Original Paper

Effect of Copper Sulphate on Lipid in Some Vital Organs of Freshwater Crab *Barytelphusa gureini*

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ABSTRACT

Toxicity of copper on some metabolic processes can lead to disturbance and imbalance of various physiological activities such as respiration, reproduction, bone formation, and metabolism of some nutrients. Copper sulphate is frequently used as a general biocide in the aquaculture industry.

The present study reflects the effect of copper sulphate on lipids in some vital organs of the freshwater crab *Barytelphusa gureini.* Crabs of equal size were treated with different concentrations of copper sulphate (0.5, 1.0, 1.5, 2.0, and 2.5 ppm) respectively. The mortality rate was noted up to 96 hours. After deducing the LC_{50}, the crabs were treated with a sub-lethal concentration of copper sulphate (1.5 ppm) for 24, 48, 72 and 96 hours respectively. Total lipid estimation was done in the case of sub-lethal concentration (1.5 ppm) of copper sulphate exposure and compared with the control group of crabs.

The results showed a significant decline in the total lipid in the following organs of *Barytelphusa gureini* at sub-lethal concentration of copper sulphate – hepatopancreas (67.7%) < muscle (58.8%) < gills (47.3%) < haemolymph (powder form) (28.8%). In contrast, there was an increase in the lipid in various organs of control crabs – hepatopancreas (0.091 mg/100 mg) > gills (0.076 mg/100 mg) > haemolymph (powder form) (0.052 mg/100 mg) > muscle (0.034 mg/100 mg).

The lipid reduction in the present study is a reflection of breakdown of lipid in stress situation.

Key Words: Freshwater crab, *Barytelphusa gureini,* Lipid, Copper sulphate

Introduction

Water is a very essential and vitally important biological solvent. Environmental poisoning by heavy metals has increased in recent years due to extensive use of heavy metals in agriculture, and chemical and industrial processes, posing a serious threat to living organisms. Among various heavy metals, copper, chromium and iron are the most important pollutants originating from industrial effluents and agricultural wastes in aquatic environment, causing significant damage to aquatic organisms, resulting in imbalance of the ecosystem.

Industrial evolution has its ultimate impact on aquatic fauna in the form of industrial effluents which are continuously polluting water bodies with toxic chemicals including heavy metals. The effect of heavy metals on aquatic organisms is currently attracting widespread attention, particularly in studies related to industrial pollution.

Heavy metals are introduced into aqueous environment through industrial and urban effluents, soil leaching, and rainfall. Aquatic animals occupy high trophic level in accumulating various xenobiotics in the aquatic environment. Several investigations have already shown that metals accumulate at higher concentrations in freshwater and marine food chain. Waste water from textile, dyeing, printing, and tannery industries are highly coloured, and can cause problems to the ecological sys-

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tem. The toxicity of chemicals in invertebrates is mainly reflected in the central nervous system, respiratory system, and various other physiological processes. Respiration is obviously the most vital of all functions, and serves as a sign of life and index of all biochemical activities that occur due to the effect of toxicants on the overall metabolism of the exposed animals. Changes in oxygen consumption due to pollution create a physiological imbalance in the organisms.

Essential heavy metals such as copper and zinc play an important role in various biological processes including oxidative phosphorylation, gene regulation, free radical formation, homeostasis, and serve as essential co-factors. However, when their concentration exceeds beyond normal requirements, they cause serious harm on metabolism by altering proteins, carbohydrates and lipids. The harmful effects increase with both the concentration and length of exposure. Copper is one of the trace metals essential to the healthy life of many living organisms. It is a constituent of haemocyanin, a metalloprotein which helps in oxygen transport, in aquatic animals, and is one of the most important elements in metabolism involving albumin, globulin, thrombin, and fibrinogen. Copper is also helpful in the formation of blood. It is also present in many enzymes, one of which is an oxidative enzyme. Copper sulphate as a salt of copper is widely used in aquaculture as an algaecide. Copper acts as a toxicant when it is in the form of sulphate or phosphate or any other similar form.

Lipids are a complex group of substances which occur as esters of long-chain fatty acids. They occur in small amounts in almost all tissues of crab, and in each cell as a constituent of plasma membrane and other important organelles. Lipids are significant not only as a potential source of energy, but also are needed for mechanical support, besides insulating against the loss of heat. The exact physiological state, nutritional habits and overall biotic and abiotic factors have a profound influence on the deposition of lipid content in the various organs of crab. Although lipids are distributed in all tissues, in some they get accumulated as depot fat. The principal sites of depot fat include muscles, hepatopancreas, subcutaneous connective tissues and certain other viscera.

A survey of literature reveals that considerable work has been done on the effect of pollutants on marine animals, but comparatively little attention has been focused on their freshwater counterparts. Hence the present investigation was undertaken to study the lipid metabolism of the fresh water crab, *Barytelphusa gureini* after exposure to copper sulphate for varying periods of time.

Materials and Methods

Adult specimens of freshwater crab *Barytelphusa gureini* were collected from the outskirts of paddy fields of Pune district (Maharashtra). They were acclimatized in the laboratory for seven days before they were used for experimentation. Only healthy crabs weighing between 30–40 grams were selected for experimentation to avoid problems of sex and size. The animals were fed with small pieces of goat flesh and uncooked oats.

Acclimated crabs of equal size were divided into five experimental groups of six crabs each, and treated with different concentrations of copper sulphate (0.5, 1.0, 1.5, 2.0, and 2.5 ppm) respectively. The mortality rate was noted up to 96 hours. The test medium and dead crabs were removed within an interval of 24 hours. The LC_{50} was calculated by using Probit analysis method.[1] After deducing the LC_{50}, the crabs were treated with a sub-lethal concentration of copper sulphate (1.5 ppm) for 24, 48, 72 and 96 hours respectively. The other group of crabs was kept as control. Each group of crabs was maintained in a plastic trough of 30 litres capacity, and was starved for 24 hours prior to, and during the course of experiment for the estimation of total lipid, which was carried out by using Barnes and Barestock method. Two-way ANOVA was used to test the differences between the two groups. Statistical significance was assessed at $p<0.05$ level. Results were found to be statistically significant.

Results

The biochemical response of copper sulphate in freshwater crab *Barytelphusa gureini* was studied at a sub-lethal concentration (1.5 ppm) for 24, 48, 72 and 96 hours of periods. The lipid was estimated and compared with the control group of crabs. **Table 1** reveals a significant decline in the total lipid in various tissues of *Barytelphusa gureini* at sub-lethal concentrations of copper sulphate, as follows: hepatopancreas (67.7%) < muscles (58.8%) < gills (47.3%) < haemolymph (powder form) (28.8%).

Figure 1 shows a significant increase in the total lipid in various tissues of control crab *Barytelphusa gureini* – hepatopancreas (0.091 mg/100 mg) > gills (0.076 mg/100 mg)

Table 1 Lipid content in the various tissues of *Barytelphusa gureini* during exposure to copper sulphate

Tissue	Control	24 hrs	48 hrs	72 hrs	96 hrs
Muscle	0.034 ± 0.11	0.026 ± 0.62	0.023 ± 0.04	0.018 ± 0.04	0.014 ± 0.22
Gills	0.076 ± 0.03	0.068 ± 0.04	0.050 ± 0.18	0.049 ± 0.00	0.040 ± 0.03
Haemolymph (PF)	0.052 ± 1.65	0.048 ± 2.43	0.043 ± 4.05	0.039 ± 1.12	0.037 ± 3.23
Hepatopancreas	0.090 ± 0.44	0.072 ± 1.71	0.061 ± 0.44	0.046 ± 0.44	0.033 ± 0.033

Values are expressed as mg lipid/100 mg wet weight of tissues. Each value is a mean of ± standard deviation of six individual observations. Experimental values are statistically different from control with statistical significance at P< 0.05 (non-significant)

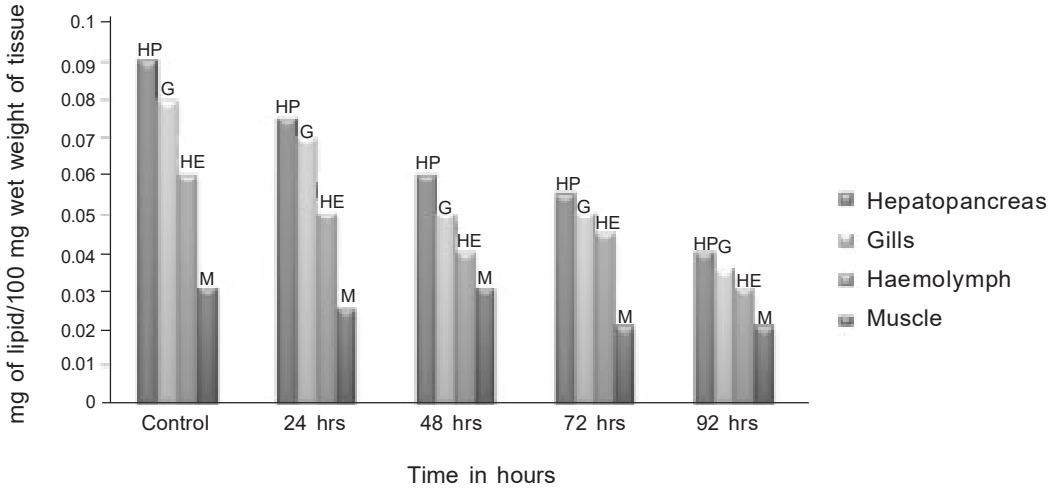

Fig. 1 Changes in lipid content in *Barytelphusa gureini* due to copper sulphate at different time periods

> haemolymph (powder form) (0.052 mg/100 mg) > muscles (0.034 mg/100 mg). The results were compared with each other, and also with the findings of other researchers.

Discussion

Environmental stressors can alter both the quantity and quality of lipids in crustaceans. Exposure to contaminants such as pesticides and heavy metals can increase or decrease total lipids and triglycerides depending on the species, concentration, and duration of contaminant exposure. The majority of crustaceans undergo a period of natural depletion for a part of their life cycle. During such environmental stress, they utilize the body energy reserve as fuel for metabolic activities and survival. The metabolic strategies employed to resist stress vary considerably from species to species. The hepatopancreas of arthropods is analogous in function to the liver and pancreas of vertebrates. In vertebrates, liver parenchymal cells synthesize plasma lipoproteins and their secretions are coordinated by the Golgi apparatus.[2,3] The results showed considerable amount of lipid (0.091 mg/100 mg) in the hepatopancreas of *Barytelphusa gureini* which indicate that during environmental stress it is preferably utilized as a reserve source material.

In one study, the total lipid content was found to be higher in the hepatopancreas of test prawns *Macrobranchium malcolmsoni* when exposed to 32.0 mg/L of endosulfan, than in the control hepatopancreas.[4] In another study, *Spirolothelphusa hydrodroma* treated with chlorpyrifos showed depletion of lipid content in hepatopancreas.[5] The decrease of lipid was high at (0.04 ppm) sub-lethal concentration of chlorpyrifos for 30 days of exposure period. Similarly, *Macrobranchium kristensis* when exposed to pesticide showed a decline in lipid content in the hepatopancreas.[6]

In the present investigation, copper sulphate elevated lipid levels in the hepatopancreas (58.8%) of *Barytelphusa gureini* at acute exposure, and lowered it at chronic exposure. It appears that at chronic exposure, lipids were used as energy source to counteract heavy metal stress. The decrease in the lipid content of hepatopancreas may be due to inhibition of lipid synthesizing capacity, or due to increase in hydrolysis of hepatic lipids to combat the stress condition. Since lipid has high calorific value than that of carbohydrates or proteins, they might have been utilized for energy production during the stress condition.

There was considerable amount of lipid in the muscle (0.034 mg/100 gram) of *Barytelphusa gureini* which indicates that during environmental stress it is preferentially utilized as reserve source material, and as a source of energy for muscle contraction. When freshwater crustaceans subjected to 1.4 ppm of copper sulphate for 60–80 days demonstrated a gradual decrease in muscle lipid, it was attributed to either oxidation or hydrolysis of these lipids.[7,8] In this study, the decrease in total lipid content in muscle (58.8%) exposed to copper sulphate suggests that lipids have been channeled for energy production during stress conditions. Depletion of lipids may be associated with meeting the extra demand of energy necessitated by the quick and brisk movement which the crab showed in its behavioural response under copper sulphate toxicity, since lipids constitute energy whose calorific value is twice that of carbohydrates and proteins.

It has been shown that the haemolymph of male and female decapod crustaceans contain a high density lipoprotein, LP1 whose concentration ranges from 1.1 to 2.0 mg/L.[9] It plays an important role in transporting lipids from the hepatopancreas to peripheral tissues such as muscles, and functions as B-1,3 glucan-binding protein in the crustacean's immune recognition.[10] In adult insects and crustaceans, most of the haemolymph lipid is associated with high-density lipoproteins, namely lipophorin in insects, and LP1 in crustaceans.[4] LP1 accounts for only 3% of the total haemolymph in crustaceans.[11] The site of lipophorin synthesis in crustaceans is the fat body. In females, lipophorin is transported by the haemolymph to the ovaries, where it is taken up by developing oocytes.[12]

In the present study, the amount of lipid in haemolymph (0.052 mg/100 mg) was considerably higher than in the muscles. The amount of lipid in the haemolymph of *Barytelphusa gureini* may be associated with high-density lipoproteins, i.e., LP1, transport of lipid from hepatopancreas to muscles, and immune recognition. Exposure to copper sulphate lowered the lipid content in the haemolymph (28.8%), which indicates low production of lipoprotein LP1 in the haemolymph of *Barytelphusa gureini* which ultimately reflects on the development of gonadal cells. The present observations are in agreement with the findings of earlier researchers.[9,12]

Gills are the major organs of the respiratory process of aquatic animals such as the crustaceans, including crabs. When toxic contaminants are waterborne, gills become the sites for damage, which can be easily assayed. The impact of copper sulphate on the gills of *Barytelphusa gureini* caused changes in the structure of gill lamellae. The present study showed a considerable amount of lipid (76 mg/gram) in the gills of *Barytelphusa gureini*, which indicates that during environmental stress, it is preferentially utilized as an energy source for the removal of toxicants, since gills act as organs of excretion and respiration. The decrease in total lipid content in gills exposed to copper sulphate suggests that lipids have been channeled for energy production during hypoxic conditions. More amount of energy is utilized for intake of oxygen and removal of contaminants to maintain homeostasis.

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