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Studies on enzyme activities in heavy metal toxicated fish *Oreochromis mossambicus* (Peters) at Pallikaranai wetland ecosystem, Chennai, Tamil Nadu, India

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***Abstract*: Wetlands provide habitat for fish and wildlife, including endangered species. Pallikaranai wetland is only surviving wetland ecosystem in the South India. Chennai City 60% of wastes is dumped in the ecologically sensitive areas of Pallikaranai wetland. These wastes have highly toxicated heavy metals and affected the physico - chemical parameters in surrounded water and metabolic activities in fishes. Present study estimated the enzyme activities of different organs (gill, liver, muscle and intestine) for toxic induced (toxicated water sample collected from Pallikaranai wetland ecosystem) freshwater fish *Oreochromis mossambicus* (Peters) in two different sublethal concentrations (1% and 3%). As the result, the enzyme profile in the four tissues *viz.*, gill, muscle, liver and intestine showed the decrease values in the activity of ALP and ACP. Enhanced activity of GOT and GPT were recorded in the gill, muscle and liver in different proportions. Glycogen phosphorylase activity showed a decline in the gill, muscle and liver tissues. The overall valuation confirmed that the fish habitat stressed from the polluted water sample. So immediately we must take action to avert the environmental pollution from surrounded industries and sewage water; and save Pallikaranai wetland ecosystem for the native animals.**

***Keywords*: Pallikaranai wetland ecosystem, heavy metals, enzyme, *Oreochromis mossambicus,* environmental pollution.**

I. INTRODUCTION

Wetland plays a vital role in aquatic ecosystem. Generally wetlands called as “natural recharge zones”, because they are improving the water quality by filtering residues and nutrients from surface water (Jayanthi *et al.*, 2012), flood protection, shoreline stabilization and stream flow conservation (Suganya Balakumar and Sukanya Das, 2015). Pallikaranai wetland is only surviving wetland ecosystem in the South India (Aravindkumar *et al.*, 2014; Antony Raj, 2015). In this wetland provide habitat for fish and wildlife, including endangered species. It is home for 10 species of mammals, 115 species of birds, 21 species of reptiles, 10 species of amphibians, 46 species of fish, 7 species of butterflies, 5 species of crustaceans and 9 species of mollusk (Jayashree Vencatesan, 2007). At the same time, Chennai City produces about 3,500 tons of discarded every day and about 60% of this is dumped in the ecologically sensitive areas of Pallikaranai wetland (Jayaprakash *et al.*, 2010). The Chennai metropolis waste dump yard and Perungudi Sewage Treatment Plant occupy about 250 acres of Pallikaranai marsh land (Nikhil Raj *et al*., 2010) (Fig.1). Commonly surface water contaminated by the runoff from the waste dump and it has organic and inorganic pollutants.

Fig 1: Dumpsite of Pallikaranai wetland ecosystem (Chennai, Tamilnadu, India)

The heavy metals are most important inorganic pollutants in aquatic regions for its toxicity and bioaccumulation behavior in aquatic animals (Sehar Afshan *et al.*, 2014). Generally less amount of heavy metals occur naturally in the ecosystem with different concentration. Some heavy metals are essential to organisms for the metabolic activities and they are high in organisms become very dangerous (Siji Thomas and Abbas Mohaideen, 2015). Progressively increased the heavy metals level in aquatic ecosystem by human activities like industrial effluents, agricultural runoff, domestic untreated sewage water etc., the surpluses contain unrestricted level of heavy metals (Punitha *et al.*, 2018). The Pallikaranai wetland water contains toxic heavy metals like chromium, copper, lead, zinc, iron, cadmium and nickel (Karpagavalli *et al.*, (2012).

Fish health is index of water quality in aquatic regions for fish thoroughly avoid toxic chemicals, low oxygen dissolved in surrounded water, extreme temperature and pH and other uninvited characters for water quality (Stoner and Livingston, 1978). Fish reflects the biological effects of environmental pollution. Most of the heavy metals are easily accumulate in fish body by feeding and absorbing (Chinnadurai Kuppusamy *et al.*, 2016) and triggered metabolic activities. Usually metabolic activities in the organisms are controlled by enzymes. Slight changes in level of enzyme activity in an organism would affect the entire metabolic activities. Estimate the level of enzyme activities in an organism it can be identified the disturbance of metabolic activities (Lakshmaiah Govindu, 2016). Accordingly fishes are widely used for aquatic toxic studies attracted and attention to many researchers in all over the world (Dutta and Dalal, 2008). The fish *Oreochromis mossambicus* is one of the bioindicator of heavy metal toxicity in the water body (Thirumavalavan, 2010 & Bhuvaneshwari *et al.*, 2012)

Present study estimated the enzymes like Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT) and Glycogen Phosphorylase activities in the freshwater fish *Oreochromis mossambicus* with heavy metal toxicated water collected from Pallikaranai wetland ecosystem.

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II. METHODOLOGY

In the present investigation live specimens of *Oreochromis mossambicus* fingerlings collected from river Cauvery, at Mohanoor, Namakkal District, Tamilnadu, India. The fish were transported to the laboratory and acclimatized in the aquarium for a period of one week prior to the experiment. The experimental water collected from Pallikaranai wetland ecosystem, Chennai, Tamilnadu, India. After a week the fish was introduced into separate groups (Group I, II and III), each group had 40 fish fingerlings of sublethal concentrations of 1% and 3%. Group I: Control (tap water free from chlorine); Group II: 1% (1liter of tap water with100ml of experimental water) and Group III: 3% (1liter of tap water with 300ml of experimental water) for 7^{th}, 15^{th} and 30^{th} day of experimental periods. Fish movement was considering for throughout the experimental periods.

The experimental fish were exposed to sublethal concentrations (1% and 3%) of the effluent for 7^{th}, 15^{th} and 30^{th} day of the experimental periods. Subsequently the organs were dissected out (each period) for using enzymes analyzes. The wet tissues of gill, muscle, liver and intestine were isolated to use for estimation of ACP and ALP (by procedure of Tenniswood *et al.*, 1976), the tissues of gill, muscle and liver were used the following estimations of GOT, and GPT (by procedure of Reitman and Frankel, 1957) and Glycogen Phosphorylase (by the method of Cori and Cori, 1952).

Statistical comparisons were made using Two way ANOVA, One way ANOVA and Turkey - HSD test (Multiple range test) with Statistical package for social science (SPSS Package).

III. RESULTS AND DISCUSSION

A. Phosphatases:

In the present study the enzyme ALP and ACP was significantly decreased in the gill, muscle, liver and intestine of *O. mossambicus* for the two different concentrations intended for 7, 15 and 30 days of treatment. The enzyme alkaline phosphatase was found to be inhibited in the gill, muscle, liver and intestine exposed to the sublethal concentrations for 30 days. In liver the ALP activity maximum decreased (5.665 µmole PNP/mg protein/hr) in 3% concentration (showed Graph.1). 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 ALP activity may have hampered glycogen and lipid metabolisms and disrupted the transfer of these catabolites of the hepatic cells and it falls in line (Anusiya Devi *et al*., 2016). Decreased the level of ALP indicates the disturbance of cell organelles like endoplasmic reticulum and membrane transport system (Yacoub and Gad 2012). Generally Phosphatase is known to play an important role in acute energy crisis and serve as markers for the evaluation of diseases or pathological conditions. ALP has been reported to be of metabolic significance and catalyses the hydrolysis of phosphorus compound (monoesters, diesters and triesters) at alkaline p^H and the transfer of phosphoryl groups (transphosphorylation) to an acceptor molecule (Fernley, 1971 and Fishman, 1974). 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).

Acid phosphatase (ALP) is a lysosomal enzyme which hydrolyses phosphorous esters in an acid medium. 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 water in the field showed inhibition of acid phosphatase. In the gill tissue the inhibition may be due to the presence of stabilizers in the effluent. ALP activity also maximum decreased (2.243 µmole PNP/mg protein/hr) in the liver when compared to the control group for 30 days of treatment (showed Graph..2).

In the liver, the inhibition of the 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 the intestine, the activity of acid phosphatase showed massive inhibition at all the concentrations. The reduction in the activity possibly indicates an impaired nutrient assimilation and absorption in the intestinal lumen. 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). Chinnadurai Kuppusamy *et al.* (2016) also similar reported to our result that the activity of acid and alkaline phosphate decreased when the concentration increased.

The enhanced enzyme activity specified the accumulation of heavy metals were concerned in tissues of *O. mossambicus.* There are indications of depressed or accelerated enzyme activity in aquatic organisms exposed to low/high concentrations of metals (Hewitt and Nicholas, 1963). Similar experiments of Jackim *et al.* (1970) confirmed the consequence of metal poisoning by liver - enzymes towards the fish mortality.

B. Transaminase:

Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) activity was significantly increased in the tissues gill, muscle and liver (Graph. 2). Transaminase enzymes are indicators of environmental stress as they undergo changes in their activity and serve as markers towards the manifestation of pathology. The GOT showed a significant increase in the activity, particularly in the tissues of gill, muscle and liver of the fish caught from the polluted

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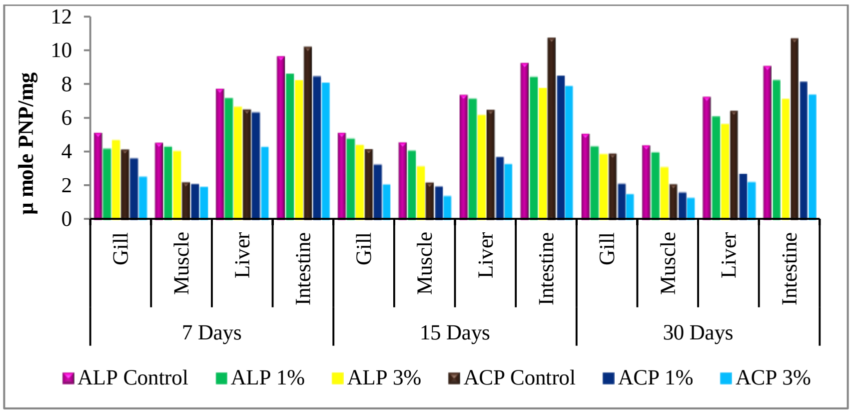
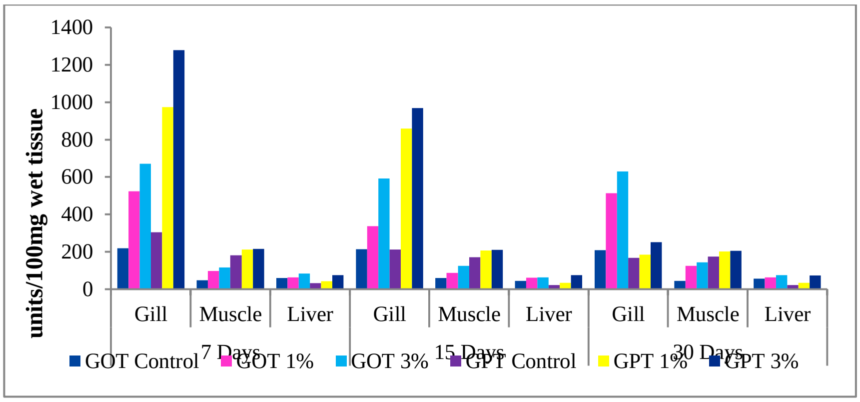
water. The present result was in accordance with Wieser and Hinterleitner, 1980 & Celine Hilda Mary *et al*. 2015. Since the transaminases activity requires pyridoxal phosphate for the conversion of amino acids into ketoacids, on the enhancement of GOT activity might be probable in the tissues which exhibited limited ammonia excretion (Anusiya Devi *et al*. 2016). In the present study the GPT activity showed a significant increase in the gill, muscle and liver exposed to the effluent (Graph. 2). The elevated transaminase activities may also be attributed as a response to provide energy to the aminase in the event of depressed oxidative metabolism. The increase in the GPT may also indicate the mobility of amino acids towards the formation of pyruvic acid as documented by Celine Hilda Mary *et al*., (2015). Enhanced transaminase activity is also suggestive of the increased catabolism of protein. It can be concluded that in fish farming, an elevation in the enzymes is indicative of acute intoxication (Kumar *et al*. 1991).

C. Phosphorylase:

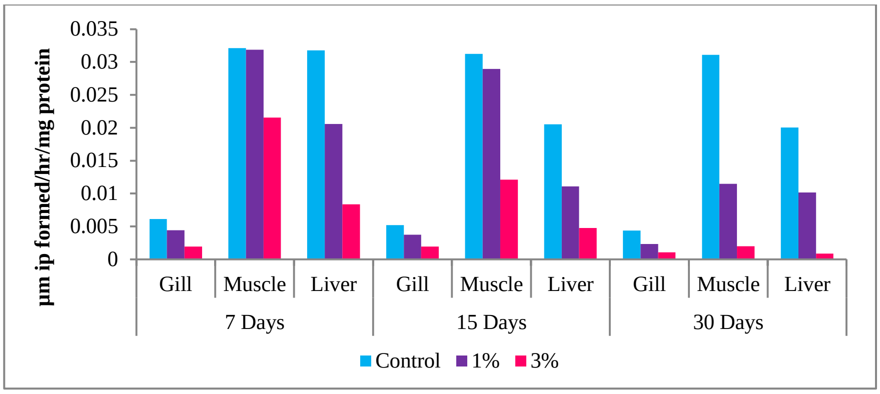
Activity of glycogen phosphorylase was decreased in all tissues and maximum decreased in liver (Graph. 3). Conformationally two forms of phosphorylases, A and B exist in the tissues and both can assume catalytically either the inactive or active confirmations. The active phosphorylase brings about the degradation of glycogen. Moreover, in vertebrates, the level of glycogen has been auto regulated by its own concentration and also by the modulation enzymes, glycogen phosphorylase and glycogen synthetase. Such regulation mechanism maintains a critical level of glycogen in the tissues which is indispensable for survival (Seeler and Weisige, 1961).The activity of glycogen phosphorylase in both the control and test groups revealed a diminution in all the tissues. Glycogen mobilization as well as its maintenance to a critical threshold level is essential to the survival of the animals. The changes in the enzyme activity in the fish tissues exposed to the effluent seem to suggest the effect of glycogenolysis probably at a lower rate than the normal tissue.



Graph 1: Analysis of Alkaline phosphatase and acid phosphatase (µ mole PNP/mg) in the tissues of *Oreochromis mossambicus* exposed to sublethal concentrations (1% & 3%) of the test sample.



Graph 2: Activity of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) (units/100 mg wet tissue)) in the tissues of *Oreochromis mossambicus* exposed to sublethal concentrations (1% & 3%) of the test sample.



Graph 3: Activity of glycogen phosphorylase (µm ip formed/hr/mg protein) in the tissues of *Oreochromis mossambicus* exposed to sublethal concentrations (1% & 3%) of the test sample.

IV. CONCLUSION

The overall assessment of water samples collected from Pallikaranai wetland ecosystem confirms that the habitat is stressed to a great extent throughout the study period. The enzyme profile in the four tissues *viz*., gill, muscle, liver and intestine of showed a decrease in the activity of ALP and ACP. Enhanced activity of GOT and GPT were recorded in the gill, muscle and liver in different proportions. Glycogen phosphorylase activity showed a decline in the gill, muscle and liver tissues.

A comprehensive ecotoxicological investigation on the Pallikaranai wetland ecosystem was pursued with reference to the water quality and the incidences of few trace heavy metals as well as the bio accumulation of these metals in the target organisms. A survey was also made to assess the number of industries, factories that are located in the vicinity of the estuary. About 12 industries were found to be located in the neighborhood of the Pallikaranai wetland ecosystem. They can simply discharge their untreated water carrying heavy metallic elements are being released per day into the estuary. The quality of the surface water in Pallikaranai wetland has not safe for aquatic and domestic life. Accordingly we must to take management actions should be taken to control the quality of the surface water of the Pallikaranai wetland ecosystem from toxicants for the native animals and number of migrated birds.

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