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Effect of tannery waste water on lactate dehydrogenase (LDH) enzyme activity of fresh water fish, *Channa punctatus*

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Abstract
Channa punctatus is the most common fish found in Indian rivers and water bodies. The present study was conducted to investigate the dilution effect of tannery waste water on the activity of serum LDH enzyme of the Indian freshwater fish *Channa punctatus*. The effect was observed based on the result of chronic toxicity and comparison of control with experimental groups. Tannery wastewater causes cellular hypoxia creating anaerobic condition and cellular damage. Investigation showed significant increase in LDH level with increase in concentration of tannery wastewater (5%, 10%, 15% and 20%TWW) i.e. 31.66 and 29.57 IU/L in 5% concentration to 50.64 and 48.25 IU/L in 20% concentration at both site 1 and 2 respectively and also with increase in exposure period in all concentrations from 15th to 45th days. The results indicate the effect of tannery wastewater stress at the organ level of fish and represent a valuable tool in biological monitoring.

Keywords: *Channa punctatus*, fresh water, LDH, tannery

1. Introduction
Fisheries provide an important source of protein minerals and vitamins as a food, employment, income, and recreation for people throughout the world [1], and nowadays fishes are exposed to biotic and abiotic stresses in wild and as well as in captivity; like environmental pollutants and various types of intensive agriculture practices. Among the aquatic fauna, fish is the most susceptible organism to heavy metal contamination than any other aquatic fauna and the environmental pollutants causing stress mainly includes changes in water quality, metals, and xenobiotics in water which cause severe stress, health hazards and ultimately resultant is a death of the fishes. Since heavy metals are non-biodegradable; they can be bioaccumulated by fish, either from the surrounding water or by ingestion of food. In addition, it indicates that when metals reached sufficiently in high concentrations in body cells they alter the physiological functioning of the fishes [2]. Fishes in captivity when exposed to certain chemicals and toxic substances also become physiologically stressed and in turn the stressed fish exhibit a generalized stress response characterized by impaired release of stress hormones as primary responses which in turn triggers a number of biochemical, physiological changes and irregularity in metabolic enzymes termed as secondary stress response[3].
The chromium (Cr) element found naturally in rocks, volcanic dust, soil, animals, plants, and gasses. In natural water, the concentration of chromium is low and is within the range [4] and one of the commonly used metals and its particulates enter in a variety of environmental media including soils, sediments, and aquatic system. It exists in two valence states, trivalent (CrIII) and hexavalent (CrVI) compounds. It is widely used in metallurgical industry, refractory, dye industry, electroplating, chemical, and tannery industries. Tanneries are the major industries that use chromium for the treatment of leather and nearly 40% of the chromium used is released into the environment as sludge which contaminates surface water as well as groundwater[5]. Chromium is an essential element in trace amounts; however, it is toxic above the permissible limits and chromium containing industrial effluents is the major source of water pollution affecting the living organisms and their distribution[6].
Enzymes are good indicators for animals exposed to contaminants such as metals and other xenobiotics[7]. Enzyme assay has recently emerged as an important diagnostic tool in the field of environmental toxicology and LDH, the terminal enzyme in vertebrate's anaerobic glycolysis, is one of the enzymes that have been employed for diagnosing hepatic and cellular

damage caused by pollutants in fish [8]. Anaerobic metabolism can be measured by LDH activity and this activity can be impaired after prolonged exposure to xenobiotics [9]. Lactate dehydrogenase is Zinc containing, the enzyme and is generally associated with cellular metabolic activities. A fish under stress preferentially meet its energy requirements through an anaerobic oxidation process and thus LDH can be used as an indicator in monitoring metal-induced toxicity in fish. Stressed fish (*O. massambicus*) exposed to phenol may continuously move opercula in need of oxygen because a lack of oxygen may be induced the anaerobic oxidation to release energy by enhanced LDH activity [10]. Abdullakareem and Owalabi, (2014) [11] have also reported enhanced the activity of LDH in hybrid catfish Heteroclarias and contaminated Heteroclarias-fed rats when exposed to sub-lethal concentrations of Monocrotophos (MCP). The increased level of lactate dehydrogenase (LDH) activity is a marker for tissue damage in fish [12], hypoxic conditions, muscular damage and serves as a good diagnostic tool in toxicology. Stress and injuries cause damage to cells and tissues of organs releasing LDH enzyme in blood. The objective of this paper is to explore the toxic effect of tannery waste water on LDH of freshwater fish *Channa punctatus,* as LDH is considered as a useful biomarker to determine the health status of the fish and pollution level of the aquatic system.

2. Materials and Methods

The dilution effect of tannery wastewater toxicity on freshwater fish *Channa punctatus* was conducted to explore the tolerance and toxic limits against a different level of diluted tannery wastewater. All the experiment on test species *Channa punctatus* was collaboratively conducted at the Laboratory Department of Environment Science, SHUATS, Allahabad and Department of Environmental Science, I.B.S.B.T. and Department of Health Sciences, C.S.J.M. University, Kanpur.

2.1 Collection of Tannery wastewater sample

Tannery waste waters were collected from the two sites located at Jajmau, Kanpur, and Banthar, Unnao in a 100 Litre of the plastic container and transported to the laboratory.

2.2 Collection and acclimatization of fish sample

The experimental fishes, *Channa punctatus* were collected with the help of fisherman from local water bodies located the nearby region of Kanpur. The fishes were washed thrice in a tap water and then disinfected with 0.02% $KMnO_4$ and 0.004% formalin solution for the removal of external infections *viz.* fungi, algae, etc. The normal healthy fishes were selected for the experiment. Fishes were acclimatized to the laboratory conditions for 15 days prior to the experimentation. The experimental fishes were fed on TOKYO fish food made in Japan twice every day and the water was changed to every second day and temperature of aquaria was maintained the 30±1 °C.

2.3 Experimental setup

For experiment 40 small size fishes with the length of from 11±2 cm and weight of 20±2 g were selected and were divided into 4 groups, consisting of 10 fishes in each and kept separately in 100 L volume glass aquaria. In group I, fishes was maintained as a control without any treatment while the group II, III and IV were exposed to different concentrations (5%, 10% and 20%) of tannery waste water (TWW) for the 15th, 30th and 45th days respectively. The waste products were removed from aquaria water by using good quality of aquaria filter.

2.4 Physico-chemical analysis of tannery effluent and water sample

Water analysis was done following the standard methods [13]. The physico-chemical characteristics such as pH, EC, TDS (total dissolved solids), Total hardness, Total alkalinity, chloride, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand). The pH and EC were measured by digital portable kit (model pp9040) portable battery operated with glass and calomel electrodes.

2.5 Enzyme Assay

Blood was collected in a plain, clean dry tube and allowed to clot. Then the separated fluid was transferred and centrifuged at a moderate speed. The clear serum obtained was transferred carefully to another tube for use. The fish blood serum was used for the estimation of Lactate dehydrogenase enzyme (LDH) by the method of Cabaud and Wroblewski, (1958) [14] and analyzed in fully automatic analyzer SN-831014123, A-25 at the Department of Health Science, C.S.J.M. University Campus, Kanpur.

3. Statistical analysis

The observed data were statistically analyzed by one-way ANOVA and standard error (S.E.) for testing the hypothesis with the help of computer software IBM SPSS Statistics 21.0 program. The data shown are the averages of three replicates ± S.E.

4. Results and Discussion

4.1 Physico- chemical characteristics of tannery waste water

The result of the physio-chemical characteristics of the tannery waste water of both site-1 and site-2 were presented in table 1. All the parameters of the tannery waste water i.e., pH, EC, TDS, Total hardness, Total alkalinity, Chloride, BOD, COD and Cr were observed much higher than the permissible limit given by BIS standard.

The study of effect of tannery wastewater on serum LDH level of fish presented in table-2 shows that the level of LDH of fish serum of both site-1 and 2 was increased significantly with increase in tannery wastewater concentration and also with exposure period in comparison to control (26.71 and 27.91 IU/L) at both site 1 and site 2. With increase in concentration of tannery waste water the level of LDH increased from 31.66 and 29.57 in 5% concentration of tannery wastewater to 50.64 and 48.25 IU/L in 20% concentration of tannery wastewater in both site 1 and site 2 respectively, and with increase in exposure period the maximum level of serum LDH was observed 57.13 ad 53.66 IU/L at 45th day in 20% concentration of tannery wastewater at both site 1 and site 2.

Table 1: Physico- chemical characteristics of tannery waste water

References		pH	EC (µS/cm)	TDS (mg/L)	TH (mg/L)	TA (mg/)	Cl (mg/L)	DO (mg/L)	BOD (mg/L)	COD (mg/L)	Cr (mg/L)
Mwinyihija *et al.*, (2006) [15]		7.66	-	-	-	-	1693.7	nil	438.5	1307.4	0.93
Singh and Rao, (2013)[16]		3.7-5.9	-	10340-10675	2400-3500	993-1436	231-293	0.4-1.8	835-1125	4390-4879	12.76-16.65
Wosnie and wondie, (2014) [17]		7.15	3953.25	2003.25	-	-	1408.125	-	342	850.75	3.535
Sugasini and Rajgopala, (2015) [18]		7.76	7879.08	5543.8	1245	665.5	1147.6	-	856.3	2281.8	-
Farahad ali *et al.*, (2015) [19]		6.56	5409.50	3516.5	-	1018.08	1333.67	Nil	754.5	2776.83	12.95
In this study	Site-1	10.18	14690	14500	1190	1366	3224	Nil	1218	3152	60.01
	Site-2	9.12	11230	9100	1060	1234	2163	Nil	890	2651	36.35

Table 2: Effect of Tannery Wastewater on LDH (IU/L)

Study area	Treatments	Exposure period			F value
		15th day	30th day	45th day	
Site 1	Control	27.58±0.25	27.91±0.56	27.72±0.28	.181
Site 2	Control	26.71±0.66	26.38±0.33	26.38±0.89	.082
Site 1	5% TWW	31.66 ^{a} ±0.40	37.65 ^{b} ±1.14	42.01 ^{c} ±0.62	43.44
Site 2	5% TWW	29.57 ^{a} ±1.22	32.94 ^{b} ±1.59	34.84 ^{b} ±0.99	8.35
Site 1	10% TWW	37.04 ^{a} ±0.63	39.86 ^{b} ±0.26	45.47 ^{c} ±0.40	87.67
Site 2	10% TWW	37.61 ^{a} ±0.41	38.95 ^{a} ±0.95	43.88 ^{b} ±1.08	14.44
Site 1	15% TWW	48.93 ^{a} ±0.73	51.00 ^{a} ±0.56	55.33 ^{b} ±1.76	8.05
Site 2	15% TWW	42.56 ^{a} ±0.33	45.96 ^{b} ±1.25	47.33 ^{b} ±0.78	7.87
Site 1	20% TWW	50.64 ^{a} ±0.95	53.80 ^{ab} ±1.87	57.13 ^{b} ±1.07	5.68
Site 2	20% TWW	48.25 ^{a} ±0.52	51.77 $^{a\,b}$ ±1.47	53.66 ^{b} ±1.20	5.80

Site -1= Kanpur, Site-2= Unnao, TWW= Tannery Waste water
LDH= Lactate Dehydrogenase; Values are mean ± SEM, n=3, Significant at p<0.05 level.

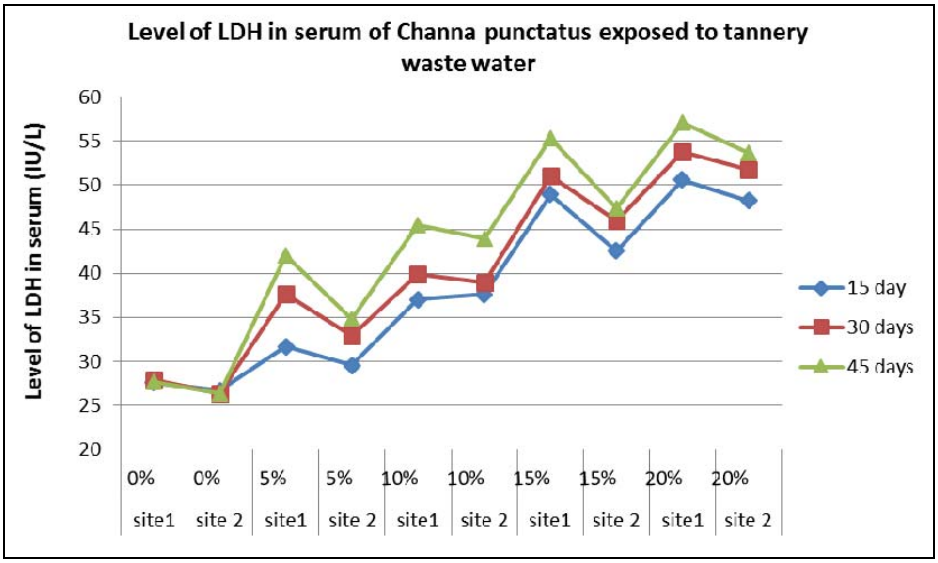

Fig 1: Level of LDH in serum of *Channa punctatus* exposed to tannery waste water

In present study increased level of serum LDH was observed with increase in tannery wastewater concentration and prolonged exposure period. The increase in TDS, chloride and metal concentration in tannery wastewater causes stress condition and damaging the gill structures which reduces the oxygen uptake by the gills required for aerobic respiration. Thus lowered amount of oxygen in tissues performs the anaerobic activity for the energy requirement which is furnished by the enhanced level of serum LDH. The similar result was observed in *Oreochromis mosambicus* on exposure of Zn and Hg (44.76 ±0.56 IU/L at 8th day of exposure period), Koundinya and Ramamurthi in *Tilapia mossambica* on exposure of sumithion (fenitriothion) [20]. Saha *et al.*, (1999) [10] also reported that a stressed fish (*O. massambicus*) exposed to phenol may continuously move opercula when in need of oxygen, which may be induced the anaerobic oxidation to release energy by enhanced LDH activity. LDH is an important glycolytic enzyme in biological systems and is inducible by oxygen stress [21]. According to Amin *et al.*, (2005) [22] K_2Cr_2O7 significantly elevated LDH activity in serum which considered as a presumptive marker of necrotic lesions. Cell necrosis leads to rising in LDH enzyme concentration in serum and tissue, provides an index of cell death and membrane permeability to LDH, and an increase in its activity in the serum occurs as a result of cell membrane disintegration and enzyme leakage.

LDH is cytoplasmic and an anaerobic enzyme involved in the conversion of pyruvate to lactate for the production of glucose. It is generally associated with a cellular metabolic activity. It is a crucial enzyme in the glycolytic pathway and

tricarboxylic acid cycle. The enzyme shows an increasing activity during a strenuous muscle exercise [23]. The liver is one of the richest sources of LDH and the leakage of an enzyme from an even small mass of damaged liver tissue can increase the observed level to a significant extent. The increase in the activity of enzymes after exposure to some pollutants was explained as a result of the destruction of liver cells and increased cell permeability leading to a leakage of the enzymes from the damaged liver cells into the serum [24].

The increased level of the lactate dehydrogenase is due to an alternative aerobic glycolytic pathway in the conversion of lactate to pyruvate for the production of glucose which is a major source of energy during stress induced by heavy metals [25]. The variation of lactate dehydrogenase activity can thus be used as another sensitive index for assessing heavy metal toxicity. Oluah *et al.,* (2005) [26] recorded increased activity of serum and liver dehydrogenase (LDH) enzyme in *C. punctactus* exposed to increasing concentrations of sub-lethal Gammalin 20 and Acetellic 25 EC. LDH is present in the cytoplasm of different cells of the body. However, an increase in the bloodstream can be related to liver or muscle damage [27, 11]. The extent of cellular injury due to CrVI toxicity was assessed by monitoring the value of LDH [28].

Thus, it is obvious that the increase of degenerative effects of Cr become more prominent with the increase in the LDH activity in serum. Increase in LDH activity in the serum of Rats exposed to K_2Cr2O_7 treated group [29]. The elevated lactate dehydrogenase (LDH) activity is a marker for tissue damage and its increased level is reported in liver necrosis.

5. Conclusion

It was concluded from the study that fishes were stressed after being exposed to different concentration of diluted tannery wastewater. The pollutants present in tannery wastewater (TDS, chloride) and metal chromium altered the activity of the enzyme LDH significantly by causing cellular damages in fish. The result thus indicates that it will be a valuable tool in biological monitoring of pollution.

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