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Impact of Zinc and Lead on Liver Enzymes of Fish *(Cirrhinus mrigala)*

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Abstract The fresh water field carp, *Cirrhinus mrigala* is an important human food source in northern part of India and the carp is constantly exposed to heavy metals. The toxic effects of zinc and lead on the experimental fish for the three treatments T_1, T_2 and T_3 containing zinc and lead at concentrations 0.01, 0.02, 0.04 ppm, respectively in triplicates was carried out. Quantitative study of biochemical changes of some enzymes like: PDH (Pyruvate dehydrogenase), SDH (Succinate dehydrogenase), GDH (Glutamate dehydrogenase) were undertaken.

Keywords Heavy metals, Zinc, Lead, Impact on enzymes, *Cirrhinus mrigala.*

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Introduction

The pollution of aquatic ecosystems by heavy metals is an important environmental problem as heavy metals constitute some of the most hazardous substances that can bioaccumulate. Bioaccumulation is a process in which a chemical pollutant enters into the body of an organism and is not excreted, but rather collected in the organism's tissues (Zweig et al. 1999). Metals that are deposited in the aquatic environment may accumulate in the food chain and cause ecological damage while also posing a threat to human health (Adams et al. 1992). These health concerns are quite considerable. For example, cancer, damage to the nervous system has all been documented in humans as a result of metal consumption (Zweig et al. 1999). The US Environmental Protection Agency conducted a national study of accumulated toxins documenting this concern (USEPA 1992). Anthropogenic impacts including industrial discharges, domestic sewage, non-point source runoff and atmospheric precipitation are the main sources of toxic heavy metals that enter aquatic systems (Langston et al. 1999). Fish species are often the top consumers in aquatic ecosystems (Dallinger et al. 1987) and thus metal concentrations in fish can act as an environmental indicator. There are two main ways heavy metals can enter the aquatic food chain, either directly through the digestive tract due to consumption of contaminated water or food, or non-dietary routes across permeable membranes such as gills (Handy 1993).

Materials and Methods

In the present study, 6-8 inch long fingerlings of *C. mrigala* were collected from the local fish farm and acclimatized for two weeks in large tanks already filled dechlorinated tap water with proper aeration under laboratory conditions. These were exposed to the sublethal concentrations (<0.1 ppm) of zinc and lead for 45 days ; in small plastic tanks of 40.1 capacity in triplicates with 10 fishes each replicate; and were fed with normal standard diet on alternate days at the rate of 2% of total fish body weight. The treatments were T_1, T_2 and T_3 having concentration of 0.01, 0.02 and 0.04 ppm of zinc and lead; while T_0 was control having dechlorinated water only. The three fishes were taken and sacrificed from each replicate at the end of 45 days and analyzed for the estimation of liver tissue total enzymes like PDH (Pyruvate dehydrogenase), SDH (Succinate dehydrogenase), GDH (Glutamate dehydrogenase).

Enzyme estimation

The tissue (muscle, liver) from the freshly dissected fish were homogenized for 1-3 minutes in motor driven ground glass homogenizer and a stock solution for the enzyme was made at a concentration of 5 mg per ml (wet weight in 0.1 M phosphate buffer (pH 7.7) in icc cold condition). The contents were centrifuged at 4^0C at 10,000 rpm and the supernatant was used stock enzyme solution. The activities of enzyme were estimated by the method given by Nachlas et al. (1960).

Table 1. Effects of lead on different dehydrogenase enzyme activities (µmol of product derived/mg protein/h) in liver of *C. mrigala* after 45 days treatment. Figures in parentheses are the percentage change over control.

Treatment dose (ppm)	Enzyme activities (µmol of product derived/mg protein/h)		
	SDH	GDH	PDH
Control	26.53 ± 1.21	25.20 ± 1.30	24.60 ± 1.16
0.01	25.43 ±0.38 (4)	25.13 ± 0.46 (2.6)	24.93 ± 2.27 (1)
0.02	24.46 ± 0.80 (7)	24.50 ±0.81 (2.7)	21.83 ±2.46 (11)
0.04	17.16 ±0.56 (35)	14.30 ± 0.60 (43)	13.33 ± 1.31 (45)
SE (m)	0.80	0.85	1.89
CD at 5%	2.61	2.79	6.17

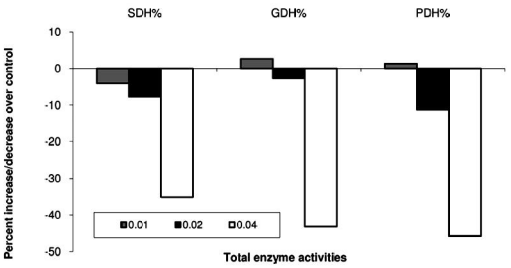

Fig. 1. The effect of lead on different dehydrogenase enzyme activities (µmol of product derived/mg protein/h) in liver of *C. mrigala* after 45 days treatment.

The obtained results were analyzed by statistically (Snedecor and Cochran 1994).

Results and Discussion

In animal body all the chemical reactions and metabolism processes are controlled by the enzymes activities. So, enzymes are very essential for body functioning. Any decrease or increase of these enzymes affects the body functioning and metabolism process.

SDH (Succinate dehydrogenase), GDH (Glutamate dehydrogenase) and PDH (Pyruvate dehydrogenase) are the key respiratory enzymes are mostly are inhibited by the action of heavy metals (zinc and lead) after 45 days treatment. In present study Table 1 and Fig. 1 shows lead treatment causes inhibition in enzymatic activities. The SDH (Succinate dehydrogenase) was 4.14% inhibition at 0.01 ppm, 7.78% at 0.02 ppm and 35.16% at 0.04 ppm dose level. In case of GDH (Glutamate dehydrogenase) it was found that 2.6% inhibition occurred at 0.01 ppm, 2.74% at 0.02 ppm and 43.25% at 0.04 ppm while in the case of PDH (Pyruvate dehydrogenase) it was found to be 1.35% inhibition at 0.01 ppm, 11.25% at 0.02 ppm and 45.80% at 0.04 ppm in the liver tissue of fish.

Table 2 (Fig. 2) shows the zinc treatment causes an inhibition in enzymatic activity of SDH by 9.55% at 0.01 ppm, 11.81% at 0.02 ppm and 13.92% at 0.04 ppm as compared to the controls. GDH activity was reduced by 7.08% at 0.01 ppm, 12.33% at 0.02 ppm and 26.92% at 0.04 ppm. PDH activity was reduced by

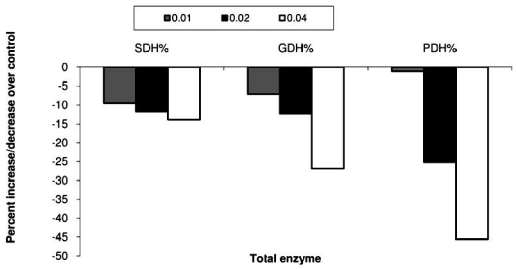

Fig. 2. The effect of zinc on different dehydrogenase enzyme activities (µmol of product derived/mg protein/h) in liver of *C. mrigala* after 45 days treatment.

1.15% at 0.01 ppm, 25.22% at 0.02 ppm and 45.57% at 0.04 ppm.

According to Passow et al. (1961) and Hodson (1988) the inhibitory action of the heavy metals on enzymes of fish was due to the binding capacity of the metals with enzyme protein. Heavy metal ions can attach with active site of the enzyme and inhibit time of enzyme activity. Further Eichhorn (1975) has pointed out heavy metals can also bind with sites other than the active sites at the enzyme molecule of fish and can produce both beneficial and adverse effects depending upon the concentration of the heavy metals. At the low concentration, heavy metals may have a beneficial effect upon the enzyme while at high concentration the excess metal ions attach to other sites there by causing inhibition.

Table 2. Effects of zinc on different dehydrogenase enzyme activities (µmol of product derived/mg protein/h) in liver of *C. mrigala* after 45 days treatment. Figures in parentheses are the percentage change over control.

Treatment dose (ppm)	Enzyme activities (µ mol of product derived/mg protein/h)		
	SDH	GDH	PDH
Control	25.13 ±1.52	25.40 ±1.25	26.46 ± 0.285
0.01	22.73 ±0.83 (9)	23.60 ± 0.60 (7)	18.40 ± 0.50 (1)
0.02	22.16 ± 0.73 (11)	22.26 ±1.36 (12)	19.80 ±2.64 (25)
0.04	21.63 ± 1.69 (13)	18.56 ±0.39 (26)	14.40 ± 0.34 (45)
SE (m)	1.26	0.99	1.21
CD at 5%	NS	3.24	4.44

James et al. (1992) studies the effects of sublethal doses (Cu, Cd, Zn) mixture on the activities of SDH, GDH and PDH in the freshwater fish *O. massambicus*. The treatment of 96 h of <50 Cu was highly toxic, followed by Zn and Cd and the trimetal combination (Cu+Zn+Cd) was extremely toxic than any other combination. The combination of Zn+Cd was least toxic. It is further evidence that the level of glycogen and pyruvic acid decreased while lactic acid showed an increase. Activities of LDH, SDH and MDH enzyme decreased, while G-6 PDH activity increased with sublethal toxicity of copper.

Sastry and Gupta (1978) showed that the chronic treatments with heavy metals appear to be more effective in inhibiting the activities of peptidases as the inhibition produced by chronic mercury and lead treatment resulted in a marked inhibition in the activity of two peptidases than acute tretment in *Channa punctatus*.

Dinodia et al. (2003) studied proteolytic enzyme activity of *C.mrigala* and *L. rohita* at two levels of the cadmium treatment and reported an inverse relationship between cadmium concentration and the proteolytic enzyme activity. A decrease in the enzyme activity was upto 56.49% in *C. mrigala*. 32.25% in *C. carpio* and 46.04% in *L. rohita* at two levels of the cadmium treatment and reported an inverse relationship between cadmium concentration and the proteolytic enzyme activity. Decrease in enzyme activity was upto 56.49% in *C. mrigala* 32.25% in *C. carpio* and 46.04% in *L. rohita* at 5.00 ppm.

The toxic effects depend upon fish species and the concentration of metals (Kuzmina et al. 2004). Thus for all the investigated species viz., carp, bream, roach more toxic effects of copper in comparison with zinc was shown. 25 mg/l of copper concentration reduces amylolytic activity in roach by 38%, while the zinc practically does not change it. The low concentration (0-1-1.0 mg/l) of these metals cause an increase in the enzyme activity significantly especially in carp fry. The increase of copper and zinc concentration gradually reduced the intestinal mucosa proteolytic activ-

ity in all investigated fish species. However, the reduction in the degree of enzymes activity at high concentration of copper (10 and 25 mg/l) for different species is variable in carp and bream by 35-40%, in perch 55-60% and 75-80% as compared to the control.

Heavy metals show the special effects on enzyme activity (Heath 1995). It was also observed in Cd treated fresh water rosy carp. Alkaline phosphatase was unaffected in liver and gills, stimulated in kidney and ovary and inhibited in the gut (Gill et al. 1991). Acid phosphatase activity was inhabited in liver, ovary and gills but the enzyme activity increased in kidney and intestine. The activity of alkaline phosphatase decreased in liver, kidney and intesting but elevation was recorded in ovary and muscles of *H. malitrix* after a chronic exposure of 0.26 mg/l of cadmium (Sastry and Subhadra 1985).

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