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Enzymatic Responses of Snake headed fish, *Channa punctatus* (Bloch,1793) Exposed to Paper Mill Effluent

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ABSTRACT

Industrial effluents are the main culprit for undesirable changes in physicochemical properties of water of fresh waterbodies. The paper mill effluent is one of the major sources of water pollution in India. The paper mill effluent are alkaline in nature and characterized by high pH, COD, BOD, TSS, temperature and low dissolved oxygen and if it releases into the natural fresh waterbodies. These pollutants directly or indirectly alter the metabolic process taking place in the body's tissues of aquatic animals. A biochemical study provides an early warning to potentially damaging alterations in stressed animals like fishes. So, the objective of this study to investigate the impact of sub lethal concentrations of paper mill effluent on the enzymes: ATPase, aspartate transaminase, alanine transaminase, acid phosphatase, alkaline phosphatase and lactate dehydrogenase of fresh water snake headed fish, Channa punctatus (Bloch). Significant fluctuationsin enzyme responses were recorded in experimental fish, however these were nither concentrations of effluent nor exposure periods dependent after exposure to paper mill effluent. Therefore enzymatic activity can be used as early biomarkers of fish heath.

Keywords:*Paper mill effluent, Channa punctatus,Enzymes, Toxicity.*

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INTRODUCTION

Environmental pollution is a major problem in both developed and developing countries like India. Anthropogenic activities (urbanization and industrialization) are producing huge amount of wastewaterswhich discharges into nearby waterbodies like rivers, streams and lakes etc. without properly treated, leading to aquatic pollution that produces deleterious effects on aquatic organisms especially fishes and human who consumed it. The industrial wastewaters contain highly toxic chemicals that may pollute the aquatic environment so the safe disposal of waste water discharges from various industries is a serious problem worldwide [1]. In India, about more than two tones industrial wastewater is discharged waterbodies annually and pollutes surface water resources [2].

The environmental pollutants may produce primary short term identifiable changes/ symptoms or secondary long term noticeable changes in exposed organism. Any alteration in the natural conditions of the aquatic environment causes several alterations and modifications in fishes. Industrial and domestic effluents are the main culprit for these undesirable changes in water quality. The industrial effluent are generally alkaline in nature and characterized by high pH, COD, BOD, TSS, conductivity, temperature and low dissolved oxygen. High pH of effluent increases the chance of solubilisation of essential elements if released in natural waterbodies and furthermore affects the aquatic life.High BOD in the wastewater leads to the decomposition of organic matter under the anaerobic condition that produces methane, ammonia and hydrogen sulphide gas [3]. The interaction of these gases and other chemicals into water may adversely affect many aquatic organisms, so to protect and maintain aquatic life, regular monitoring of the physico-chemical parameters is necessary. However, the disposal of industrial effluents in the waterbodies could pose a threat to aquatic animals particularly fish because they live in very intimate contact with their environment, and are therefore very susceptible to alterations in water quality criteria that may be reflected in their blood and tissue components [4].

Enzymes play an important role in digestion of food in an animal and maintain its body's metabolic processes. They are exceedingly efficient and very specific in terms of nature of reaction catalysed and the substrate utilized. Pollutants or xenobiotics or toxicant present in the form of macromolecules in the waterbodies altered the membrane permeability of cells and may bind to enzyme or change the concentrations of co factors or reactants and indirectly affecting enzyme activity [5] High conductivity

and low dissolved oxygen of industrial effluent changes the activity of hydrolytic enzymes like esterases and transminases of the fish exposed to them [6].Enzymes are sensitive biomarkers in toxicological study as they provide an early information regarding potentially hazardous changes in aquatic organisms inhibited in polluted water [7]. Metabolic enzymes are used as clinical biomarkers that can give an estimate of relative damage caused to the physiology of fish on chronic exposure to toxicant [8]. In fishes, enzymes also serve as useful biomarkers to assess the in-vivo environmental exposures [9]. Estimation of enzymes such as ALP, AST, ALP, ACP and LDH help to detect any abnormalities in fishes [10-11].Therefore, the aim of the present work to assess the changes in the activity of enzymes: ATPase, aspartate transaminase, alanine transaminase, acid phosphatase, alkaline phosphatase and lactate dehydrogenase in serum of fresh water snake headed fish, *Channa punctatus* exposed to paper mill effluent.

MATERIAL AND METHODS

Procurement of test fish: The snake headed fish, *Channa punctatus* (45±5 g & 12±5 cm) were collected from local fresh waterbodies and transported to laboratory without any mechanical injury.The fish were treated with 0.1 % KMNO4 solution to get rid of dermal infection than healthy fishes were kept in plastic jar containing 50L of clean tap water and acclimatized for 15 days to the laboratory conditions, during that period they were fed on boiled egg yolk and commercial fish food.

Collection of paper mill effluent: For the present study, the treated paper mill effluent was collected from Yes paper mill Ltd. Darshan Nagar, Ayodhya in polyethylene container. The percent concentration of test solution has been calculated by using the formula: Volume % = V_E / (V_E + V_{DW}) X 100; Where, V_E = Volume of effluent , V_{DW} = Volume of dilution water.

Plan of Experiment: The 96h LC_{50} of *Channa punctatus* for treated paper mill effluent was 15% [12] so, the fishes were exposed to 5% concentrations for three weeks. A control group in dechlorinated water was also maintained in the same environment for same duration. Three replicates for each treatment with ten fish of equal size were maintained for two weeks along with control. The fishes of both groups were regularly fed with commercial food and the medium was exchanged daily. The fishes of both groups were sacrificed at the end of first and second weeks than the blood and desired tissues (Liver, kidney, gills and muscles) were dissected out for the estimation of enzymes.

Analysis of Enzymes: At the end of exposure periods *i.e.* first and second weeks, the blood samples were collected from live fishes of both control and experimental groups through cardiac puncture. The collected blood was kept it stand for some time and thereafter, centrifuged at 3000 rpm for 10 minutes to obtain serum. The fishes of both groups were sacrificed and the desired tissues were dissected out, homogenized and mixed the 5ml of deionized water than centrifuged at 3000 rpm for 10 minutes to obtained supernatant.

The serum and supernatantwas analyzed for enzymes. The activity of enzymes, ATPase, aspartate transaminase (AST) & alanine transaminase (ALP), acid phosphatase (ACP) & alkaline phosphatase(ALP) and lactate dehydrogenase were determined by followingstandard method [13-16].

Analysis of Data: The data in this paper have been presented with mean ± mean standard error and the statistical significance of difference between control and experimental group was calculated by student's t- test.

RESULTS AND DISCUSSION

The fluctuations observed in concentrations of various enzymes in blood and other tissues of *Channa punctatus* exposed sublethal concentration of paper mill effluent are given in Tables 1-6.

Fishes try to maintain the equilibrium in the presence of any toxicants or pollutants that are known to disrupt physiological and biochemical processes by changing the activity of enzymes involved in metabolic processes [17]. According to Mayer *et al.*[18]basically there are four different processes that may decide the responses of enzymes to specific or non-specific chemical stress. They are: 1. direct enzyme inhibition 2. enzyme induction by specific classes of chemicals 3. elevation of serum enzymes via tissue damage and 4. alterations in enzyme activity as a result of changes in metabolic pathways or fluxes. The aspartate transaminase (AST) and alanine transaminase (ALT) are the key enzymes of nitrogen metabolism and are important to energy mobilization [19].

Table1. Fluctuation in Enzymes, Aspartate transaminase (IU/L) of *Channa punctatus*exposed to sublethal concentrations of Paper mill effluent (mean±SD)

Tissues	Exposure for One Week			Exposure for Two Week		
	Control	5%	10%	Control	5%	10%
Blood	6.80±1.47	13.15±1.12*	18.45±1.34**	6.84±1.57	10.25±1.12	12.87±1.42*
Gill	45.25±7.14	42.50±5.45	46.28±4.12	46.25±5.65	50.24±3.25*	65.14±2.24**
Muscles	144.26±15.21	125.54±4.12	111.12±3.15*	146.28±4.65	131.24±2.25	105.26±4.11**
Liver	60.21±22.21	58.15±3.25	74.12±4.25*	62.15±3.25	72.14±2.25*	91.54±1.25**
Kidney	30.25±2.25	41.26±1.47*	68.25±2.17*8	32.24±2.58	48.25±3.15*	65.25±2.47**
Significant at *p<0.05; **p<0.001						

In the aspartate transaminase (AST)activity in serumand kidney was decreased where as in gill, muscles and liver its activitywasdecreasedin the paper mill effluent exposed fish, *Channa punctatus*(Table 1) is indicated that fish try to maintain the equilibrium in the presence of pollutants which are known to disrupt the rate of metabolic processes of the body that is controlled by these vital tissues (Liver, Kidney and Gill) and thus any change occurring in these tissues is a reflection of cellular damage to the fish. In the present study, increased activity of AST in these tissues indicates the increased rate of transamination as a result protein breakdown to free amino acids for subsequent utilization in glycogenic pathway [9]. Thus the significant increase in AST activity in paper mill effluent exposed fishes may be attributed to increased autolysis in tissues due to cytotoxicity.

Table2. Fluctuation in Enzymes, Alanine transaminase (IU/L) of *Channa punctatus*exposed to sublethal concentrations of Paper mill effluent (mean±SD)

Tissues	Exposure for One Week			Exposure for Two Week		
	Control	5%	10%	Control	5%	10%
Blood	3.75±1.14	5.25 ±1.23	6.50±1.32**	3.85±1.25	5.14±1.26	6.10±1.36*
Gill	22.10±2.54	18.30±2.14	15.20±1.58*	20.70±1.67	17.20±2.32	13.50±2.15**
Muscles	15.50±1.55	18.10±2.11	22.40±3.10*	14.90±1.25	17.10±1.22	20.10±1.32*
Liver	17.20±1.54	18.50±1.32	20.30±1.41*	17.50±1.47	19.40±1.25	22.10±1.47*
Kidney	18.30±2.12	16.80±2.14	17.25±2.13	18.40±2.22	16.90±2.11	17.20±2.22*
Significant at *p<0.05; **p<0.001						

The alkaline phosphatase(ALP) is a multifunctional enzyme that acts as transphosphorylase at alkaline medium and also plays an important role in membrane transport activities and mineralization of the skeleton [7].The fluctuation in alanine transaminase (ALT) activity reflects the change in endoplasmic reticulum, cell membrane and may be involved in metabolic activities [4]. In the present study, the responses of ALT among the treated group were variable in different manner. The ALT activity increased in gill and kidney where as its activity in serum, muscles and liver was decreased in effluent exposed fishes. ALP is capable of inactivating phosphorylase enzyme necessary for glycogen breakdown so in the present study, decrease in serum, muscles and liver alkaline phosphatase activity probably facilitates the increased activity of phosphorylase enzyme and subsequent breakdown of tissues glycogen into glucose to produce energy during present stress condition of paper mill effluent exposed fishes [20].

Enzyme acid and alkaline phosphatases are membrane-bound lysosomal enzymes and the sensitive biomarkers in toxicological study as they provide an early information regarding potentially hazardous changes in aquatic organisms inhibited in contaminated water. Both enzymes are concerned with the biosynthesis of fibrous proteins and mucopolysaccharides, or they may serve as regulators of intracellular phosphatase concentration [7].

Table3. Fluctuation in Enzymes, Acid phosphatase (IU/L) of *Channa punctatus*exposed to sublethal concentrations of Paper mill effluent (mean±SD)

Tissues	Exposure for One Week			Exposure for Two Week		
	Control	5%	10%	Control	5%	10%
Blood	4.12±0.88	5.21±0.27*	5.46±0.32**	4.18±0.54	5.10±0.25	5.33±0.24*
Gill	18.25±1.32	15.45±0.36	13.54±0.54*	19.54±2.11	16.54±1.78	11.54±1.44**
Muscles	5.10±0.25	6.25±0.15*	7.15±0.22**	5.15±0.25	6.75±0.32*	7.75±0.28**
Liver	21.25±1.54	19.26±1.34	17.11±1.14*	22.55±2.14	18.74±1.59*	15.54±1.22**
Kidney	23.47±2.11	20.41±1.74	20.31±1.12*	24.16±2.14	20.12±1.11	18.15±2.14**
Significant at *p<0.05; **p<0.001						

Phosphatases has important role in carbohydrate metabolism in animals. Acid phosphatase is lysosomal enzyme and plays an important role in autolytic degradation of the tissue and dissociation of dead cells. In the present study, ACP activity in gill, liver and kidney was increased where as its activity in serum and muscles in industrial effluents exposed fish and suggested that these tissues of the exposed fish have been impaired by industrial effluent.In the present study, the ACP activity decreased in the serum and muscles may be due to decreased biosynthetic activities in these tissues and anaerobic capacity of industrial exposed fish, while an elevation in ACP activity in gill, liver and kidney, suggests an increase in lysosomal mobilization and cell necrosis due to industrial effluent toxicity [4]. Thus it can be concluded that this fluctuation in ACP activity may eventually result in a shift in biosynthesis and the energy metabolism pathway of the toxicant exposed fish.

Table4. Fluctuation in Enzymes, Alkaline phosphatase (IU/L) of *Channa punctatus*exposed to sublethal concentrations of Paper mill effluent (mean±SD)

Tissues	Exposure for One Week			Exposure for Two Week		
	Control	5%	10%	Control	5%	10%
Blood	40.25±1.79	35.25±2.11	30.25±1.18*	41.15±2.31	32.25±1.57*	27.16±1.28**
Gill	78.54±3.58	67.25±3.15	58.28±4.12*	80.18±2.89	61.28±3.28*	50.18±2.98**
Muscles	42.25±2.35	51.12±2.37	59.55±4.11*	43.54±3.15	58.66±2.58*	67.14±2.47**
Liver	95.15±3.58	105.24±4.22	119.58±5.47*	104.11±4.11	126.85±3.58**	111.54±4.21*
Kidney	188.21±3.25	275.24±4.87	3.92±5.25*	197.11±2.47	375.14±7.11*	457.24±8.14**
Significant at *p<0.05; **p<0.001						

Alkaline phosphatase (ALP) is present on all cell membranes where active transport occurs, and hydrolase and transphosphorylase in function. Alkaline phosphatase(ALP) is a multifunctional enzyme that acts as transphosphorylase at alkaline medium and also plays an important role in membrane transport activities and mineralization of the skeleton [7]. In the present study, elevation of this enzyme in muscles, liver and kidney indicate the pathological condition of these tissues such as musclesdisruption, liverimpairment and kidney disfunction [9]. ALP is capable of inactivating phosphorylase enzyme necessary for glycogen breakdown so in the present study, decrease the level of alkaline phosphatase in serum and gill probably due to increased activity of phosphorylase enzyme and subsequent breakdown of tissues glycogen into glucose to produce energy during stress condition imposed by paper mill effluent in exposed fishes[20]. Since ALP is a membrane bound enzyme, therefore in the present study, exposure of fish *Channa punctatus* to industrial effluent causes disruption of its tissue membrane and change in their properties that could changes the ALP activity.

Table5. Fluctuation in Enzymes, Lactate dehydrogenase (IU/L) of *Channa punctatus*exposed to sublethal concentrations of Paper mill effluent (mean±SD)

Tissues	Exposure for One Week			Exposure for Two Week		
	Control	5%	10%	Control	5%	10%
Blood	475.24±8.14	415.25±7.21	372.54±6.52*	495.55±7.16	387.15±6.47*	354.54±5.87**
Gill	754.25±8.41	654.25±4.58	591.65±5.87*	789.24±7.45	611.25±4.56*	501.47±5.68**
Muscles	874.00±5.41	751.23±4.14	658.26±5.15*	888.50±6.44	579.54±5.87*	498.58±6.47**
Liver	947.58±6.18	898.45±3.47	769.18±7.14*	975.64±4.74	775.58±4.98*	672.85±5.78**
Kidney	1187.25±8.58	897.87±7.85*	754.85±8.54**	1211.12±7.59	825.48±8.87*	711.24±6.98**
Significant at *p<0.05; **p<0.001						

Lactate dehydrogenase (LDH) is an enzyme found in nearly all cell and convert the lactate into pyruvate. In the present study its activity increased in all the tissues of effluent exposed fishes as compared to control. The possible reason of increase the LDH activity in these tissues is the inhibition of glycolytic process that lowered the metabolic rate in presence of the toxicant in the effluent. In hypoxic environment as exhibited by the industrial effluent LDH activity increases as a result fishes shift towards anaerobic respiration [4].Increased activity in tissues indicate metabolic changes in chemically stressed fish, considered as a good indicator of anaerobic activity in a tissue [21]. Thus in the present investigation, the increase in LDH activity may reflect an increased dependence on anaerobic carbohydrate metabolism in the tissues of the fish on exposure to paper mill effluent.

Table6. Fluctuation in Enzymes, ATPase (IU/L) of *Channa punctatus* exposed to sublethal concentrations of Paper mill effluent (mean±SD)

Tissues	Exposure for One Week			Exposure for Two Week		
	Control	5%	10%	Control	5%	10%
Blood	3.15±0.54	2.85±1.22	2.68±1.02*	3.22±1.12	2.37±1.51*	2.14±1.24**
Gill	1.78±0.08	1.81±0.07	1.90±0.06	1.81±0.07	1.89±0.05	2.11±0.08*
Muscles	1.75±0.25	2.74±0.15	3.11±0.74*	1.86±0.31	3.02±0.54*	4.12±0.24**
Liver	2.02±0.09	2.87±0.8	3.32±0.7	2.54±0.58	3.41±0.57*	4.84±0.08**
Kidney	1.65±0.25	1.75±0.24	1.91±0.25*	1.74±0.06	1.95±0.04*	2.21±0.08*
Significant at *p<0.05; **p<0.001						

ATPase is a mitochondrial enzyme and it carries out oxidative phosphorylation, *i.e.* it catalyses the hydrolysis of ATP to ADP and phosphoric acid and release enormous energy. It is also involve in osmoregulation and intracellular function like sodium pump [20]. Inhibition or stimulation of ATPase activity could be expected to have metabolic or ionic effect in fishes in relation to osmoregulation as stated by Verma *et al*.[22].In the present study, the serum ATPase activity was increased with slight decreased its activity in rest of tissues of effluent exposed fish, *Channa punctatus* in relation to the control due to increase its activity to compensate some inhibitory effects (hormesis) as reported in fish by Yang and Randall [23].The effluent can interact directly with the enzyme or alter ATPase activity due to disruption of energy producing metabolic pathways [24]. The stored ATP reduces to fulfill energy demand in response to a physiologically stressful condition. Thus estimation of ATPase is a potentially useful indicator of pollution in aquatic animals.

However in the present study, the alterations in enzyme responses in effluent exposed fishes were neither neither effluent concentrations nor exposure periods dependent but these can be used as major biomarker indicating the impact of toxicant or pollutant on fish heath.

CONCLUSION

ALT, AST, ALP are non plasma specific enzymes that are localized in tissue cells of liver, heart, gills, kidneys, muscles and other organs and their presence in the blood may give specific information about organ dysfunction [55].The result of present study confirms that sublethal concentration of paper mill effluent induces significant alterations in the metabolic processes and disruptive changes in the organ of the fish as evident by fluctuation in enzyme activities. Therefore enzymatic activity can be used as early biomarkers of fish heath and *Channa punctatus* as a good model of environmental exposure.This study provides scope to study biochemical and histological assessment of industrial wastewater on freshwater food fishes under study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest among themselves or with any other individual, organization etc. throughout entire duration of this study.

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