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Impact of Heavy Metals on Digestive Enzyme Activity in *Cirrhinus mrigala*

Dr.Sarita

Associate Professor, Department of zoology, Govt. College Hisar. Haryana, India

Abstract: The study used commonly cultivated, healthy *C. mrigala* that was subjected to As, Hg, Ni, and Cr alone and As in combination with Hg, Ni, and Cr at two sublethal dose levels (0.025 and 0.05 ppm). All heavy metal treatments reduced the activity of the digestive enzymes' protease, amylase, and lipase. Different heavy metal combinations have more toxic effects than heavy metal alone. These studies clearly evidenced enzyme activity measurements as direct index of metal toxicity in fishes.

Keywords: Heavy metal toxicity, *Cirrhinus mrigala*, Digestive enzymes.

I. INTRODUCTION

Environmental pollution today, has become an international problem. Everyday tons of chemicals are released into the environment, thus disturbing the delicate ecological balance. Pollution by heavy metals is of prime importance as they are frequently discharged into water by industrial effluents. They exert toxic effect in the organisms at tissue, cellular and molecular level. They mainly affect the permeability of the cell membrane and disturb the energy metabolism including the activity of enzymes due to their binding with proteins or metal replacement by heavy metals. This study is thus aimed to highlight the effects of certain commonly available heavy metal pollutant in water, affecting digestive enzyme functions in locally cultured fish *C. mrigala*, which could constitute important biomarkers as clinical test for determining heavy metal toxicity in fishes.

II. MATERIALS AND METHODS

C.mrigala weighing 90±15 were procured from local fresh water ponds and were acclimatized in tank filled with well aerated water for seven days. These were treated with As, Hg, Ni and Cr individually and As in combination with Hg, Ni and Cr at 0.025 and 0.05 ppm concentrations in plastic tubs of 40 litre capacity. After 45 days treatment, the fishes were dissected to remove their intestine for the estimation of digestive. The enzyme protease was analysed following method of Walter (1984a) using BSA as substrate, amylase enzyme was analysed by Bernfeld (1955) method using starch as substrate and the enzyme lipase was determined using vegetable oil mixed with sodium taurocholate and gum arabic as substrate (Walter, 1984b).

III. RESULT AND DISCUSSION

Digestive enzymes are responsible for the breakdown of food particles in the alimentary canal. These also influence growth and food utilization by fishes. Alterations in the activities of protease, amylase and lipase induced by different heavy metals were studies in *Cirrhinus mrigala*. The activities of all the enzymes were found to be inhibited (Table 1). Arsenic (0.05 ppm) registered maximum reduction in the activity of all the enzymes on their exposure to heavy metals. The reduction was 23.1, %, 17.2% and 19.1% for protease, amylase and lipase respectively. Nickel showed minimum reduction ranging in between 2.3% to 7.9%.

A pursual of results in both the fish species, indicate that the toxic effects of different combination of heavy metals were more severe than single heavy metal treatment. Hg in combination with As at 0.05 ppm showed maximum reduction of 28.3 and 31.1 per cent respectively of protease and amylase enzyme while activity of lipase enzyme was reduced to the maximum by Cr in combination with As at 0.05 ppm, followed by As + Hg and As + Ni combinations. Activity of all the digestive enzymes was inversely proportional to concentration of heavy metal.

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Essentially all chemical reactions in cells are catalyzed by enzymes, thus the action of a foreign chemical in the cell almost involves disturbances in enzyme functions. Nevertheless, the direction of change (i.e. increase or decrease in activity) and the relative extent of the effect make these studies further fruitful.

According to Hodson (1988) the inhibitory action of the heavy metals on enzyme is due to the binding of the metal with enzyme protein. Heavy metal ions can displace metals situated at the active site of the enzyme and inhibit the enzyme activity. Further, Eichhorn (1975) has pointed out that heavy metals can also bind with sites other than the active sites of the enzyme molecule and can produce both beneficial and adverse effects depending upon the concentration of the heavy metal. At low concentration, heavy metals may have a beneficial effect upon the enzyme, and at high concentration the excess metal ions attach to other sites, causing inhibition. 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 marked inhibition in the activity of two peptidases, than acute treatment in *Channa punctatus* (Sastry and Gupta, 1978a, b).

Dinodia *et al.* (2003) studied proteolytic enzyme activity in *C. mrigala* and *L. rohita* at two cadmium treatment levels and reported 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. Sastry and Gupta (1979b) reported inhibition in trypsin activity in the digestive system of *H. fossilis* after cadmium chloride intoxication. This study also revealed similar results.

The effects of heavy metals (Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}) on activities of carp trypsin, alpha-chymotrypsin carboxypeptidase A and lipase were studied (Kotorman, 2000). The enzymes were isolated from the gastrointestinal tract and the effects of metal ions were investigated during incubation for 5 minutes. The presence of Cd^{2+} did not influence the activities of CPA and trypsin and 10-20% inhibition was observed with alpha-chymotrypsin and lipase. Cu^{2+} only slightly influenced the trypsin and lipase activities, whereas alpha-chymotrypsin activity was decreased. All enzyme activities decreased at higher Zn^{2+} concentrations. With the exception of trypsin, Pb^{2+} also inhibited the activities of the investigated enzymes. Dongmei Xie et al (2019) observed decrease in intestinal digestive enzyme activity in yellow catfish *Palteobagrus fulvidraca*, when exposed to cadmium for a period of 8 weeks. After exposure, significant growth retardation and Cd accumulation were observed, and obvious histopathological lesions in the intestine such as increased goblet cells, excessive mucus, vacuolization and thickened lamina propria were also detected.

Golovanova *et al.* (1999) studied in vitro effects of cadmium (0.5-50 mg/l) and DDVP (dischlorvos, at 0.2-100 mg/l) on the total amylolytic, sucrase and proteinase activities of intestinal mucosa in eleven fresh water teleosts. Total amylolytic activity in burbot (*Lota lota*), crucian carp (*C. auratus*) and common carp (*C. carpio*), sucrase activity in blue bream and total proteolytic activity in burbot and pipe were significantly decreased by cadmium at 50 mg/l, DDVP (at 0.2 mg/l) caused a significant decrease in total proteolytic activity in pipe and had no effect on either protease or carbohydrase activities in other fish species.

Different copper ($CuSO_4$) and Zinc ($ZnSO_4$) concentrations (0.01-100 mg metal/l) influence carbohydrase activity of fish intestinal mucosa *in vitro*. Toxical effects depends on fish species and concentration of metal (Kuzmina *et al.*, 2004). Thus, for all investigated species (carp, bream, roach) more toxical effect of copper in comparison with zinc was shown. 25 mg/l of copper concentration reduces amylolytic activity in roach by 38%, the zinc practically does not change it. The low concentration (0.1-1.0 mg/l) of these metals cause an increase in enzyme activity, especially significant in carp fry. The increase of copper and zinc concentration gradually reduced the intestinal mucosa proteolytic activity in all investigated fish species. However, the degree of enzyme activity reduction at high concentration of copper (10 and 25 mg/l) for different species is variable; in carp and bream 35-40%, in perch 55-60%, in pike 75-80% from control. These results testify that even allowable concentrations of copper and zinc in food are considerably to reduce activity of enzyme.

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Various histochemical studies (Khillare and Wagh, 1988; Khare, 1993) and findings of Sastry and Subhadra *et al.* (1982) have further suggested that ruptured cells and deformation of tissues on account of heavy metal toxicity affect the functional activity of the digestive enzyme and this may interfere with digestion. Mehrotra (1996) reported a decrease in absorption rate due to damaged villi of intestine. According to Kuzmina (1996) in the fresh water teleost, digestive enzyme activity appeared affected by feeding behaviour, biochemical composition and ambient stress. So, another reason for decrease in digestive enzyme activity may be stress conditions i.e., under heavy metal toxicity stress, the fishes did not feed well (Dinodia *et al.*, 2003).

IV. CONCLUSION

This study indicates that these enzymes estimates could be a good index of the nutritional status as well as a marker of fish health on account of their exposure to pollutants particularly the heavy metal.

Table 1: Alterations in digestive enzyme activity in the intestine of *C. mrigala* exposed to different heavy metals

Enzyme	Control	Treatments (ppm)													
		Hg		Ni		Cr		As		As+Hg		As+Ni		As+Cr	
		0.025	0.05	0.025	0.05	0.025	0.05	0.025	0.05	0.025	0.05	0.025	0.05	0.025	0.05
Protease	1.52±0.03	1.36±0.02	1.26±0.02	1.43±0.03	1.40±0.04	1.37±0.02	1.30±0.04	1.30±0.02	1.17±0.04	1.20±0.02	1.09±0.05	1.30±0.04	1.21±0.03	1.26±0.03	1.15±0.03
% alteration		-10.6	-17.2	-6.0	-7.9	-9.9	-14.5	-14.5	-23.1	-21.1	-28.3	-15.5	-20.4	-17.2	-24.4
Amylase	1.32±0.02	1.17±0.03	1.12±0.02	1.29±0.04	1.26±0.02	1.20±0.02	1.18±0.01	1.13±0.02	1.09±0.03	1.03±0.01	0.91±0.02	1.12±0.03	1.09±0.01	1.08±0.02	1.01±0.01
% alteration		-11.4	-15.2	-2.3	-4.6	-9.1	-10.7	-14.4	-17.2	-22.0	-31.1	-15.2	-17.2	-18.1	-23.5
Lipase	1.42±0.06	1.30±0.06	1.21±0.06	1.39±0.07	1.32±0.06	1.18±0.05	1.30±0.04	1.15±0.05	1.30±0.06	1.26±0.06	1.12±0.05	1.32±0.05	1.15±0.06	1.21±0.04	1.06±0.04
% alteration		-8.5	-14.8	-2.2	-7.1	-7.1	-16.0	-8.5	-19.1	-11.3	-21.2	-7.1	-19.1	-14.8	-25.4

Values are mean of 3 samples ±S.E.
- indicate decrease in enzyme activity over the control.
CD = 0.05 (P<0.05)

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