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A STUDY ON THE IMPACT OF TEXTILE DYE EFFLUENT AND PHYTOREMEDIATED TEXTILE DYE EFFLUENT ON MARKER ENZYMES AND ANTIOXIDANT STATUS OF FRESH WATER FISH, *Clarias batrachus*

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ABSTRACT

The industrial wastes and effluents are increasing sharply in recent years and discharging of all by products, wastage and effluents on soil, canal river and water cause serious environmental pollution. Catfishes (order Siluriformes) are a diverse group of ray- finned fish. The investigations were conducted on fresh water fish *Clarias batrachus* to check out the effect of textile dye effluent on marker enzymes such as Aspartate transaminase (AST), Alanine transaminase (ALT) and Lactate dehydrogenase (LDH), antioxidant status such as Glutathione – S – transferase, Glutathione reductase, reduced glutathione and vitamin E. From the present study it was observed that there is a significant change in the levels of marker enzymes, antioxidant status of the groups of fish treated with textile dye effluent and phytoremediated textile effluent. This may be an indication that the *Calotropis procera* is useful in the study of phytoremediation.

KEY WORDS: ***Clarias batrachus*, phytoremediation, textile effluent, *Calotropis procera***

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INTRODUCTION

The industrial wastes and effluents are increasing sharply in recent years and discharging of all by products on soil, canals, rivers and water cause serious environmental pollution. They pollute productive soils, natural water systems as well as ground water. The industrial by products, wastes, effluents contain high level of heavy metals such as As, Cd, Cr, Fe, Hg, Mn, Ni, Pb and Zn. The higher amount of lead and cadmium is very dangerous to human body and plants. Heavy metal tolerance is a relatively rare trait found only in a few highly adapted plant species. These plants are capable of growing especially at sites with normally or artificially elevated levels of heavy metals. Some plant species are not only tolerant to Zn, Cd, Ni but they can accumulate heavy metals in the shoots[46]. The dye present in the effluent should be removed before discharging them to the environment to avoid the health hazards and destruction of ecosystem. Study of alterations in enzyme activities in the organs of fish is one of the most reliable means of assessing effects of toxicants[1]. The variation in enzyme activities in the freshwater fish exposed to various pollutants and heavy metals, in particular, have been reported[2,3,4,5]. Changes in enzymes profile are important pollution indices and hence enzyme activity and other biomarkers have been studied as possible tools in aquatic toxicological research[6,7,8]. Many reports on the study of activity of AST, ALT and LDH due to the impact of different pollutants such as heavy metals, [9,10,11] phenolic compounds[12],tannery effluents[13], and textile dye effluent[14]in freshwater fish have been observed. Heavy metals can removed by using plant materials such as palm pressed fibres and coconut husk[15] water fern *Azolla filiculoids*[16], peat moss[17]. Heavy metal pollution is the commonest form of environmental pollution. *Calotropis procera* showed a higher capacity in Ni and Cd accumulation. Ni, Cd and Fe in organs of *C.procera* and *C.colocynthis* and those in sediment, indicating the potential use of the two plants for pollution monitoring of these metals[18]. Leaves of *Calotropis procera* were found to accumulate various heavy metals[19]. The present study was carried out to investigate the effect of textile dye effluent and recovery due to phytoremediated textile dye effluent on the liver and muscle tissues of fresh water fish, *Clarias batrachus*.

MATERIALS AND METHODS

Fish are collected from a fish farm near Poondi, Thiruvallur, Tamilnadu. Fishes were grown in 5 tanks. Fishes were acclimatized for 10 days. Water was changed alternative days and the fishes were fed twice daily. Textile effluent was collected from the textile mill industry near Iyyaampattai, Kanchipuram District, Tamilnadu.

1. GROUP1-Control
2. GROUP 2 – Fishes treated 1ppm textile dye effluent
3. GROUP 3 – Fishes treated 2ppm textile dye effluent
4. GROUP 4a –Fishes transferred from group 3 into textile dye effluent phytoremediated with aqueous extract of *Calotropis procera*
5. GROUP 4b –Fishes transferred from group 3 into textile dye effluent phytoremediated with lipid extract of *Calotropis procera*.

After 10 days of treatment with textile effluent, the fishes were netted, sacrificed and tissues such as liver and muscle were dissected out for homogenization. Liver and muscle tissues were homogenized with 10 ml of saline and the obtained homogenate was analysed for marker enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH), and antioxidant status such as glutathione S transferase (GST), glutathione reductase (GR), reduced glutathione and vitamin E. Calotropis leaves were collected from the road side area from Tiruvellore. Collected plant materials were shade dried at room temperature, in order to prevent the exhaust of essential oils and compounds present within the leaves, when exposed to sunlight and also to remove the unwanted compounds such as chlorophyll, which do not have any antimicrobial property. Leaves were ground with hand grinder to

particle size of 300 µm approximately. An aqueous extract was prepared by boiling 10 % wt/wt of the air dried powdered plant part is sterile distilled water for 10 min and then cooled to room temperature overnight[47] . The aqueous extracts were filtered using a Millipore filters to remove particulate matter. The finite volume of each filtrate was made up to 100 ml with distilled water with 0.2% Tween 80 to account for the evaporated water during boiling. Lipid extract preparation was carried out by maceration technique (cold extraction process). It is technically, chemically defined as, the preparation of an extract by soaking the containing material in an organic solvent. The ground leaf materials were taken in three aspirator bottles separately, containing about 500 gms in each bottle. Then three organic solvents ranging from low polarity to high polarity i.e., hexane, chloroform, methanol was added to aspirator bottles respectively. The bottles were shaken thoroughly after every six hours. After 72 hours the solvent was decanted, filter with Whatman filter paper and then allowed to evaporate. Finally the extract was obtained in a semisolid state (paste). To 5 litre of textile effluent, 50 ml of aqueous and lipid extract of *Calotropis procera* was added and incubated for 3 days. Textile dye effluent and phytoremediated textile effluent was analysed for total dissolved solids, total suspended solids, salinity, qualitative analysis such as ferric ions, ammonium, ammonia, nitrite, sulphide, sulphate. The liver and muscle tissue samples were dissected out for group of fish treated under phytoremediated textile dye effluent and the tissues were analysed for marker enzymes such as aspartate transaminase, alanine transaminase, Lactate dehydrogenase, antioxidant status such as Glutathione S transferase (GST), Glutathione reductase (GR), Reduced glutathione and vitamin E.

RESULTS

It was observed that the activity of AST and ALT does not significantly altered in liver and muscle of fish treated with textile dye effluent with a slight decrease was observed. LDH activity was significantly reduced in the liver of fish treated with textile dye effluent on comparison with control group. There was no significant change in LDH activity of liver. LDH activity in liver and muscle was found to increase and reached nearly normal value as that in the control group this is suggestive of recovery of fish sample from aerobic stress. On comparison of aqueous and lipid extract treated effluent, lipid extract was found to give better phytoremediation.

Table 1
Physiochemical properties of textile effluent.

S.NO	PARAMETER	BEFORE PHYTOREMEDIATION	AFTER PHYTOREMEDIATION	
			AQUEOUS	LIPID
1	TSS	0.091	0.037	0.039
2	TDS	10400000	10750000	9100000
3	Salinity	4.5	11	5.5
4	Sulphide	P	A	A
5	Sulphate	A	P	P
6	Ferric ions	A	A	A
7.	Ammonia & Ammonium	P	P	P
8.	Nitrite	A	A	A

P- DENOTES PRESENT
A – DENOTES ABSENT

Table 2
Activity of marker enzymes and antioxidant in liver of fish treated with textile dye effluent and phytoremediated textile dye effluent

S.NO	PARAMETER	CONTROL	TEXTILE EFFLUENT		PHYTOREMEDIATED TEXTILE EFFLUENT		P - VALUE
			1PPM	2PPM	AQUEOUS	LIPID	
1	AST	4.9659 ± 1.5302	4.6806 ± 1.9387	3.2063 ± 2.1317	3.306 ± 2.0183	3.8151 ±2.3950	0.2530
2	ALT	4.924 ± 2.7452	4.631 ± 2.8535	4.750 ± 3.1237	5.7 ± 3.1237	3.89 ± 1.81	0.4885
3	LDH	6.32 ± 2.6657	2.0387 ± 0.2551	2.26 ± 0.5237	3.75 ± 1.63	6.72 ± 1.81	0.0004
4	GST	5.1468 ± 1.470	4.138 ±1.5962	4.0396 ± 2.259	5.9067 ± 2.6704	6.1072 ± 3.1005	0.5170
5	GR	6.3632 ± 1.7864	3.0848 ±1.948	4.891 ± 0.6815	4.334 ± 0.9	5.004 ± 1.330	0.0090
6	REDUCED GLUTATHIONE	4.1176 ± 0.5882	1.7 ± 0.7137	1.9608 ± 1.7538	2.3528 ± 2.03771	3.3646 ± 0.7198	0.0042
7	VITAMIN-E	10.68 ± 2.1	1.300 ± 0.011	3.304 ± 0.002	19.3 ± 4.8	22.3 ± 6.7	<0.0001

Table 3
Activity of marker enzymes in muscle of fish treated with textile dye effluent and phytoremediated textile dye effluent

S.NO	PARAMETER	CONTROL	TEXTILE EFFLUENT		PHYTOREMEDIATED TEXTILE EFFLUENT		P - VALUE
			1PPM	2PPM	AQUEOUS	LIPID	
1	AST	5.9863 ± 2.8471	4.445 ± 3.032	5.221 ± 2.562	5.469 ± 2.0019	4.7435 ± 2.4131	0.6483
2	ALT	3.5 ± 1.3912	4.7244 ± 1.216	4.75 ± 3.124	3.122 ± 2.3925	2.9656 ± 1.0118	0.5708
3	LDH	4.132 ± 3.3052	4.450 ± 3.0322	5.221 ± 2.563	2.9903 ± 1.8257	3.8361 ± 2.0540	0.8120
4	GST	4.0035 ± 1.0462	2.66 ± 0.5803	4.3298 ± 2.5605	5.249 ± 0.020	3.7086 ± 1.1797	0.2047
5	GR	4.283± 3.0809	4.7808 ±1.3537	3.0443 ±1.6226	3.6362 ± 1.871	4.6498 ± 2.1155	0.3804
6	REDUCED GLUTATHIONE	4.0196 ± 0.4046	3.03872 ± 2.1044	2.9409 ±1.3586	3.6469 ± 2.1175	2.394 ± 0.4956	0.3925
7	VITAMIN-E	11.2 ± 0.1	2.4 ± 0.03	0.7098 ± 0.02	15.6 ± 2.4	9.445 ± 1.7	<0.0001

The GST activity has not significantly changed in the liver of fish treated with textile dye effluent when compared with control. But a slight decrease was observed with levels of GST. The GR activity has significantly decreased in the liver of fish treated with textile dye effluent in comparison with control. There was a significant decrease in the activity of GST of muscle of fish treated with textile dye effluent when compared to control group.But there was no significant change in the activity of GR in muscle of both the groups of fish. The GST and GR activity in liver was found to increase due to challenge of textile effluent and recovery by aqueous and lipid extract of Calotropis. There is a significant recovery in the activity of GST, GR in muscle from group of fish treated with phytoremediated textile effluent with aqueous and lipid extract of Calotropis. Reduced glutathione level and Vitamin –E level has significantly decreased in the liver of fish treated with textile dye effluent on comparison with control. Reduced glutathione level and Vitamin –E level has significantly decreased in the muscle of fish treated with textile dye effluent on comparison with control. Reduced glutathione level and Vitamin-E level in liver was found to be increased due to challenge of textile effluent and recovery by aqueous and

lipid extract of Calotropis. Reduced glutathione level and Vitamin –E level in muscle significantly recovered from the group of fish treated with phytoremediated textile dye effluent with aqueous and lipid extract of Calotropis.

DISCUSSION

AST and ALT activities were found to be elevated in liver and muscle of *Tilapia mossambica* under the stress of arsenite[20]. AST and ALT activities were increased in the edible crab *Scylla serrate* under the stress of sublethal concentration of cadmium chloride[21]. AST and ALT activities were increased in the liver of rosy barb (*Puntius conchonious*) exposed to low concentration of nonyl phenol[22]. The activities of transaminases (AST and ALT) were increased in the liver of *Brycon cephalus* juveniles exposed to phenol[23]. AST and ALT activities were increased significantly in liver of Grass carp exposed to adiquat[24]. Significant stimulation in the activity of AST and ALT was observed in the liver of *Notopterus notopterus* exposed to phenolic compounds[12]. The activities of AST and ALT were increased in the liver tissue of *Notopterus notopterus* exposed to phenol[25]. Decreased levels of AST and ALT were due to its sharing in transforming proteins to glycogen. The decreased activities of AST and ALT indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system. Such damage to cell organelles has been reported in various studies[26,27]. Lactate dehydrogenase is the key enzyme in the process of glycolysis as it plays a crucial role in the conversion of pyruvate to lactate during anaerobic glycolysis. Pollutants stress triggers the anaerobic metabolism, resulting in marked increase of LDH activity in fish. This elevation of LDH activity indicates the dependence of anaerobic metabolism in effluent exposed to fish to meet the energy demands. Impaired respiratory function of gills leads to hypoxia and favouring of anaerobic metabolism in fishes under pollutant stress. Increased LDH activity may reflect energy demand for anaerobic burst[28,29]. It was stated that the activity of LDH decreased in the liver of teleost fish, *Channa punctatus* exposed to cadmium[30]. LDH activity was found to be decreased in white muscle of *Oreochromis niloticus* after cadmium treatment indicating decreases in the capacity of glycolysis of the tissue[31]. Decreased LDH activity was observed in the liver tissue of *Sarotherodon mossambicus* due to exposure of mercury[32]. Decrease in LDH activity was observed in the edible crab, *Scylla serrate* exposed to cadmium[21]. Significant decrease of about 78% in the activity of LDH was observed in the muscle of freshwater teleost, *Channa striatus* exposed to 7% of fertilizer industry effluent[33]. Glutathione – S – transferase (GST) is an enzyme which catalyses conjugation of reduced glutathione to electrophilic substrates and this enzyme is known to be involved in detoxification during stress by pollutants. Similar results were observed in the previous investigations reported by many authors. Increase of GST activity was observed in the liver of *Brycon amazonicus* exposed to mercuric chloride[34]. Increased GST activity was observed in the muscle of tilapia, *Oreochromis niloticus* exposed to total ammonia nitrogen[35]. GST activity was induced in hepatic tissues of green – liped mussels due to polycyclic aromatic hydrocarbons[36]. The glutathione – S – transferase (GST) significantly high in the liver of *Clarias gariepinus* collected from Ogun River contaminated with heavy metals[37]. GST activity was found to be increased in the liver of goldfish under the exposure of HC Orange No. 1[38]. Significant elevations in the activity of GST was recorded in liver of freshwater teleost, *Oreochromis mossambicus* due to the stress induced by cadmium[39]. An increasing trend of GST activity was observed in the liver of freshwater teleost, *Ictalurus melas*[40]. Glutathione reductase (GR) plays a crucial role in reducing oxidized glutathione in order to maintain optimum levels of intracellular reduced glutathione. The present findings were in accordance with the observed increased activity of glutathione reductase in the liver of *Brycon amazonicus*exposed to mercuric chloride[34] and in liver and muscle of tilapia, *Oreochromis niloticus* exposed to total ammonia nitrogen[35]. But in contrast, significantly reduced glutathione reductase activity was observed in the white muscle *Brycon amazonicus* exposed to mercuric

chloride[34]and in liver of *Sparus aurata* due to exposure of cadmium[41]. Tissue GSH is known to be involved in the metabolism and detoxification of exogenous and endogenous substances[42]. During a rapid phase reaction or prolonged exposure, GSH depletion accompanies increased lipid peroxidation. The time – dependent antioxidant effect of GSH can be explained on the basis of the initial presence of some labile GSH – dependent or microsomal factors i.e GSH transferases or cytochrome P – 450 that can provide protection. Reduced glutathione maintains a reduced intra cellular environment and act as a primary defense against excessive generation of harmful ROS[43]. Increase in the activities of ALT and AST were observed in the serum of *Oreochromis niloticus* exposed to zinc, cadmium and both[44]. *Cyprinus carpio* showed increased activity of AST and ALT in response to cadmium[45].

CONCLUSION

From the present study it was observed that there is a significant change in the studied marker enzymes, antioxidant status and electrolytes when the groups of fish treated with textile effluent and phytoremediated textile effluent. This may be an indication that the fish serve as an indicative tool to assess the contamination in the aquatic system and the Calotropis is useful in bioaccumulation and phytoremediation.

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