



Research Article

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Mercury chloride and copper sulphate induced alterations in enzyme secretory activity of fresh water crab, *Barytelphusa guerini*.

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ABSTRACT

Fresh water edible crab, *Barytelphusa guerini* were exposed to acute dose of mercury chloride and copper sulphate. The aim of investigation was to evaluate the effect of heavy metal on the enzyme secretory activity in hepatopancreas of *B. guerini*. The effects were studied and observed seasonally into two groups as control and experimental. The experimental groups were exposed to HgCl_2 and CuSO_4 at different concentration as for 24, 48, 72 and 96 hrs. At the end of 96hrs the crabs were removed and hepatopancreatic tissue was separated and enzyme extract was prepared for assay purpose. The results are reported in mg of maltose/gm of tissue/hr. In the present investigation, the amylase was significantly altered and progressive decrease in amylase activity was observed due to inhibitory action of HgCl_2 and CuSO_4 . Among the exposed mercuric chloride was found to be the potent inhibitors of amylase activity as compared to control and copper sulphate. From the results, it is also observed that the enzyme activity is time dependent.

Keywords: Hepatopancreas, digestive enzyme, Mercury chloride, copper sulphate, *B. guerini*.

INTRODUCTION

Enzymes are referred as highly specialized proteins. Enzymes are the reaction catalyst of biological systems. They have extraordinary catalytic power. They have a high degree of specificity for their substrates, and they functions in aqueous solutions under very mild temperature and pH. Enzymes are one of the keys to understanding how cell survive and proliferate.

The use of heavy metals such as mercury, copper, lead, cadmium etc. in agriculture and industrial areas has been increased tremendously. Since different environmental pollutants are likely to affect biological systems in different ways according to their respective properties, the sum of physiological changes created by a particular pollutant is likely to be the characteristics of that pollutant. The reason for such studies is that, the heavy metals have specific binding affinity to sulfhydryl group of the enzymes. This would naturally alter the activity of the enzymes that may range from activation to total inhibition. The metal induced alteration in enzyme activity may be taken as more or less accurate indicator of metal toxicity. The change in enzyme activity further provides significant information about sub cellular biochemical adjustment and consequent ability of animals to adapt any environmental change.

The higher concentration of toxicants brings the adverse effect on aquatic organism at cellular level or molecular level. Ultimately it leads to disorder in biochemical composition, alteration in the functional efficiency of the nervous system on exposure to heavy metal causes change in the enzyme activity level of carbohydrate and protein

metabolism of the organism [1, 2, 3, 4 & 5]. Moreover, many other investigators have used enzymological techniques to evaluate the sub lethal stress induced by mercury and other metal pollutants on animals [6, 7, 8 & 9]. Hence an attempt has been made to study the effect of mercury and copper on the enzyme secretory activity related to energy yielding processes at cellular level of freshwater crab, *Barytelphusa guerini* with respect to change in level of digestive enzyme amylase.

EXPERIMENTAL SECTION

The crab *Barytelphusa guerini* were collected from fresh water reservoir i.e. Paithan dam near Aurangabad and were brought to the laboratory without any mechanical injury. The crabs were maintained in laboratory for 2-4 days in plastic trough to acclimatize. During acclimatization the crabs were fed with small pieces of bivalve and earthworm. Only healthy, same size, same weight crabs were selected for experimental purpose. Two groups of crabs were formed. One trough was maintained as control and second was considered as experimental.

The experimental group of crabs was exposed to heavy metals such as HgCl_2 and CuSO_4 for 24, 48, 72 and 96 hrs short duration respectively. The water from control and experimental trough was renewed after every 24 hour and dead animals were discarded. The animals were starved a day before experimentation to avoid metabolic differences, if any, due to differential feeding.

Amylase activity was estimated by the method of Noelting and Bernfeld [10]. Standard was prepared by using maltose. The reaction mixture contains 1 gm of starch; 1 ml of properly diluted enzyme with phosphate buffer of pH 6.9 was incubated at 20°C . The enzyme reaction was interrupted by the addition of 2 ml dinitrosalicylic acid reagent. The tube containing this mixture was heated for 5 minutes in boiling water and then cooled in running tap water. After addition of 20 ml of glass distilled water, the optical density of the solution containing the brown reduction product is determined calorimetrically by means of green filter (i.e. 540 nm), and blank is prepared without enzyme extract and adjust zero before estimation.

Table -1: Amylase activity in hepatopancreas of *Barytelphusa guerini* during acute exposure to HgCl_2 and CuSO_4 in monsoon season

Treatment	Control	24 hrs	48 hrs	72 hrs	96 hrs
HgCl_2	4.116 ± 0.015	3.675 ± 0.001 (10.71%)*	3.216 ± 0.02 (21.86%)*	2.675 ± 0.002 (35.0%)*	2.23 ± 0.015 (45.82%)*
CuSO_4	4.116 ± 0.015	3.850 ± 0.02 (6.46%)*	3.560 ± 0.015 (13.50%)*	3.420 ± 0.0015 (16.90%)*	3.220 ± 0.02 (21.76%)*

Enzyme activity is expressed as mg of maltose/gm of tissue/hr at 37°C .

Each value is the mean of three observations \pm S.D.

Values are significant at *** $P < 0.001$

Bracket values indicate percent variation over control.

Table- 2: Amylase activity in hepatopancreas of *Barytelphusa guerini* during acute exposure to HgCl_2 and CuSO_4 in winter season

Treatment	Control	24 hrs	48 hrs	72 hrs	96 hrs
HgCl_2	3.940 ± 0.0015	3.620 ± 0.0025 (8.12%)*	3.124 ± 0.001 (20.71%)*	2.640 ± 0.0015 (32.99%)*	2.190 ± 0.0015 (44.41%)*
CuSO_4	3.940 ± 0.0015	3.72 ± 0.015 (5.58%)*	3.340 ± 0.025 (15.22%)*	2.875 ± 0.002 (27.03%)*	2.480 ± 0.01 (37.05%)*

Enzyme activity is expressed as mg of maltose/gm of tissue/hr at 37°C .

Each value is the mean of three observations \pm S.D.

Values are significant at *** $P < 0.001$

Bracket values indicate percent variation over control.

Table- 3: Amylase activity in hepatopancreas of *Barytelphusa guerini* during acute exposure to HgCl₂ and CuSO₄ in summer season

Treatment	Control	24 hrs	48 hrs	72 hrs	96 hrs
HgCl ₂	4.270 ±0.0015	3.391 ±0.0015 (20.58%)*	3.575 ±0.001 (16.27%)*	3.320 ±0.02 (22.24%)*	2.980 ±0.005 (32.31%)*
CuSO ₄	4.270 ±0.0015	3.990 ±0.0005 (6.55%)*	3.750 ±0.025 (12.17%)*	3.60 ±0.02 (15.59%)*	3.31 ±0.015 (22.48%)*

Enzyme activity is expressed as mg of maltose/gm of tissue/hr at 37°C.

Each value is the mean of three observations ± S.D.

Values are significant at *** P < 0.001

Bracket values indicate percent variation over control.

RESULTS AND DISCUSSION

The seasonal effects of mercuric chloride and copper sulphate on amylase activity in digestive gland of *Barytelphusa guerini* after acute exposure are summarized in table 1-3.

After acute exposure to mercuric chloride and copper sulphate the amylase activity of control group was (4.116 ± 0.015) mg of maltose/gm of tissue/ hr. In monsoon season amylase activity of the crabs exposed to mercuric chloride after 24, 48, 72 and 96 hrs showed (3.675 ± 0.001; P<0.001); (3.216 ± 0.02; P<0.001); (2.675 ± 0.002; P<0.001) and (2.23 ± 0.015; P<0.001). Similarly amylase activity of the crabs exposed to copper sulphate showed (3.850 ± 0.02; P<0.001); (3.560 ± 0.015; P<0.001); (3.420 ± 0.0015; P<0.001) and (3.220 ± 0.02; P<0.001).

In winter season the amylase activity of control, crabs showed (3.940 ± 0.0015) mg of maltose/ gm of tissue/ hr. In winter season amylase activity of the crabs exposed to mercuric chloride after 24, 48, 72 and 96 hrs showed (3.620 ± 0.0025; P<0.001); (3.124 ± 0.001; P<0.001); (2.640 ± 0.0015; P<0.001) and (2.190 ± 0.0015; P<0.001). Similarly amylase activity of the crabs exposed to copper sulphate showed (3.720 ± 0.015; P<0.001); (3.340 ± 0.025; P<0.001); (2.875 ± 0.002; P<0.001) and (2.480 ± 0.01; P<0.001).

In summer season the amylase activity of control, crabs showed (4.270 ± 0.0015) mg of maltose/ gm of tissue/ hr. In summer season the amylase activity of the crabs exposed to mercuric chloride after 24, 48, 72 and 96 hrs showed (3.391 ± 0.0015; P<0.001); (3.575 ± 0.001; P<0.001); (3.320 ± 0.02; P<0.001) and (2.980 ± 0.005; P<0.001). Similarly amylase activity of the crabs exposed to copper sulphate showed (3.990 ± 0.0005; P<0.001); (3.750 ± 0.025; P<0.001); (3.60 ± 0.02; P<0.001) and (3.31 ± 0.015; P<0.001).

It was observed that mercuric chloride is highly toxic than copper sulphate and found to be more potent enzyme inhibitor in all seasons. [11] studied the effect of copper chloride on the enzyme activity of the crab, *Sesarma quadratum*. [12] reported decrease in amylase activity in *L. marginalis* and explained that mercury chloride and copper chloride are potent inhibitors of amylase activity. [13] studied the lipase activity level in the freshwater crab, *Barytelphusa guerini* after exposure to two pesticides (Sevin and DDT), reported decrease in the activity level of lipase when compared with the control.

From the above results it was concluded that the decrease activity was time dependent. Similar results have been reported by [14] observed decreased amylase activity due to heavy metal in freshwater bivalve, *Parreysia favidens*. Similar results have been reported by many workers [15] in *Parreysia corrugata*; [16] in *Lamellidens marginalis* and [17] in *Corbicula striatella*. [18] studied the alterations in the level of dehydrogenase in a freshwater fish, *Tilapia mossambica* exposed to arsenic toxicity. [19] reported CdCl₂ inhibit and decrease the enzyme activity in rat. In the present investigation the crab was affected severely and observed significant decrease in amylase activity in different seasons after acute exposure to HgCl₂ and CuSO₄.

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