophenyl phosphate) and 0.5 mlof 0.1 N citrate buffer were kept in a water bath maintained at 37°C for half an hour and 1 ml of tissue extract was added to each test tube and incubated as before. The reaction was arrested in the extract by adding 3.8 ml of 0.1 N sodium hydroxide. The colour formed at the end of the reaction was

read at 415 nm in Spectronic 21 Spectrophotometer. The acid phosphatase activity is expressed in µ mole PNP/mg protein/hr in wet tissue.

Alkaline Phosphatase

Similarly 100 mg of wet tissues (gill, muscle, liver and intestine) were homogenized as before and six test tubes each having 0.5 ml of the substrate solution (P - nitrophenyl phosphate) and 0.5 ml of glycine buffer were incubated for 5 minutes in a water bath maintained at 37°C and 1 ml of tissue extract was added to each. The test tubes with the tissue extract were then kept in water bath at 37°C for half an hour and the reaction was arrested in the extract by adding 10 ml of 0.02 N sodium hydroxide. The colour formed at the end of the reaction was read at 415 nm and expressed in µ mole PNP/mg protein/hr in wet tissue

3.RESULTS

Activity of acid phosphatase in various tissues

The levels of acid phosphatase activity in the gill, muscle, liver and intestine of *Arius maculatus* exposed to the sublethal concentrations of the effluent illustrated (Table1), the following trends.

Muscle

The activity of Acid phosphatase in the muscle of the control sample (muscle) revealed a mean value of 1.330, 1.295 and 1.138 µmole PNP/mg protein/hr for the exposure periods of 7, 15 and 30 days respectively (Table 31). In the lower sublethal concentration (2%) at different exposure periods showed a declined value of 1.052 (for 7 days), 0.963 (for 15 days) and 0.538 µmole PNP/mg protein/hr (for 30 days). Similar trend was observed at higher sublethal concentration (6%) and the values were 0.965 (for 7 days), 0.438 (for 15 days) and 0.345 µmole PNP/mg protein/hr for 30 days (Table 1).

Intestine

The activity of Acid phosphatase in the intestine of the control sample revealed a mean value of 9.793, 9.765 and 9.703 µmole PNP/mg protein/hr for the exposure periods of 7, 15 and 30 days respectively (Table 1). In the lower sublethal concentration (2%) for the different exposure periods showed a lower value of 7.612 for 7 days, 7.590 for 15 days and 7.090 µmole PNP/mg protein/hr for 30 days. Similar trend was also observed at higher sublethal concentration (6%) and the values were 7.122 for 7 days, 6.903 for 15 days and 6.400 for 30 days µmole PNP/mg protein/hr (Table1).

The levels of Acid phosphatase activity in the tissue of field specimen (gill, muscle, liver and intestine) of *Arius maculatus* showed a decreased value of 0.410, 0.803, 1.623 and 5.390 µ mole PNP/mg protein/hr with respect to the control value (3.100, 1.483, 5.603 and 9.590 µmole PNP/mg protein/hr) (Table1).

The Two way ANOVA for Acid phosphatase activity in all the tissues showed significant values at 1% level between the exposure periods and the two sublethal concentrations (Table 3.56). The One way analysis of variance, showed a significant value at 1% level between the control and two sublethal concentrations (Table1). The intestine showed an insignificant value between the periods of exposure and the multiple range test on gill, muscle and liver showed a significant value at 5% level particularly for 7 and 30 days of exposures (Table 3.58). A significant value in the Acid phosphatase at 1% level in the field samples was observed between the control and test samples (Table1).

Table 1 Activity of acid phosphatase activity (µ mole PNP/mg protein/hr) in the tissues of *Arius maculatus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the effluent

Period of exposure	Tissues	Concentration level (% 96 hr LC_{50})		
		Control	2%	6%
7 Days	Muscle	1.330 ± 0.026	1.052 ± 0.022	0.965 ± 0.013
	Intestine	9.739 ± 0.028	7.612 ± 0.025	7.122 ± 0.039
15 Days	Muscle	1.295 ± 0.024	0.963 ± 0.017	0.438 ± 0.046
	Intestine	9.765 ± 0.069	7.590 ± 0.026	6.903 ± 0.046
30 Days	Muscle	1.138 ± 0.017	0.538 ± 0.040	0.345 ± 0.051
	Intestine	9.703 ± 0.017	7.090 ± 0.026	6.400 ± 0.037

Values are mean of five replications ± SD

Activity of alkaline phosphatase in various tissues

The levels of alkaline phosphatase activity in the gill, muscle, liver and intestine of *Arius maculatus* exposed to sublethal concentrations of the effluent illustrated the following trends (Table 2).

Muscle

The levels of Alkaline phosphatase activity in the muscle of the control sample revealed a value of 3.683, 3.655 and 3.505 µmole PNP/mg protein/hr for the exposure periods of 7, 15 and 30 days respectively (Table 2). In the lower sublethal concentration (2%) at different exposure periods showed a lower value of 3.410 (for 7 days), 3.195 (for 15 days) and 3.068 µmole PNP/mg protein/hr (for 30 days). Similar trend was shown at higher sublethal concentration (6%) and the values were recorded as 3.198 for 7 days, 2.280 for 15 days and 2.213 µmole PNP/mg protein/hr for 30 days (Table 2).

Intestine

The levels of Alkaline phosphatase activity in the intestine of the control sample revealed a value of 8.768, 8.385 and 8.215 µmole PNP/mg protein/hr for the exposure periods of 7, 15 and 30 days respectively (Table 2). In the lower sublethal concentration (2 %) at different exposure periods showed a lower value of 7.715 for 7 days, 7.665 for 15 days and 7.425 µmole PNP/mg protein/hr for 30 day). Similar trend was repeated at higher sublethal concentration (6%) and the values were 7.320 for 7 days, 6.990 for 15 days and 6.385 µmole PNP/mg protein/hr for 30 days (2).

The levels of alkaline phosphatase activity in the tissues of field specimen (gill, muscle, liver and intestine) of *Arius*

maculatus showed a decreased value of 3.092, 1.485, 4.803 and 7.188 µmole PNP/mg protein/hr with respect to the control group (4.090, 4.113, 7.188 and 8.248 µmole PNP/mg protein/hr) respectively (Table 3.54 and Graph 28).

The Two way ANOVA for Alkaline phosphatase activity showed a significant value at 1% level for the periods of exposure and the two sublethal concentrations. Similarly the One way analysis of variance confirmed a significant value at 1% level between the control and the two sublethal concentrations. The Multiple range test also evidenced a significant value at 5% level. However, the muscle and intestine showed an insignificant value between the various periods of exposure. The multiple range test for the gill and liver showed a significant value at 5% level for 7 and 30 days of exposure . A significant value in the alkaline phosphatase activity at 1% level in the field samples were observed between the control and test samples.

Table 3.50 Analysis of alkaline phosphatase activity (µ mole PNP/mg protein/hr) in the tissues of *Arius maculatus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the effluent

Period of exposure	Tissues	Concentration level (% 96 hr LC_{50})		
		Control	2%	6%
7 Days	Muscle	3.683 ± 0.033	3.410 ± 0.093	3.198 ± 0.017
	Intestine	8.768 ± 0.017	7.715 ± 0.013	7.320 ± 0.082
15 Days	Muscle	3.655 ± 0.013	3.195 ± 0.013	2.280 ± 0.348
	Intestine	8.385 ± 0.013	7.665 ± 0.013	6.990 ± 0.265
30 Days	Muscle	3.505 ± 0.013	3.068 ± 0.238	2.213 ± 0.258
	Intestine	8.215 ± 0.013	7.425 ± 0.013	6.385 ± 0.013

Values are mean of five replications ± SD

4.DISCUSSION

Aacid and alkaline phosphatrase activity

Phosphatase are known to play an important role in acute energy crisis (De Duve, 1963; Reddy and Rao, 1986) and serve as markers for the evaluation of diseases or pathological conditions (Murti *et al.,* 1984). Acid and alkaline phosphatases are known as an inducible enzymes and their activity goes up when the tissues were intoxicated with a variety of toxicants. At the time of intoxication, the enzymes begin to counteract the toxic effect (Leland, 1883). These activities also serve as diagnostic tool to assess toxicity stress of chemicals in the living organisms (Harper, 1991). Acid phosphatase is a lysosomal enzyme, which hydrolyses the ester linkage of phosphate ester and helps in autolysis of the cell after its death (Novikoff, 1961). Alkaline phosphatase is a brush border enzyme, splits various phosphate esters at an alkaline pH and mediates membrane transport (Shakoori *et al.,* 1992). Generally phosphatase activity is carried out in clinical and ecotoxicological studies as it serves as a good indicator of intoxication because of its sensitivity to metallic salts (Boge *et al.,* 1992). In view of this, the present study has been designed to evaluate the acid and alkaline phosphatase activities in the selected tissues of fish, *Arius maculates* treated with sub lethal concentration of effluents.

In the present study the alkaline phosphatase was found to be inhibited in the gill, muscle, liver and intestine exposed to the sublethal concentrations and also to the raw effluent for 30 days. The inhibition may be due to altered membrane permeability which is brought about by the binding of the heavy metal ions present in the effluent to the enzyme configuration. The present results are coincide with the report of Karuppasamy (2000a) in *C. punctatus* treated with phenyl mercuric acetate. Furthermore, the inhibition of alkaline phosphatase activity may have hampered glycogen and lipid metabolisms and disrupted the transfer of these catabolites of the hepatic cells and it falls in line with the report of Chhaya *et al.* (1997). Moreover the inhibition of the activity of alkaline phosphatase could lead to disrupt the process of oxidative phosphorylation (Dalela *et al.,* 1980).

Acid phosphatase is a lysosomal enzyme which hydrolyses phosphorous esters in an acid medium (De Duve, 1963). It is non - specific in its site of action as well as to substrates (esterified sugars). Fish exposed to the two sublethal concentrations and raw effluent in the field showed inhibition of acid phosphatase. In the gill tissue the inhibition may be due to the presence of labilizers in the effluent.

In the liver, the inhibition of enzyme may be due to disruption in the membrane permeability of the hepatic cells which ultimately affects other functions of the liver (Dalela *et al.,* 1980). In the case of intestine, the activity of acid phosphatase showed massive inhibition in all the concentrations. The reduction in the activity possibly indicates an impaired nutrient assimilation and absorption in the intestinal lumen (Hinton and Koening, 1975). The activity of acid phosphatase may be due to the alteration in the membrane structure caused by toxic metals or organic compound which might have caused leakage in the lysosomal membrane thereby releasing all hydrolytic enzymes as reported by Hossain and Dutta (1986), Karuppasamy (2000a). The significant inhibition of both alkaline and acid phosphatases possibly hampered the active transport across the muscle fibre leading to impaired cellular metabolism (Sahana *et al.,* 1986).

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