ASIAN JOURNAL
ISSN: 0976-3376

Available Online at http://www.journalajst.com

Asian Journal of Science and Technology
Vol. 1, Issue 11, pp.070-075, October, 2011

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

IMPACT OF INDUSTRIAL EFFLUENTS ON SOME BIOMARKER ENZYMES IN SELECTED TISSUES OF *ARIUS MACULATES* FROM UPPANAR ESTUARY, CUDDALORE DISTRICT, TAMILNADU

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Received 25th May, 2011; Received in revised form; 18th June, 2011; Accepted 19th July, 2011; Published online 30th October, 2011

ABSTRACT

The present study is aimed to investigate enzyme studies in the gill and liver tissue of estuary fish *Arius maculates* exposed to sublethal concentration of efflunts. In the present study, the activity of Acid phosphatase (ACP), alkaline phosphatase(ALP), Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) were observed in the gill and liver tissue. During the sublethal concentration of effluents, the Acid phosphatase, alkaline phosphatase, Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) were increased in the liver tissue. This result indicates the concentration dependent enzyme alterations in both gill and liver tissue of *Arius maculates.*

Key words: Effluents Bioenzyme Arius *maculates* Gill Liver

INTRODUCTION

The pollution of ecosystems by heavy metal is a worldwide problem. Industrialization, Population growth and the resulting waste material lead to pollution of the environment in air, water, soil and living organisms. The chief sources of the waste matter are automobile emissions, industrial effluent, household chemicals released into sewage system etc. These chemicals entering the ecosystem affect man, animal life, plant life and materials and exert serious health and ecological problems (Ober *et al*., 1987). Effluents are often present at elevated concentrations in aquatic ecosystems due to the rapid growth in population (Biney *et al.*, 1994; Seymore, 1994), the increase in industrialization (Biney *et al.*, 1994; Pelgrom *et al.*, 1994), the increase of urbanization and socio-economic activities, exploration and exploitation of natural resources, extension of irrigation and other modern agricultural practices and the lack of environmental regulations (Biney *et al.*, 1994). Increased application of chemicals, pesticides, herbicide and fungicides to ameliorate the agricultural crop from the damage caused by the pest resulted in a corrollary problem of pollution of freshwater ponds, lakes and rivers as these pesticides washed away (either by rain or draining of pesticides with excess water after irrigation) finally into the water bodies affecting the ecosystem and its fauna and flora including fishes. Water pollution by discharging of effluents from various industries had posed a serious problem, in many rivers and ponds and it exerts harmful effects on the inhabitants especially fishes, tadpoles and aquatic insects (Balasubramanian *et al*., 1986). Water pollution occurs due to the presence of dissolved inorganic and organic materials such as proteins, fats, carbohydrates and other substances found in domestic and industrial water and physical factors such as turbidity, colour, temperature, associated radioactivity etc. Both metals and potential pollutants are affecting the *ichthyofauna* either directly or indirectly (Kumar and Pant, 1981). Most of the industries discharge their waste water into water courses without proper treatment which cause changes in the physical, chemical and biological characteristics of water. The release of untreated industrial effluents into aquatic systems seriously affect the aquatic biota and their production (Srinivas *et al*., 1984). The effluents released by various industries are causing a lot of problems and their disposal involves a complicated task. Many industries do not have proper facilities to treat the effluents and about 68.5 cubic million litres of industrial effluents are discharged as such into the environment. Alterations in the chemical composition of the aquatic environment usually affect behavioural and physiological activities of the inhabitants, particularly the fish population (O' Brien, 1967). Uppanar estuary is a good source of fisheries, particularly mullets and catfish. Extensive beds of the edible oyster, silver bellies, horse mackerels, gizzard shad, whiting, *Etroplus, Therapon, Gerres, Chanos chanos, Elops saureus, Polynemus, Teuthis* and jewfish are inhabiting in smaller numbers almost throughout the year. Prawns and crabs form about 5.0 and 2.5%, respectively of the total catches. The town of Cuddalore is endowed with three rivers: the uppanar, Gaddilum and Ponnai River, of which the Uppanar has been

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degraded as a 'black spot' in spite of the fact that it was once known as the centre piece of the 'garden town'. Industrial effluents consist of a variety of substances of either known or unknown lethality. Most water sources will receive a number of industrial effluents either directly or indirectly. 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). The fishes which are largely being used foe the assessment of the quality of the aquatic environment and can cause bioindicator of environmental pollution (Dautrempuits *et al*., 2004; Lopes *et al*., 2001).

The gills are the first target organ in the toxic accumulation because they are directly in contact with water (Dubale andShah,1979).Fish gills, which serve as the primary uptake site in fish for metals, represent the most important targets when exposed to elevated levels of ambient metals (Newman and Jagoe, 1994). Liver is one of the most multifaceted and active organs in higher animals. In a vertebrate body, the liver is the most important target organ as it is the chief metabolic and detoxification center (Bhattacharya and Mukherjee, 1976). It is the site for numerous and varied metabolic activities, including synthesis of bile which contains bile salts, bile pigments, cholesterol and lecithin.

MATERIALS AND METHODS

Procurement 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 River by operating cast net. The fish were acclimatized in the aquaria of 120 liters capacity containing well aerated sea water (salinity 28 ppt; 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 on chopped 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 were used as control and Uppanar brackish water area. Live fish were also collected from the experimental station, which carried effluents or discharges from the surrounding industries. The fish were transported to the laboratory and maintained in the same way as the control fish collected from Uppanar river. The water was renewed once in two days.

Experimental design: Fishes were divided into two equal groups each comprising of 20 fishes. Each group was kept in separate plastic tanks. The first group was kept as negative control; the fishes were maintained in water containing normal water without any treatment. The fishes of two groups were exposed to a sub-lethal concentration of effluent s (1% and 3 %) added in the water for 30 days. Solutions were renewed once daily after exposure period, animals (n=20/group) were sacrificed and the tissues were removed, homogenized and stored at −80 °C for further biochemical analyses. After experiment, the fish each from the respective experimental as well as control groups were sacrificed. The gill and liver were isolated from the fish and used for various study. Solutions were renewed once daily after exposure period, animals (n=20/group) were sacrificed and the gills and liver tissues were removed, homogenized and stored at −80 °C for enzyme analyses (Acid and alkaline phoshatase tenniswood *et al*., 1976 and Glutamate Oxalateacetate Transaminase, Glutamata Pyruvate Transaminase, Reitman and Frankel, 1957).

Statistical analysis: Data from the present studies were subjected to Standard deviation and the significance of difference obtained was assessed by ANOVA for the study between the various periods of exposure as well as concentrations. The significant difference within the groups of exposure periods and within the concentrations were separately assessed by ANOV (Multiple range test).

RESULTS

Activity of Acid phosphatase in various tissues: The activity of Acid phosphatase in the gill of the control sample revealed a value of 3.917 µmole PNP/mg protein/hr in the control gill for the exposure periods of 30 days. At the lower sublethal concentration (1%) for the different exposure periods showed a decline in the values as 2.162 µmole PNP/mg protein/hr (for 30 days). Similar trend was repeated at higher sublethal concentration (3%) as well and the values were 1.59511 µmole PNP/mg protein/hr (for 30 days). The activity of Acid phosphatase in the liver of the control sample revealed a mean value of 6.493 µmole PNP/mg protein/hr for the exposure periods of 30 days. In the lower sublethal concentration (1%) for the different exposure periods showed a lesser value of 2.883 µmole PNP/mg protein/hr (for 30 days). Similar trend was recorded at higher sublethal concentration (3%) and the values were 2.393 µmole PNP/mg protein/hr for 30 days.

Activity of alkaline phosphatase in various tissues: The levels of alkaline phosphatase activity in the gill and liver of *Arius maculatus* exposed to sublethal concentrations of the effluent for 30 days. The activity of Alkaline phosphatase in the gill of the control sample revealed a mean value of 5.065 µmole PNP/mg protein/hr for the exposure period of 30 days. In the lower sublethal concentration (1%) for the different exposure periods showed a lower value of 4.235 µmole PNP/mg protein/hr (for 30 days). Similar trend was shown at higher sublethal concentration (3%) as well and the values were 3.838 µmole PNP/mg protein/hr for 30 days.

The levels of Alkaline phosphatase activity in the liver of the control sample revealed a value of 7.493 µmole PNP/mg protein/hr for the exposure periods of 30 days. In the lower sublethal concentration (1%) at different exposure periods showed a lower value of 6.233 µmole PNP/mg protein/hr (for 30 days). Similar trend was shown at higher sublethal concentration (3%) as 5.950 µmole PNP/mg protein/hr for 30 days.

Activity of Glutamate Oxaloacetate Transaminase (GOT) in various tissues

The level of Glutamate oxaloacetate transaminase activity in the gill and liver of *Arius maculatus* exposed to sublethal concentrations of the effluent. The activity of Glutamate oxaloacetate transaminase in the control sample (gill) recorded the mean values of 204.340 Units/100 mg of wet tissue. But the activity in the gill at 1% sublethal concentration revealed an increase in the mean value 520.150 Units/100 mg of wet tissue (for 30 days). But at 3%, the activity for 30 days further

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

Exposure Periods	Concentration levels (% 96 hrs LC_{50})			
	Tissues	Control	1%	3%
30 Days	Gill	3.917 ± 0052	2.162 ± 0.080*	1.595 ± 0.024*
	Liver	6.493 ± 0.028	2.883 ± 0.039*	2.393 ± 0.028*

Values represents mean ± SE. * significance 5% level of significance (ANOVA)

Table 2. Activity of Alkaline phosphatise (µ mole PNP/mg) in the tissues of *Arius macculatus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the effluent

Exposure Periods	Concentration levels (% 96 hrs LC_{50})			
	Tissues	Control	1%	3%
30 Days	Gill	5.065 ± 0.013	4.235 ± 0.013*	3.838 ± 0.129*
	Liver	7.493 ± 0.017	6.233 ± 0.017*	5.950 ± 0.187*

Values represents mean ± SE. * significance 5% level of significance (ANOVA)

Table 3. Activity of glutamate oxaloacetate transaminase (GOT (units/100 mg of tissues)) in the tissues of *Arius macculatus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the effluent

Exposure Periods	Concentration levels (% 96 hrs LC_{50})			
	Tissues	Control	1%	3%
30 Days	Gill	204.340 ± 0.034	520.150 ± 0.026*	660.423 ± 0.026*
	Liver	56.317 ± 0.024	60.445 ± 0.034*	76.445 ± 0.034*

Values represents mean ± SE. * significance 5% level of significance (ANOVA)

Table 4. Activity of glutamate pyruvate transaminase (GPT (units/100 mg wet tissue)) in the tissues of *Arius macculatus* exposed to sublethal concentrations (% 96 hr

Exposure Periods	Concentration levels (% 96 hrs LC_{50})			
	Tissues	Control	1%	3%
30 Days	Gill	164.085 ± 4.042	183.155 ± 2.522*	250.390 ± 25.830*
	Gill	164.085 ± 4.042	183.155 ± 2.522*	250.390 ± 25.830*

Values represents mean ± SE. * significance 5% level of significance (ANOVA)

increased its values as 660.423 Units/100 mg of wet tissue respectively.The activity of Glutamate oxaloacetate transaminase in the liver of the control sample revealed a mean value of 56.317 Units/100 mg of wet tissue. At 1% sublethal concentration revealed an increased mean value of 60.445 Units/100 mg of wet tissue (for 30 days). Further at 3%, the activity for 30 days increased its value as 84.268, 64.405 and 76.445 units/100 mg of wet tissue respectively.

Activity of Glutamate Pyruvate Transaminase (GPT) in various tissues: The level of Glutamate pyruvate transaminase activity in the gill and liver of *Arius maculatus* exposed to sublethal concentrations of the effluent. The activity of Glutamate pyruvate transaminase in the gill of the sample revealed a mean value of 164.085 Units/100 mg of wet tissue. But the Glutamate pyruvate transaminase activity in the gill at 1% sublethal concentration revealed an enhanced (mean) value 183.155 Units/100 mg wet tissue (for 30 days). Furthermore, at 3%, the activity of the enzyme during the exposure of 7, 15 and 30 days increased its level as 250.390 Units/l00 mg of wet tissue. The activity of Glutamate pyruvate transaminase in the liver of the control sample showed a mean value of 21.425 Units/100 mg of wet tissue. At 1% sublethal concentration, it revealed an enhanced mean value of 32.925 Units/l00 mg wet tissue (for 30 days). Similarly at 3% the activity of the enzyme during the exposure of 30 days increased as 72.255 Units/l00 mg wet tissue

DISCUSSION

The toxic medium may cause injury to the organisms and the damaged tissues shall dysfunction, which result in altered enzyme activity. Thus enzyme bioassay can provide diagnostic tool to assess a change or damage caused to organism due to administration of heavy metals(Harper *et al*., 1978). 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 enzymes, splits various phosphate esters at an alkaline pH and mediates membrane transport (Smith *et al*., 1983).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 and liver exposed to the sublethal concentrations of 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. 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 Jignasa Dalela *et al* ., (1980). 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). 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).

In the present study, the level of acid and alkaline phosphatase activity increased in the gill, liver and kidney tissue of *Arius maculates* when exposed with effluents. This result suggests that increased level of acid and alkaline phosphatase might be due to the toxicity effect of cadmium chloride. These increased activities can be attributed to the destruction of cell membrane and lysosomes, which in turn leads to hepatic damage. The increased level of acid phosphatase activity suggested the involvement of lysosomes in metal toxicity. Alkaline phosphatase is involved in the synthesis of nuclear protein, nucleic acid and phspholipids. These enzymes are associated with transmembrane transport mechanism, ion transport, maintenance of ionic strength and cell growth in the organ (Moog, 1946). A significant increase of acid phosphatase and alkaline phsophatase activities were reported in *Cirrhinus mrigala* exposed to lead acetate [Ramalingam *et al*., 2002]. Changes in acid and alkaline phsophatase activities were observed in *Channa punctatus* exposed to mercuric chloride (Jeelani and Shaffi, 1989). James *et al*., (1992) reported that the increased in acid phosphatase activity has been reported in the gill, liver tissues of *Oreochromis mossambicus* exposed to heavy metal. Ramesh *et al.* (1993) reported the level of acid phosphatase increased in the gill and liver tissue of *Oreochromis mossambicus* exposed to nickel electroplating effluent. Mumtaj, (1986) reported that a significant increase in the level of acid and alkaline phosphatase activities in the *Channa punctatus* exposed to mercuric chlorides.

Transaminases are enzymes, which play vital role in the metabolism of non-essential amino acids. These enzymes are commonly employed as diagnostic tools in the assessement of liver damage in clinical practice (Goetz, 1980) and cellular damage of vital organs when treated with toxicants (Moss *et al*., 1986; Sankarsamipillai and Jagadeesan, 2005). During cellular damage three enzymes are leaked into the serum and hence elevation of the activities of these enzymes in serum is considered as a sensitive indicator of even minor cell damage because the levels of these enzymes exceed those of extra cellular fluid by more than three fold increase (Moss *et al*., 1986). In the present study, the level of GOT and GPT activity increased in the gill and liver tissues of *Arius maculates* exposed to effluents for 30 days. This result may be due to necrosis, which causes increase in the permeability of cell membrane resulting in the damage of tissues. Similar results made reported by Hwang and Wang [2001]. They reported that the level of AST and ALT activities are increase due to heavy metals in chronic liver damage. The activity of AST and ALT can be used to indicate the tissue damage of liver and kidney (Nemsoc and Boross, 1982). Hori *et al*., (2006) have observed the level of AST in the liver tissue of *Brycon cephalus* exposed with phenol. Alteration in the activity of AST and ALT will be reflected nitrogen metabolism on the energy yielding TCA cycle(Beyer *et al*., 1996). Gupta and Paul, (1978) and Palanivelu, (2005) reported that the Increases in GOT and GPT levels after 15 and 60 days of exposure to monocrotophos also indicate liver damage, since increases in the activities of blood transaminase have been attributed to tissue damage, particularly the liver of *O.mossambicus*. Sastry and Sharma (1980) observed an increase in GOT and GPT in the blood of *C. punctatus* following the treatment of mercuric chloride. Liver damage has also been observed in *Clarias batrachus* following chronic exposure of carbofuran. It is generally accepted that an increase of these enzyme activities in the extracellular fluid or plasma is a sensitive indicator of even minor cellular damage (Palanivelu,2005). Agrahari (2007) reported that the measurement of transaminase and phosphatases activities in fish of *Channa punctatus*. Vutukuru *et al.*, (2007) reported that Significant increase in transaminases (AST and ALT) activity in fish exposed to arsenic could be due to possible leakage of enzymes across damaged plasma membranes and/or the increased synthesis of enzymes by the liver in *Labeo rohita*. Jen lee et bal. (2003) demonstrated an increased activity of ALT and AST and hepatocyte ultra structure of common carp, *Cyprinus carpio* after gallium exposure. Several investigators also reported that heavy metal intoxication showed a significant increase in AST and ALT activities in the liver tissue of animals (Rana *et al*.,1996; Khandelwel *et al*.,2002). The hepatocellular necrosis is generally associated with alterations in the liver tissue and serum (Zimmerman,1978). The elevated level of AST and ALT indicate stopped up transmutation where feeding of amino acids into the TCA cycle occurs in order to cope up the energy crisis during cypermethrin toxicity [Philip *et al*., 1995]. The significant increase of these enzyme in the tissues seems to indicate possible dysfunction, taking place in the tissues of animals (Casilla *et al*., 1983). Sharma [1999] has reported that similar pattern of increase in AST and ALT in the liver tissue of *Channa Batrachus* exposed to pesticides. Mary chandravathy and Reddy (1991) have reported that the elevation of AST and ALT in the gill and brain tissues of *Anabas scandens* exposed to lead nitrate. Usha and Raj (1993) have reported the increase in the lever of AST and ALT in the animals exposed to vanadium. Mukhopadhyay *et al*., (1982) have observed that an increase level of AST and ALT activities in the liver tissue of *Clarius batrachus* exposed to carbofuron. Similar results observed by Ganguli *et al*., (1997). They reported that level of these enzyme increase in the gill, liver and kidney tissues of *Anabas testudineus* exposed to lindene, and furandan.

Acknowledgement

The authors are thankful to Professor and Head, Department of Zoology, Annamalai university for providing necessary lab facilities to carry out the work successfully.

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