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Effect of Copper Chloride on the Enzyme Activities of the Crab *Sesarma quadratum* (Fabricius)

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Abstract: A series of experimental studies indicated that copper as a toxicant causes a disturbance in enzyme activity. Stress conditions impose forced anaerobic conditions in the muscle, gill and hepatopancreas tissues of the crab *Sesarma quadratum* when exposed to two sublethal concentrations of 1/10 [2.8 ppm (Exp. 1)] and 1/3 [9.3 ppm (Exp. 2)] copper chloride for 21 d. Lactate dehydrogenase (LDH) activity was significantly elevated in muscle and hepatopancreas tissues whereas succinate dehydrogenase (SDH) activity was suppressed in the crab tissues of the muscle, gills and hepatopancreas for 21 d.

Key Words: Copper chloride, SDH, LDH, *Sesarma quadratum*

Yengeç Türü *Sesarma quadratum* (Fabricius)'un Enzim Etkinliği Üzerinde Bakır Klorid'in Etkisi

Özet: Bir dizi deneysel çalışma, bakırın enzim etkinliği üzerinde bozucu etki gösteren bir zehir olduğunu göstermiştir. Stres koşulları, *Sesrama quadratum* adlı yengeç türü 21gün süreyle 1/10 [2.8 ppm (Deney 1) ve 1/3 [9.3 ppm (Deney 2)] oranlarında iki subletal bakır klorid konsantrasyonuna maruz bırakıldığında, yengeçlerin kas, solungaç ve hepatopankreas dokularında zamanla anaerobik koşullar yaratır. Laktat dehidrojenaz (LDH) etkinliği kas ve hepatopankreas dokularında anlamlı biçimde arttığı halde suksinat dehidrojenaz (SDH) etkinliği kas, solungaç ve hepatopankreas dokularında azaldı.

Anahtar Sözcükler: Bakır klorid, SDH, LDH, *Sesarma quadratum*

Introduction

Copper and its compounds have been used by man since prehistoric times. There are several sources of copper emission into the atmosphere. Bertine and Goldberg (1) have estimated that approximately 80,000 to 2,50,000 tonnes of copper enter the environment per annum as a result of weathering mobilisation. Copper reaches the aquatic environment through wet or dry deposition, mining activities, land runoff and industrial, domestic and agricultural waste disposal. Among the industrial sources, the main contribution comes from metal pickling baths, copper and plating baths, waste waters from alkaline rayon processing, pulp and paper mills, petroleum refineries, metal works and foundries, mine water, mine tailing ponds and acid mine drainage (from lead mining). Sittig (2) has summarised the copper concentration found in the waste waters from these sources. They can reach averages of up to 900 ppm in waste waters from processes such as brass rod and wire and copper wire mills.

Various chemicals entering the aquatic ecosystem through human activities, either accidently or by design, may cause adverse effects on the aquatic biota, including deleterious changes which disrupt metabolic activity at the biochemical level (3). Verma et al. (4) reported on the toxic effects of sublethal concentrations of copper sulphate, a heavy metal, on certain biologically important enzymes. Enzymes catalyse physiological reactions by lowering the activation energy level that the reactants (substrates) must reach for the reaction to occur. The effect of toxicants on enzymatic activity is one of the most important biochemical parameters which is affected under stress. When an organ is diseased due to the effect of a toxicant, enzyme activity appears to be increased or it may be inhibited due to the active site being either denatured or distorted. Since some enzymes catalyse some steps in the metabolism of carbohydrates and protein, they are present in most tissues. The increase or decrease in their level may be sufficient to provide information of diagnostic value.

To study the strategy of energy production adopted by *Sesarma quadratum*, changes in the activities of two enzymes, the lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH), were assayed as they are very sensitive to environmental pollutants (5). LDH catalyses the conversion of pyruvic acid to lactic acid in anaerobic conditions and the activity of SDH may be taken as an indication of the level of operation of the TAC cycle (6).

The species *S. quadratum* is a commonly available crustacean at the Adyar estuary of the Madras coast. Since the Adyar estuarine region is highly polluted with copper (94.24 ppb) (7), an attempt has been made to investigate copper chloride induced alterations of activities of LDH and SDH in various tissues like the muscle, gills and hepatopancreas of this crab.

Materials and Methods

For the enzymes assay studies, healthy specimens of the common estuarine mud crab *S. quadratum*, in the intermoult stage, were used. Specimens weighing approximately 3 to 4 g (carapace length 1.4 to 1.6 cm) were maintained under laboratory conditions in 17 ppt of 50% seawater (as that of the habitat estuarine water) of 30 to 40 cm standing height. The crabs used in these experiments were maintained under laboratory conditions without food for three successive days with daily changes of water before use.

In the present study, two sublethal concentrations of copper chloride were used, about 1/10 [2.8 ppm (Exp. 1)] and 1/3 [9.3 ppm (Exp. 2)] values were taken from LC_{50} (28 ppm) value. The LC_{50} value was calculated through a probit analysis of log transformed data. The probit line was fitted with log toxicants against mortality and the LC_{50} was calculated where the probit line crossed 50% mortality (8). Static renewal tests were conducted as outlined in APHA (9). These two experiments were repeated using seawater with two different sublethal concentrations of copper chloride for 1, 7, 14 and 21 d. Simultaneously, controls were maintained. Each experiment was repeated three times with different individuals and the mean value was taken.

The animal were sacrificed between 8:00 and 10:00 AM. The muscle, gill and hepatopancreas were sampled from the control and experimental group animals to assess change in enzyme activities. Animals were dissected over an ice-cooled glass tray. Muscle, gill and hepatopancreas tissues were dissected out rapidly and weighed. They were kept at 20 ºC until further analysis.

The activity of LDH was measured by the method of Wacker et al. (10). LDH activity was expressed as units/mg protein. SDH activity was determined by the method described by Slater and Bonner (11). SDH activity was expressed as nanomoles of succinate oxidised/min /mg protein.

The values obtained from the experiments are given as mean and standard error. The significance of the difference between the mean values of the control and experimental crabs tissues were analysed using Student's t test.

Results and Discussion

Crabs were exposed to two different concentrations of copper chloride for 21 d. Tissues (muscle, gills and hepatopancreas) were assayed for LDH activity after 1, 7, 14 and 21 d exposure. The results are represented in Figure 1(A). Muscle, gill and hepatopancreas tissues when exposed to copper chloride (two sublethal) did not show much variation on day 1. Increases in LDH activity, compared with the LDH activity of the control group, on all the other sampling days in the muscle (7, 14 and 21 d) were noted. Results were statistically significant for LDH activity in the muscle ($P < 0.001$) and hepatopancreas ($P < 0.05$), but the values obtained for gill tissues were not significant for 21 d when exposed to high concentrations (Exp. 2). While, SDH activity, in the various tissues of *S. quadratum*, was found to decrease following exposure to sublethal concentrations of copper chloride [Figure 1(B)], SDH activity was statistically significant in the muscle ($P < 0.001$), gills ($P < 0.05$) and hepatopancreas ($P < 0.002$) for 21 d at a high sublethal concentration of 9.3 ppm (Exp. 2).

Toxicants cause a disturbance in the physiological state of the animal which affects enzyme activity. Toxicants bring about distortions in the cell organelles, which may bring about elevation or inhibition in the activity of various enzymes.

Mitochondrial damage leads to decreased respiration and a partial uncoupling of oxidative phosphorylation (12). Sastry et al. (13) also observed an increase in the LDH activity in the tissues of the fish *Channa punctatus* when they were exposed to cadmium and copper. The

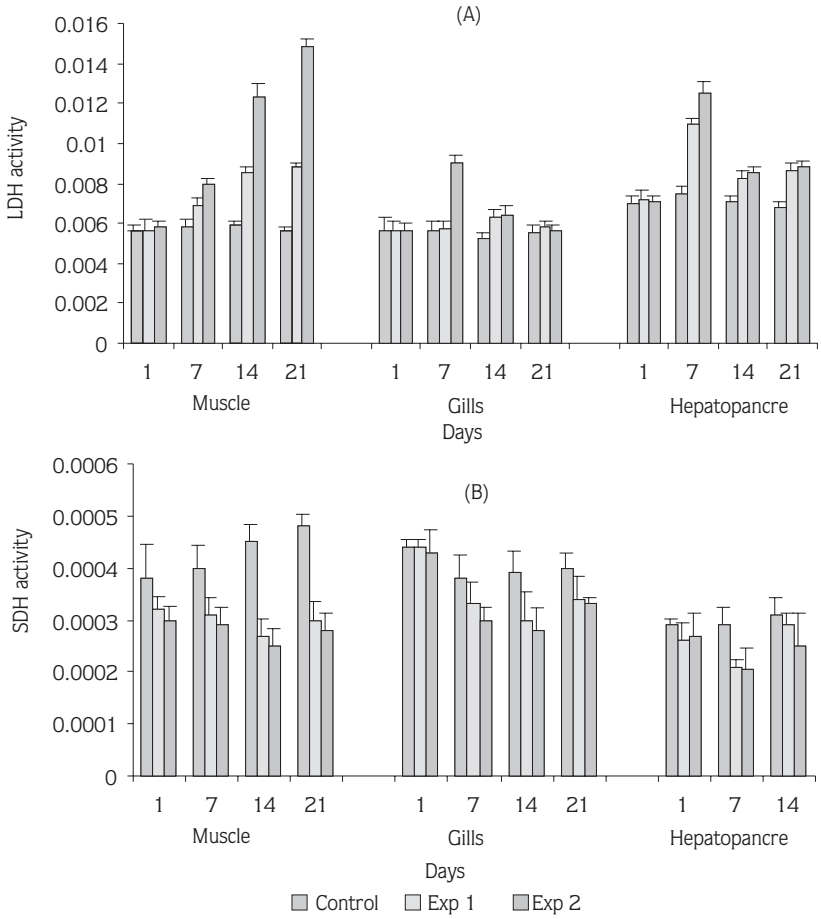

Figure 1. LDH (A) and SDH (B) activity in various tissues of Sesarma quadratum on exposure to sublethal concentrations of copper chloride.

increase may be correlated to cadmium- and copper-induced damage in the gill epithelium, which can reduce oxygen uptake capacity and bring about hypoxia in organs of vital importance (13). Such a view is supported by lower oxygen consumption in the experimental fish *Gambusia affinis* (14). According to Zalme et al. (15), inhibition of the activity of the dehydrogenase may be due to changes in the activity of mitochondrial membrane function.

The activity of LDH, which is a cytoplasmic enzyme, shows a marked elevation in activity in the muscle, gills and hepatopancreas. LDH is generally associated with cellular metabolic activity. It acts as a pivotal enzyme between the glycolytic pathway and the tricarboxylic acid cycle. Thus, the nonavailability of oxygen, inhibition of SDH and simultaneous elevation of LDH may suggest a bias towards the anaerobic glycolytic pathway.

Ramanibai (16) exposed *Scylla serrata,* the estuarine crab, to copper and found that in the muscle, gills and hepatopancreas the activity of succinate SDH was decreased, whereas LDH activity showed an increase. Manjula Devi et al. (17) reported that the LDH activity increased in the abdominal muscle of cadmium-exposed fiddler crabs. Recently, Blier et al. (18) studied the metabolic and digest enzymes activities in the Coho salmon (*Oncorhynchus kisutch*), which were engineered to have transgenic growth hormones, as well as in non-transgenic fishes. The transgenic salmon showed higher LDH activities in the muscle. This report suggests that LDH activity is a sensitive index to measure the influence of external factors. The work of Valarmathi and Azariah (19) indicated that LDH levels were significantly elevated and SDH activity was suppressed in the muscle, gill and hepatopancreas tissues of the crab *S. quadratum* when exposed to two sublethal concentrations of chlorine. Supporting evidence that LDH activity may be enhanced due to sublethal effect of aquatic pollutant (11 in number) in the case of the African sharptooth catfish,

Clarias gariepinus inhabitating Lake Maryut in Egypt is provided in the work of Adham (20). Under anaerobic laboratory conditions, the enzyme activities of LDH and octopine dehydrogenase in the heart tissue of the crab *Petrolisthes laevigatus* were also elevated (5). Similarly, in the present study LDH activity also increased, whereas SDH activity decreased in the muscle, gills and hepatopancreas of the crab (*S. quadratum*) when exposed to copper chloride. The increase in LDH activity may reflect an increased dependance on anaerobic carbohydrate metabolism by the muscle, gills and hepatopancreas of crabs that were exposed to such a toxicant (copper chloride). Thus, sublethal levels of copper chloride affect the efficiency of tissue metabolites and cause pathological changes in the muscle, gills and hepatopancreas.

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