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Impact of dye effluent on Enzymological changes in freshwater fish *Oreochromis mossambicus*

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Abstract: *Enzymes play an important role in metabolism. They are exceedingly efficient and very specific in terms of nature of reduction catalyzed and the substrate utilized. Enzymes are very essential to catalyze all chemical reaction in the cells of organism, thus the action of a foreign contaminant in the cell usually involves disruption in enzyme function. The study was carried out to investigate the acute and sublethal toxicity of dye effluent on enzymological variables of a fresh water fish, Oreochromis mossambicus under laboratory conditions. The 96 hrs LC_{50} value of dye effluent to the fish, Oreochromis mossambicus was estimated by Probit analysis method and was found to be with 95% confidence limits during acute treatment 96 hrs, enzymological variables like GOT, GPT and LDH were significantly ($P < 0.05$) increased in fish exposed to dye effluent. However, a significant ($P < 0.05$) decrease in ACP and ALP was observed in the exposed fish during above exposure period when to that of the control groups. Activity levels of dye effluent, dye effluent were studied in the different tissues of fresh water fish, Oreochromis mossmbicus exposed to concentration of dye effluent (1.3 mg/l) during different exposure periods of 24 hrs, 48 hrs, 72 hrs and 96 hrs. The levels of enzymes like GOT, GPT and LDH were noticed in gill, muscle, liver and kidney, tissues with timely course of study. The fish treated with dye effluent showed greater inhibition of enzymes during the study period.*
Key words: *GOT, GPT, LDH, dye effluent, Oreochromis mossambicus*

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I. Introduction

Textile dying and printing industries use a variety of chemical compounds, a major portion of which occurs in the effluent. The effluent is reported to be complex in nature rich in dissolved and suspended solids, various organic compounds and very high pH. Textile effluents are known toxicants, which causes acute disorders in aquatic organisms. Uptake of textile effluents through food chain in aquatic organisms may cause various physiological disorders like hypertension, sporadic fever, and renal damage, cramps etc., Enzymes are biological catalyst and increase the reaction taking place within living cells without themselves undergoing any overall change. Enzymes play a major role in metabolism they are exceeding efficient and very specific in term of nature of reaction catalyzed and final concentration of enzymes is under genetic control and is greatly influenced by very small molecules of substrates these cellular catalysts control the formation of biochemical and physiological function.

Enzymes are indicators of stress due to impaired metabolism. The toxic metals may include both lethal and sublethal concentrations, which may change the growth rate, development, reproduction, histopathology, biochemistry, physiology and behavior. GOT (Glutamic Oxaloacetic Transaminase) and GPT (Glutamic Pyruvic Transaminase) are important diagnostic tools in medicine and are used to detect adverse effects produced by various pollutants [2]. Gills serve as a good indicator of water quality.

Enzymes are biochemical macromolecules that control metabolic processes of organisms, thus a slight variation in enzyme activities would affect the organism [2].Evaluation of enzyme activities in the tissue and organs of aquatic organs in the diagnosis of the effects of pollutants is one of the emerging areas in toxicological monitoring and remediation programs [3].

II. Materials and Method

The fresh water fish, *Oreochromis mossambicus* (body length 7-8 cm, body weight 6-7 gm) were collected from Aliyar Dam and acclimatized to laboratory condition for 2 weeks in a large cement tanks (6 x 4 x 3) at (24 ± 3^{o}C). The fishes were fed regularly with conventional diet rice bran and oil cake 1 : 1 ratio feeding was stopped one day prior to the start of the experiment .Technical grade of Dye effluent was used in the investigation. Bathes of 10 healthy fishes were exposed to different concentration of the Dye effluent. LC_{50} value for 96 hrs was calculated by using probity analysis [4].

Five groups of fishes were exposed to 0.13 mg/l (sublethal concentration) of 96 hrs LC_{50} valued concentration of the dye effluent 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively. Another group was maintained as control at the end of each exposure period. Fishes were sacrificed and tissues such as liver, kidney, muscle and gill were dissected and removed. The tissues were homogenized with 80% methanol centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for analysis of different parameters. The results were expressed as mg/g wet weight of the tissue. Different enzymological parameters like GOT, GPT, LDH, ACP and ALP were analysed.

III. Results

The fish belonging to the species to the species *Oreochromis mossambicus* exposed to sublethal concentration (0.13mg/l) of dye effluent for 24, 48, 72 and 96 hours in short time period. Various enzymatic parameters like GOT, GPT, LDH, ACP and ALP were analysed in gill, liver, kidney and muscle samples. The values were presented in the form of tables (1 to 5)

Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT):

Changes in the GOT activity in the gill, liver, kidney and muscle of fish, *Oreochromis mossambicus* exposed to dye effluent were presented in the table (1). During the above treatment liver showed a maximum percentage increase (250) during 96 hour exposure time followed by gill (210.34) during 96 hours and kidney followed by (111.66) during 96 hours and muscle showed maximum percentage decreased (82.35) during 96 hours. The values are significant at 0.01% and 0.05% level. (Table: 1)

The GPT activity in the organs of fish, *Oreochromis mossambicus* when treated with dye effluent in short term exposure period. During dye effluent treatment the enzyme activity was increased in kidney, muscle, gill and liver. The fish showing a percentage increased of 273.52, 228.57, 100 and 74.46 during the period of 96 hours respectively. The values are significant at 0.01 % level. (Table: 2)

[5] reported the increased activity of GOT and GPT in the intestine tissues of the fish, *Oreochromis mossambicus* on exposed to Triazophos. GOT and GPT are the key enzymes of nitrogen metabolism and are important in energy metabolization. GOT is very active and widely distributed of the transaminases. GOT and GPT are important diagnostic tools in medicine are used to detect adverse effects produced by various pollutants.

In the present study, increase in GOT and GPT transaminases might be attributed to tissue damage particularly liver. GPT is much more abundant in tissue like hepatopancrease than any other tissues and consequently an altered activity of this enzyme points to some disorder in those tissues.

GOT and GPT enzymes activity were found to increase in response to dye effluents in *Oreochromis mossambicus* fish. The transaminases, serum GOT and GPT are two enzymes considered as a sensitive measure to evaluate hepatocellular damage and some hepatic diseases [6]. GOT and GPT enzymes active were found to increase in response to heavy metals in different fish species [8]. The increase in GOT and GPT activity may due to decrease in metabolic activity disruption of enzyme system by blocking active sites and tissue damage.

Lactate Dehydrogenase (LDH):

During short time exposure period of *Oreochromis mossambicus* to dye effluent, the LDH activity was increased in kidney a percent increased of (432.71) during 96 hours exposure time followed by muscle (289.80) during 96 hours followed by liver (132.98) and gill showed maximum percentage decreased in (128.87) during 96 hours. The values are significant at 0.01% and 0.05% level. (Table: 3)

The increase in LDH level indicates a metabolic change that is the glycogen metabolism and glucose shift towards the formation of lactase in stressed fish, *Oreochromis mossambicus* primarily the muscle tissue [8]. LDH is hydrogen transferring enzyme that catalyzes the oxidation L-Lactase to pyruvate with the mediation of NAD^+ as hydrogen acceptor. LDH an indicator of anaerobic metabolisms, expected to exhibit increased activity at lower oxygen levels.

[9] Reported that LDH is an oxidoreductase, which catalyzes the inter conversion of lactate and pyruvate depending on the availability of NAD (co-enzymes). The decrease in LDH activity suggests a reduction in the conversation of lactate to pyruvate, thereby leading to the accumulation of lactic acid. The decrease in LDH activity with a consequent increase in the levels of lactic acid suggests the predominance of anaerobic segment, glycolysis.

Acid Phosphatase (ACP):

The ACP activity in the organs of fish, *Oreochromis mossambicus* when treated with dye effluent in short time exposure period. During dye effluent treatment the enzyme activity was increased in muscle, liver,

gill and kidney. The showing percentage increased of -88.88, -91.22, -91.90 and -93.75 during the period of 96 hours. The values are significant at 0.01% and 0.05% level. (Table: 4)

The decreased activities of ACP indicate disturbance in cell organells like endoplasmic reticulum and membrane transport system. Similar findings decreased ACP were reported in *Labeo rohita* on exposure to Arsenic was studied by [10].

Phosphatases are linked with growth as related with carbohydrate metabolism in organisms. Acid Phosphatase is lysosomal enzyme has important role to play in autolytic degradation of the tissue and dissociation of dead cells. Acid phosphatase with free attached phosphate groups from other molecules during digestion. ACP is mainly involved in the catabolic and autophagic processes in the cells. The decreased activities of ACP enzyme indicate disturbance in the structure and integrity of cell organells like, endoplasmic reticulum and membrane transport system

Alkaline Phosphatase (ALP):

During short term exposure period of *Oreochromis mossambicus* to dye effluent, the ALP was increased in muscle, liver, kidney and gill. The percentage increase of -55.58, -60.67, -65.80 and -72.28 during the period of 96 hours. The values are significant at 0.01% level (Table5).

Alkaline phosphatase is involved in carbohydrate metabolism, growth and diffentiation, synthesis of certain enzymes, secretion activity and transport to phosphorylated intermediates across the cell membrane. The levels of metabolic marker enzymes in the tissues of the carp *Oreochromis mossambicus* exposed to a Dye effluent for a period of 24, 48, 72 and 96 hours. Alkaline phosphatase decrease in the first day compared to control. The defensive surface proteins antagonize the toxic radicals resulting in elimination of protein from the liver cells. The lowered level of total protein in plasma, muscle and liver reflects the capacity of protein synthesis and denote the osmolarity of the blood and liver impairments. Hence, it is valuable indicator in the diagnosis of toxicity in fish. [11].

Enzymes are fragile substances with a tendency to undergo denaturation and inactivation under unsuitable conditions. The activity of ALP enzyme in liver of freshwater fish, *Oreochromis mossambicus* exposed to dye effluent was studied in the present investigation. The enzyme acid phosphatase is present in almost all the tissues. It is a hydrolytic enzyme concerned with the process of transphosphorylation and has an important role in the general energetic of an organism. It is associated with the transport of metabolites, with metabolism of phospholipids, phosphoproteins, nucleotides, carbohydrate and synthesis of proteins [12].

V Conclusion

From the results obtained, can be concluded that the dye effluent is toxic to *Oreochromis mossambicus.* Bioremediation have been founded useful in the decolourization of dye effluent before letting into aquatic ecosystem. The dye effluent induced alterations in the activities of the enzymes like GOT, GPT and LDH and these enzymes may be used as logical candidates to monitor the toxic level of effluent and its impact on aquatic organisms.

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IV. Table

Table 1. Effect of dye effluent on the GOT content (IU/L) in the gill, liver, kidney and muscle of fish, *Oreochromis mossambicus* exposed for different periods.

Sample (mg/g wet tissue)	Exposure Periods				
	Control	**24 hours**	**48 hours**	**72 hours**	**96 hours**
GILL	2.9 ± 0.30	3.2±0.11	5.4±0.21	7.3±0.05	9.0±0.07
't' value		0.929ns	1.181ns	2.3*	2.525*
% change		10.34	86.20	151.72	210.34
LIVER	6.2±0.10	9.4±0.18	16.6±0.05	19.4±0.03	21.7±0.11
't' value		1.472ns	4.048**	9.347**	9.15**
% change		51.61	167.74	212.90	250
KIDNEY	6.0±0.18	8.3±0.09	10.4±0.12	11.6±0.05	12.7±0.15
't' value		1.283ns	5.861**	13.01**	10.53**
% change		38.33	73.33	93.33	111.66
MUSCLE	3.40±0.09	2.9±0.12	3.4±0.04	5.3±0.12	6.2±0.03
't' value		2.889*	8.621**	6.53**	11.97**
% change		-14.70	0	55.88	82.35

Values are mean± SD, n=5, Figures in parenthesis decrease over control.
*- significant at 5% (t<0.05) **-significant at 1% (t<0.01) NS- Non Significant

Table 2. Effect of dye effluent on the GPT content (IU/L) in the gill, liver, kidney and muscle of fish, *Oreochromis mossambicus* exposed for different periods.

Sample (mg/g wet tissue)	Exposure Periods				
	Control	**24 hours**	**48 hours**	**72 hours**	**96 hours**
GILL	3.8 ± 0.30	4.3±0.11	4.7±0.21	6.2±0.05	7.6±0.07
't' value		1.92*	2.67*	5.32**	12.56**
% change		13.15	23.68	63.15	100
LIVER	9.4±0.10	11.7±0.84	13.2±0.85	14.6±1.03	16.4±1.21
't' value		6.41**	7.08**	8.37**	13.62**
% change		24.46	40.42	55.31	74.46
KIDNEY	3.4±0.18	7.2±0.49	8.6±0.92	10.2±0.45	12.7±0.75
't' value		7.23ns	11.81**	22.01**	20.53**
% change		-94.70	152.94	199.99	273.52
MUSCLE	2.1±0.09	3.4±0.32	4.7±0.24	5.8±0.62	6.9±0.73
't' value		4.93*	8.22**	9.35**	10.73**
% change		61.90	123.80	176.19	228.57

Values are mean± SD, n=5, Figures in parenthesis decrease over control.
*- significant at 5% (t<0.05) **-significant at 1% (t<0.01) NS- Non Significant

Table 3. Effect of dye effluent on the LDH content (IU/L) in the gill, liver, kidney and muscle of fish, *Oreochromis mossambicus* exposed for different periods.

Sample (mg/g wet tissue)	Exposure Periods				
	Control	**24 hours**	**48 hours**	**72 hours**	**96 hours**
GILL	14.2±1.62	17.4±0.61	20.0±1.21	27.3±2.05	32.5±2.07
't' value		6.29**	10.81**	18.32**	32.24**
% change		22.53	40.84	92.25	128.87
LIVER	38.2±2.27	54.5±4.18	67.0±5.05	78.4±7.03	89.0±5.12
't' value		45.72**	64.048**	87.42**	75.55**
% change		42.67	75.39	105.23	132.98
KIDNEY	10.7±1.28	24.2±1.09	35.0±1.32	43.6±2.05	57.0±4.15
't' value		23.37ns	55.68**	73.51**	91.74**
% change		126.16	227.10	307.47	432.71
MUSCLE	15.7±1.19	21.4±1.12	32.5±1.34	47.0±2.12	61.2±3.43
't' value		8.92*	48.21**	66.73**	91.74**
% change		36.30	107.00	199.36	289.80

Values are mean± SD, n=5, Figures in parenthesis decrease over control.
*- significant at 5% (t<0.05) **-significant at 1% (t<0.01) NS- Non Significant

Table 4. Effect of dye effluent on the ACP content (IU/L) in the gill, liver, kidney and muscle of fish, *Oreochromis mossambicus* exposed for different periods.

Sample (mg/g wet tissue)	Exposure Periods				
	Control	**24 hours**	**48 hours**	**72 hours**	**96 hours**
GILL	42±0.50	3.4±0.31	2.1±0.41	0.72±0.15	0.34±0.17
't' value		3.27*	6.11**	32.25*	62.23*
% change		-19.04	-50	-82.85	-91.90
LIVER	5.7±0.72	4.2±0.53	3.0±0.23	1.8±0.53	0.5±0.14
't' value		11.42**	7.88**	13.32**	19.85**
% change		-26.31	-47.36	-68.42	-91.22
KIDNEY	3.2±0.68	2.0±0.62	1.2±0.47	0.7±0.34	0.2±0.07
't' value		4.28**	9.61**	43.54**	50.27**
% change		-37.50	-62.5	-78.125	-93.75
MUSCLE	2.7±0.79	2.0±0.62	1.4±0.64	0.9±0.32	0.3±0.09
't' value		2.48*	6.13**	26.52**	31.75**
% change		-25.92	-48.14	-66.66	-88.88

Values are mean± SD, n=5, Figures in parenthesis decrease over control.
*- significant at 5% (t<0.05) **-significant at 1% (t<0.01) NS- Non Significant

Table 5. Effect of dye effluent on the ALP content (IU/L) in the gill, liver, kidney and muscle of fish, *Oreochromis mossambicus* exposed for different periods.

Sample (mg/g wet tissue)	Exposure Periods				
	Control	**24 hours**	**48 hours**	**72 hours**	**96 hours**
GILL	55.2±2.64	37.2±1.56	20.8±2.15	17.6±1.05	15.2±1.24
't' value		57.99	78.15**	72.24**	82.57**
% change		-32.60	-62.31	-68.11	-72.28
LIVER	59.0±4.81	42.3±2.45	37.4±2.72	30.0±3.64	23.2±1.21
't' value		21.42**	34.87**	109.37**	129.54**
% change		-28.30	-36.61	-49.15	-60.67
KIDNEY	42.4±3.65	37.1±3.19	32.0±3.56	20.0±1.25	14.5±1.02
't' value		82.36**	55.67**	113.48**	110.73**
% change		-12.49	-24.52	-52.83	-65.80
MUSCLE	36.7±2.46	28.2±1.16	22.1±1.04	19.0±1.15	16.3±1.34
't' value		12.89**	19.64**	86.23**	81.17**
% change		-23.16	-39.78	-48.22	-55.58

Values are mean± SD, n=5, Figures in parenthesis decrease over control.
*- significant at 5% (t<0.05) **-significant at 1% (t<0.01) NS- Non Significant

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