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Physiological Stress Induced by Vegetable Oil Factory Effluent in *Channa punctatus* (Bloch): Measurement of Hepatic Dehydrogenases

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Industrialization affects water, air and soil, based on the type of industry and the manufacturing process involved (Billings and De-Hass 1971; Hodges 1973). In the aquatic ecosystems, industrial effluents enter either by their direct disposal or via runoff of rain water, where they adversely affect the fish and other living organisms at morphological and physiological levels, finally having an influence on their fecundity (Shaffi 1981; Oikari and Nakari 1982; Saxena et al. 1982; Kondal et al. 1984).

Dehydrogenases are the enzymes involved in the energy release by the biological oxidation of food stuff inside the mitochondria, and also in the production of reduced potential (NADPH) required in the biosynthetic and detoxification mechanisms. Since liver is the principal organ for biosynthesis, metabolism and detoxification of various endogenous waste products, it is natural to expect alterations in it when fish are exposed to environmental stresses (Saxena et al. 1982; Sastry and Rao 1984). The present investigation was undertaken to study in vivo the effects of vegetable oil factory effluent on hepatic dehydrogenases viz. glucose-6-phosphate dehydrogenase (EC 1.1.1.49; G-6-PDH), lactate dehydrogenase (EC 1.1.1.27; LDH), glutamate dehydrogenase (EC 1.1.1.42; GDH), isocitrate dehydrogenase (EC 1.1.1.43; ICDH), malate dehydrogenase (EC 1.1.1.37; MDH) and malic enzyme (EC 1.1.1.40; ME) in a freshwater teleost, Channa punctatus (Bloch) when exposed for 28 days.

MATERIALS AND METHODS

Live C. punctatus between 12.0 and 17.0 cm (24.0-32.0 g) were collected from Buddah Nullah, a freshwater

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tributary of river Satluj in Ludhiana city. The fish were washed with a dilute solution (1.0 ppm) of potassium permanganate ($KMnO_4$) to remove bacteria, then rinsed in freshwater repeatedly, and acclimatized to the laboratory conditions in glass aquaria (92X46X 46 cm) for fifteen days before the commencement of experiment. Physiological experiments were performed with vegetable oil factory effluent collected from M/s Markfed Vanaspati and Allied Industries, Khanna (District:Ludhiana). Tap water and vegetable oil factory effluent were subjected to physico-chemical analysis as per methods of American Public Health Association Inc. (1975). Fish in groups of twenty five each were transferred to the experimental aquaria containing different sublethal concentrations (2.5, 5.0 and 7.5 v/v) of vegetable oil factory effluent (96 h LC_{50}=16.10 v/v). During acclimatization and exposure periods, fish were fed ad libitum on goat liver three times a week followed by the immediate renewal of test media. Controls were maintained simultaneously in tap water alone.

Six specimens in groups of two each were sacrificed from each treated and control aquaria at seven days interval for 28 days, and the liver was taken out, washed, cut into pieces and weighed. For each group homogenate was prepared separately in 0.5M Tris-HCl buffer (pH=7.6) using Potter Elvehjem Teflon Pestle homogenizer keeping the homogenizing tube in ice bath. The homogenates were centrifuged at 100Xg for 10 min in a refrigerated centrifuge (Model Janetzke-K-24) to remove oil debris and the unbroken cells. In supernatants enzymes were assayed following a change in optical density at 340 nm using Ca-Biochem Ic-340-B-photometer, resulting from the reduction of $NAD^+/NADP^+$ or oxidation of NADH/NADPH at 30 sec interval for 3.0 min (Bergmeyer 1974). Statistical significance of the difference between control and experimental values was calculated by Students' 't' test (Fisher 1950).

RESULTS AND DISCUSSION

Report of physico-chemical analysis of tap water and the vegetable oil factory effluent is given in Table 1. Values of enzyme activity in liver of control and treated fish alongwith the mean standard error, and the values of per cent inhibition/stimulation in enzyme activity in treated fish are given in Tables 2 and 3. It is clear from Tables 2 and 3 that vegetable oil factory effluent has biphasic effects on these enzymes. G-6-PDH (except at 2.5 and 5.0 v/v after 7 days), ICDH, MDH, (except at 2.5 v/v after 7 days) and malic enzyme were found to be inhibited, whereas LDH and GDH were

Table 1 Analytical report of tap water and vegetable oil factory effluent

S.No.	Parameters	Values*	
		Tap water	Oil factory effluent
1.	Temperature (^{o}C)	20.0	20.0
2.	pH	7.2	9.5
3.	Colour	Colour	Brown
4.	Suspended solids		
	a) Fixed	12.1	280.0
	b) Volatile	4.1	110.0
	c) Total	16.2	390.0
5.	Dissolved solids		
	a) Fixed	4.8	5575.0
	b) Volatile	1.5	1025.0
	c) Total	6.3	6600.0
6.	Ammonical Nitrogen(N)	Nil	12.5
7.	Dissolved Oxygen (DO)	6.7	0.0
8.	Biochemical Oxygen Demand (BOD)	2.75	2900.0
9.	Chemical Oxygen Demand (COD)	3.12	4800.0
10.	Oil & Grease	Nil	336.0
11.	Chloride (Cl^{1-})	Nil	2000.0
12.	Sulphate (SO_4^{2-})	9.20	843.0
13.	Phosphate (PO_4^{3-})	Nil	0.58

*Values except pH, colour and temperature are expressed in ppm

stimulated at all concentrations. Further, inhibition or stimulation in enzyme activity was found to be dose and duration dependent. The alterations were maximum and significant ($p < 0.01$; $p < 0.001$) in fish exposed at 7.5 v/v concentration at 28 days interval (except LDH where alterations were maximum at 21 days), but minimum and insignificant at 2.5 v/v (except in G-6-PDH at 5.0 v/v) at seven days interval.

Table 2 Effect of vegetable oil factory effluent on enzyme activity in liver of *C. punctatus*.

Enzyme	Duration of exposure (days)	Control (Enzyme activity)[a]	Treated 2.5 v/v Enzyme activity	2.5 v/v % alterations[b]	5.0 v/v Enzyme activity	5.0 v/v % alterations	7.5 v/v Enzyme activity	7.5 v/v % alterations
G-6-PDH	7	58.8±3.9	66.2±5.1	+12.6	62.0±5.3	+ 5.4	49.6±5.5	-15.6
	14	62.6±4.8	58.5±8.2	- 6.6	47.7±2.7	-23.9*	40.7±3.3	-35.0*
	21	63.2±3.0	51.0±4.1	-19.2	38.1±2.0	-39.7**	31.8±2.9	-49.7**
	28	65.1±3.0	47.2±3.3	-27.5*	33.3±3.1	-48.5**	29.1±2.7	-55.3***
LDH	7	3250.5± 112.6	3583.6± 345.6	+10.3	3971.1± 312.1	+22.2	5003.8± 278.9	-53.9**
	14	3213.9± 145.5	4672.4± 210.0	+45.4**	4893.5± 221.7	+52.3**	7856.4± 422.7	+144.5***
	21	3298.2± 208.8	5494.4± 269.0	+66.5**	5891.5± 310.3	+78.6**	9550.9± 299.2	+189.6***
	28	3339.3± 200.2	5857.9± 316.6	+75.4**	5691.6± 296.5	+70.4**	7859.8± 410.2	+135.4***
GDH	7	16.8±1.2	18.5±3.7	+10.1	21.0±2.9	+25.3	21.6±1.3	+28.5
	14	17.9±1.3	24.4±1.6	+34.5*	24.7±1.8	+38.4*	24.9±2.1	+39.3*
	21	18.0±1.2	28.4±2.2	+57.7**	29.8±2.2	+65.6**	30.7±2.3	+70.2**
	28	17.6±1.1	26.5±2.9	+50.5*	28.6±2.2	+62.7**	36.0±2.1	+104.4***

a Enzyme activity is expressed as mμ per mg of protein

b (+) values indicate per cent stimulation; (-) values indicate per cent inhibition
Values are statistically significant at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$
(Fisher 1950)

Table 3 Effect of vegetable oil factory effluent on enzymes activity in liver of *C. punctatus*.

Enzyme	Duration of exposure (days)	Control (Enzyme activity)[a]	Treated					
			2.5 v/v		5.0 v/v		7.5 v/v	
			Enzyme activity	% alterations[b]	Enzyme activity	% alterations	Enzyme activity	% alterations
ICDH	7	21.7±1.9	18.5±2.2	-14.4	16.7±2.3	-22.9	13.4±1.2	-38.0*
	14	22.2±1.7	17.3±1.9	-21.9	14.1±1.2	-36.5*	11.2±1.2	-49.5*
	21	21.7±1.7	13.1±1.2	-39.4*	10.7±0.9	-50.8**	9.6±0.8	-55.7**
	28	21.9±1.8	13.8±0.9	-36.9**	8.9±1.1	-59.2**	7.7±0.6	-64.8***
MDH	7	7.6±0.5	8.2±0.6	+ 8.4	6.8±0.6	-10.3	5.7±0.3	-24.7*
	14	7.7±0.5	6.6±0.5	-14.4	5.4±0.3	-29.6*	4.6±0.3	-39.6**
	21	7.5±0.4	5.4±0.5	-27.4*	3.9±0.3	-47.9**	3.5±0.4	-52.6**
	28	7.3±0.5	5.9±0.6	-19.0	3.7±0.4	-49.7**	2.9±0.4	-59.4**
ME	7	3.4±0.3	3.2±0.4	- 4.6	2.9±0.4	-14.3	2.7±0.2	-20.9
	14	3.5±0.2	2.9±0.3	-17.5	2.5±0.2	-28.7*	2.1±0.2	-39.7**
	21	3.8±0.3	2.6±0.2	-29.8*	2.3±0.3	-37.9*	1.7±0.2	-54.7**
	28	3.6±0.4	2.7±0.4	-24.7	2.0±0.2	-44.5*	1.6±0.2	-55.2**

a Enzyme activity is expressed as mμ per mg of protein

b (+) values indicate per cent stimulation; (-) values indicate per cent inhibition. Values are statistically significant at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (Fisher 1950)

Vegetable oil factory effluent is a complex effluent containing high amounts of ammonical nitrogen, chlorides, phosphates and sulphates in dissolved and suspended form, and the oil and grease in emulsified form (Table 1). Its toxicity to fish was a combined effect of different components. The toxicity was further found to be affected by low dissolved oxygen, and high pH, biochemical oxygen demand (BOD) and chemical oxygen demand (COD). It has been observed that in the oxygen deficient environment, fish must pass more water over gills in order to meet the oxygen requirements. This leads to rapid absorption of toxic components via gill epithelium, causing tissue anoxia (Lloyd 1961) followed by a rise in blood pressure and quickening of heart beat (Mott 1957). This results in an increased rate of blood circulation through gills and around the body thus, to some extent responsible for rapid effect of poisons under the conditions of oxygen deficiency (Mott 1957; Hughes and Shelton 1958).

G-6-PDH is involved in the pentose phosphate pathway, which is an alternate route for glucose metabolism. This enzyme catalyses the conversion of β-D-glucono-6-phosphate to 6-phosphoglucono-δ-lactone and this reaction is the major step for generation of reduced potential (NADPH) required in the biosynthetic and detoxification mechanisms. Decrease in the G-6-PDH activity in liver indicates the decreased production of NADPH thus, resulting in the decreased biosynthetic and detoxification mechanisms. This could lead to increased fish mortality thus, influencing their fecundity. LDH is an enzyme of universal distribution which catalyses the reversible transformation of pyruvate to lactate. Under anaerobic conditions, it converts the pyruvate to lactate. Increased activity of LDH in liver could be due to the adaptation of fish to meet the energy requirements when TCA cycle is not fully operative. Saxena et al. (1982) in C. punctatus, and Gupta (1984) in Notopterus notopterus also observed a significant decline in LDH activity in liver following exposure to vegetable oil factory effluent and phenolic compounds, respectively, thus supporting the findings of this investigation. Enzyme GDH is involved in the aminoacid metabolism and catalyses the reversible conversion of L-glutamic acid to α-ketoglutarate. In liver, this enzyme functions in the presence of either NAD^+ or $NADP^+$ and is localized in mitochondria. The increased GDH activity suggests an enhanced breakdown of protein due to aminoacid catabolism. Sastry and Rao (1984) also observed an increased GDH activity in liver of C. punctatus following sublethal exposure to mercuric

chloride for 120 days.

ICDH is an enzyme which catalyses the oxidative β-decarboxylation of isocitric acid to ∝-ketoglutaric acid and carbondioxide in the presence of divalent cations (Mg^+ or Mn^+). Most tissues contain two kinds of isocitrate dehydrogenases. One of these requires NAD^+ and Mg^+, and is localized in mitochondria only. The other kind of isocitrate dehydrogenase requires $NADP^+$, and occurs both in mitochondria and cytoplasm. The NAD^+ specific enzyme is involved in the working of TCA cycle. While mitochondrial $NADP^+$ requiring enzyme is associated with other anabolic activities of TCA cycle. Hence, the decreased ICDH activity in liver might be associated with decreased conversion of isocitric acid to ∝- ketoglutaric acid, indicating inhibition of TCA cycle, i.e., cellular oxidation in liver of fish exposed to industrial effluent. Enzyme MDH catalyses the conversion of malic acid to oxaloacetate in TCA cycle. Decreased MDH activity in liver, thus suggests inhibition of TCA cycle in effluent exposed fish.

Malic enzyme (ME) catalyses the reversible formation of malate from pyruvate and carbondioxide and also the production of NADPH required for the biosynthetic processes. Decline in the activity of malic enzyme in liver has been associated with low rate of conversion of pyruvate to l-malate which in turn results in decreased fatty acid synthesis as has been observed by the author (unpublished data). Thus, it is likely that inhibition of G-6-PDH, ICDH, MDH and ME, and stimulation of LDH and GDH in liver caused by vegetable oil factory effluent would creat conditions unfavourable for oxidative metabolism of glucose and finally may influence the whole metabolism of body.

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