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Effect of Heavy Metals on some Metabolically Important Enzymes of *Oreochromis mossambicus*

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The effect of sublethal levels of heavy metals like zinc and mercury on the activities of the enzymes, acid phosphatase, aspartate aminotransferase and glutamate dehydrogenase in *Oreochromis mossambicus* was investigated. The activities of the enzymes varied depending on the duration of exposure to the toxicants. The feasibility of measuring the enzyme activities for monitoring sublethal metal poisoning is also discussed.

Key words: Heavy metal, acid phosphatase, aspartate aminotransferase, glutamate dehydrogenase, *Oreochromis mossambicus*

The effects of toxicants on the key enzymes of metabolism have become a topic of interest to toxicologists and biochemists. Studies have demonstrated that structural and other properties of enzyme as well as specific activities can be affected by exposure of animals to pollutants, which may possibly lead to loss of metabolic flexibility (Gould, 1977; Gould *et al.*, 1976; Thurberg *et al.*, 1977). Response of enzymes also varies with rate and magnitude of absorption of toxicant.

Acid phosphatase (EC 3.1.3.2) is the marker enzyme of lysosomes and exists in a latent form. Stimulation or inhibition of this enzyme can result in the disturbance of metabolism and hence is a good indicator of stress in the biological system (Gupta *et al.*, 1975; Verma *et al.*, 1980). Enhanced activity of acid phosphatase, in particular due to any stress, seems to be characteristic of tissue damage and have become a useful diagnostic and experimental tool (Tietz, 1970).

Aspartate aminotransferase (EC 2.6.1.1) catalyzes the process of bilogical transamination and represents a link between carbohydrate, fat and protein metabolism (Cohen & Sallach, 1961). This enzyme is widely distributed in the animal kingdom and serves as an indicator of cell destruction and altered physiological or stress condition (Knox & Greengard, 1965). The use of transaminases in diagnosis of tissue damage in fish was investigated by various workers (Bell, 1968; Mollander *et al.*, 1955; Wroblewski & La Due, 1956; Wroblewski *et al.*, 1956).

Glutamate dehydrogenase (EC 1.4.1.2) is a mitochondrial enzyme containing zinc. It serves as a link between the metabolism of amino acids and carbohydrates. Role of glutamate dehydrogenase has been proposed to explain the sparing effects exerted by carbohydrates and or fats in higher animals (Munro, 1961).

The present study is undertaken to examine the effect of toxicity due to zinc and mercury on the activities of the enzymes, acid phosphatase, asparatate aminotransferase and glutamate dehydrogenase in *Oreochromis mossambicus.*

Materials and Methods

Male specimens of *O. mossambicus* of an average length of 10 cm were collected from

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Rice Research Institute, Vyttila, Cochin and acclimated in the aquarium tanks maintained at pH 7.0, temperature 29±1°C and salinity 0 ppt. The effect of Hg^{2+} and Zn^{2+} on the enzymes, acid phosphatase, aspartate aminotransferase and glutamate dehydrogenase in *O. mossambicus* was studied by rearing the fish in water containing Hg^{2+} or Zn^{2+} at concentrations of 1/10 of 96 h LC 50 (Hg^{2+} 0.1 ppm and Zn^{2+} 0.42 ppm). Stocking density was 1 fish for 5 l of the medium. A control experiment was also run in parallel. Medium in the tank was changed daily, maintaining the toxicant concentration constant after each replacement. Feeding was suspended 24 h prior to the sampling of the fish for assay of enzyme activity. Fish was collected from each tank after 2,4,6 and 8 days and liver was excised causing minimum disturbance to the specimen. The tissue was blotted to remove the adhering fluids and weighed accurately.

For determination of acid phosphatase, the tissue was homogenized in citrate buffer (pH 4.8) and centrifuged at 20,000 x g for 30 min in a refrigerated centrifuge at 0°C. The enzyme activity of the supernatant was assayed following the procedure of Anon, (1963).

Aspartate aminotransferase was determined by homogenizing the tissue in 50 mM phosphate buffer (pH 7.5) and centrifuging the homogenate at 20,000 g for 30 min in a refrigerated centrifuge at 0°C (Wootton, 1964). The activity of the supernatant was assayed following the method of Reitman & Frankel (1957). Activity was expressed as micromoles of pyruvate formed per hour per gram protein.

A homogenate of tissue in 5 mM Tris-HCl buffer (pH 7.8) containing 0.1% (v/v) Triton X-100 was centrifuged at 20,000 g for 30 min in a refrigerated centrifuge at 0°C (Hayashi, 1987) and the supernatant was used for the assay of glutamate dehydrogenase activity following the method of Reiss *et al.* (1977). Activity was expressed as micromoles of NADH oxidized per hour per gram protein.

Protein content of the extract for all the enzymes were estimated by the procedure of Lowry *et al.* (1951) with bovine serum albumin as standard.

The data obtained were analysed using ANOVA technique. Wherever the effects were found to be significant, least significant difference at 5% level was calculated.

Results and Discussion

The effects of zinc and mercury on the activity of acid phosphatase are summarized in Table 1. The difference between zinc and mercury on the activities of acid phosphatase was not significant. The duration of exposure was found to influence the activity of this enzyme. The activating effect produced by mercury was maximum at 6 days of exposure and was different from that at 2 days of exposure. But these differences were not statistically significant. No marked variation was observed after 6 days. The stimulatory effect produced by zinc on acid phosphatase decreased as the period of exposure increased until the 6th day of exposure. On 8th day, the activity was again increasing.

Table 1. Effect on Zn and Hg on the activity of acid phosphatase (μ mol p-nitrophenol/h/g protein)

Time Days	Control	Mercury	Zinc
2	09.58±0.06	10.18±0.05	16.24±0.05
4	11.42±0.21	12.88±0.10	14.30±0.05
6	16.10±1.16	18.49±0.59	14.96±0.08
8	12.32±0.09	14.11±0.03	12.87±0.03

Difference between groups - not significant
Different between exposure period - not significant

Data on the effect of Zn^{2+} and Hg^{2+} on the activity of aspartate aminotransferase are presented in Table 2. The difference between the inhibitory effects produced by zinc and mercury was not significant at 5% level. Duration of exposure was found to have no influence on the inhibitory effects caused by mercury. Zinc on the other hand, had an activating effect at 2 days and inhibitory effect on further exposure and the difference in activity was significant ($p<0.05$).

Table 2. Effect on Zn and Hg on the activity of aspartate amino transferase (μ mol pyruvate/h/g protein)

Time Days	Control	Mercury	Zinc
2	71.56±5.37	59.08±3.64	111.46±2.98
4	47.59±0.00	40.98±5.01	28.28±10.4
6	75.56±0.00	72.89±8.50	73.98±2.18
8	91.92±5.16	76.64±6.02	84.68±0.00

Difference between groups - not significant
Different between exposure period - $p < 0.05$
Least significant difference - 27.3

The effects of zinc and mercury on the activity of glutamate dehydrogenase are shown in Table 3. The difference in activity produced by the two toxicants was not significant at 5% level. But the difference in activity produced by different durations of exposure was significant ($p<0.001$). Enzyme activity during exposure to mercury had a sigmoidal curve; maximum activity being obtained on 6th day of exposure. On 4th and 6th days it was stimulatory whereas on 2nd and 8th days it was inhibitory. Zinc had got a significant stimulatory effect on the 2nd day of exposure and after that the stimulatory effect diminished till the 6th day of exposure.

Table 3. Effect on Zn and Hg on the activity of glutamate dehydrogenase (μ mole NADH oxidized/h/g protein)

Time Days	Control	Mercury	Zinc
2	299.55±22.6	291.88±36.8	440.47±49.2
4	888.48±30.2	950.97±14.7	963.83±21.9
6	616.22±62.7	714.33±14.9	519.80±36.3
8	634.89±35.2	553.50±29.8	654.03±59.6

Difference between groups - not significant
Different between exposure period - $p < 0.001$
Least significant difference - 140.31

The elevation in the acid phosphatase activity could be attributed to the heavy metal induced changes in lysosomal latency (Rema, 1995). Several mechanisms have been put forward to explain the increase in the acid phosphatase activity. A direct positive correlation was noticed between the duration of exposure to mercury and the activity of acid phosphatase. This indicates that the increased internal content of the toxicant on prolonged exposure causes greater tissue damage. Mercury-induced enhancement of acid phosphatase activity has been reported earlier also (Hossain & Dutta, 1986; Hinton & Koening, 1975; Jackim *et al.*, 1970).

A gradual reduction in acid phosphatase activity from the 2nd day was noticed in the case of zinc. It has been explained that on prolonged exposure the organism might counteract the deleterious effects of zinc at the cellular level and the activity of acid phosphatase becomes more or less similar to that of control. The reduction in acid phosphatase activity may be due to the direct effect of zinc on the enzyme or stabilisation of the lysosomal membrane by zinc (Rema & Philip, 1995).

Mercury had an inhibitory effect on aspartate aminotransferase. It is probable that the inhibitory effect of the toxicant on aspartate aminotransferase is at the point of pyridoxal phosphate synthesis (Zubay, 1988) which is an absolute requirement for amino transferase activity. Tissue extract was the sole source of pyridoxal phosphate in this system. Other possible reasons for decreased

aspartate amino transferase activity may be the damage caused to mitochondrial membranes, loss of matrix and swelling of mitochondria which contains aspartate amino transferase, the decreased availability of aspartate aminotransferase or the direct inhibition of the enzyme by mercury (Rema & Philip, 1995).

Exposure to zinc caused an initial induction, followed by a progressive inhibition of the activity of aspartate aminotransferase. The initial stimulation of aspartate aminotransferase observed on 2nd day possibly reflects the increased conversion of amino acids into keto acids and vice versa, thus resulting in the disturbance of protein metabolism. The decline in the activity from 4th day onwards may be attributed to the direct inhibition of the enzyme by zinc, as noticed in the present study. Such a response of aspartate aminotransferase to the toxicant has also been demonstrated by Jackim *et al.* (1970) and Christensen *et al.* (1972).

Enhanced activity of glutamate dehydrogenase may suggest an increased amino acid metabolism and gluconeogenesis. The excess carbohydrates serve as a source of energy for metabolic requirements during stress. At the same time, they exert a sparing effect on protein. The increase in glutamate dehydrogenase activity may also be due to the rupture of mitochondria which contains the major amount of glutamate dehydrogenase in the cell. An elevated glutamate dehydrogenase activity in response to mercuric chloride has been reported in *Channa punctatus* (Sastry & Rao, 1982). Prolonged exposure to both zinc and mercury however causes a decrease in the activity of glutamate dehydrogenase.

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