

Bioaccumulation, Recovery and Toxicity of Copper in Some Vital Organs of Freshwater Crab *Barytelphusa gureini*

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ABSTRACT

Observation and results are presented of a detailed study of accumulation of copper measured from gills, muscles, hepatopancreas and small intestine of crab *Barytelphusa gureini*. The results indicate marked differences in the accumulation of copper in the above organs. Maximum accumulation of metal was observed in the gills and muscle as compared to hepatopancreas and small intestine. After recovery, the levels of copper in the various organs of the crab were below the maximum permissible limits, and appear to be safe for human consumption.

Key Words: Freshwater crab; *Barytelphusa gureini*; Copper; Bioaccumulation

Introduction

Various activities by man in recent years have increased the quantity and distribution of heavy metals in the atmosphere, land, and water bodies. The extent of this widespread but diffuse contamination has raised concerns about their hazards on living organisms including man. The flesh (meat) of various animals are consumed by man as protein-rich food. However, the fleshy tissues of aquatic animals are known accumulators of heavy metals, and the nutritional implication of this is that consumers of animals may be exposed to heavy metal toxicity if bioaccumulation results due to regular consumption.

Heavy metal studies in aquatic organisms along with bio-concentration have been carried out in various parts of

the world. According to several researchers, heavy metal studies in aquatic biota indicate that heavy metals in aquatic organisms could be more reliable water quality indicators than chemical analysis of water column and sediment.^{1,2} Heavy metal content in aquatic organisms has also been successfully used in evaluation of heavy metal input into water bodies. Existing literature describes diverse approaches for choosing the most appropriate fish group, species and tissue for these kinds of monitoring studies.

Copper, like other heavy metals, has been the subject of increasing research activities to determine and ultimately to control its concentrations in estuaries and coastal marine habitats. Due to its persistence in the environment, it is toxic at high concentrations and also has a tendency to accumulate in the biota with potential hazards to man. Copper in trace quantities, is an essential metal for metabolic processes. However, it has detrimental effects when it is present in excess quantity.

Since copper has industrial applications, and is also an important metabolic component of aquatic animals, it was thought worthwhile to study the impact of excessive copper in aquatic environment. Therefore, the present study was done to estimate the bioaccumulation and recovery of copper, and its effect on various organs of the freshwater Indian crab *Barytelphusa gureini*.

Materials and Methods

Specimens of the freshwater Indian crab *Barytelphusa gureini* were procured from the local market at Ganesh

Peth, Pune, Maharashtra, and were transferred to the laboratory in large plastic troughs. These were maintained at $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$ under normal day and night light (13:11D). The crabs were fed earthworms with an interval of 12 hours (morning and evening). All crabs taken for the study were sorted to ensure approximately the same body weight (40-50 gm) and carapace width (30-40 mm).

Toxicity Evaluation: An aqueous stock solution of copper sulphate was used to test the toxicity with appropriate dilution by tap water. A group of ten crabs were exposed to six different concentrations ranging from 3.0 to 6.0 ppm of copper sulphate. The mortality rate was noted up to 10 days, and the test medium and dead crabs were removed with an interval of two days. The LC_{50} was calculated by using Probit analysis method.³ After determining the LC_{50} , one set of six crabs was treated with a sub-lethal concentration of copper sulphate (4.5 ppm) for 2, 4, 6, 8 and 10 days respectively. The other set of six crabs were kept as control under similar conditions without exposure to the toxicant. Each set of crabs was maintained in plastic troughs of 30-litre capacity, and deprived of food during the exposure period.

Copper Bioaccumulation: The crabs were exposed to 4.5 ppm sub-lethal concentration of copper sulphate for periods of 2, 4, 6, 8 and 10 days to determine copper bioaccumulation in different organs such as gills, muscles, hepatopancreas and intestine. The organs were dissected out at the end of each exposure period and dried in an oven at 70°C for 3 days. The dried samples were powdered using mortar and pestle, and 100 mg dried powder was taken in a beaker and 10 ml of concentrated nitric acid was added to it. The mixture was shaken well and kept on a hot plate to evaporate the solution. After its complete evaporation, 10 ml of nitric acid and 2 ml of 5N perchloric acid was added. The solution was mixed well and kept on the hot plate till it evaporated and became colourless. Again 10 ml of concentrated nitric acid was added, and the mixture was placed on the hot plate for digestion till 5 ml remained in the beaker. This was cooled and made up to 25 ml with 2M solution of concentrated nitric acid, which was then used for metal estimation directly following the nitric acid digestion method of APHA and the PC-based Atomic Absorption Spectrophotometer (AA1275BD Varian Techtron, USA) at 228.9 nm wavelength. Values have been expressed in micrograms of copper per 100 milligram weight of sample.

Recovery of Bioaccumulation: For recovery studies, copper sulphate-exposed crabs were transferred to clear

water without toxicant. During recovery span on 2nd, 4th, 6th, 8th and 10th day, the crabs were dissected, and the above mentioned methodology was followed for the estimation of copper in different organs.

Statistical Analysis: Two-way ANOVA was used to test the differences between the two groups of crabs. The statistical analysis significance was assessed at $p < 0.05$ level. Thus the result was found to be not statistically significant.

Results

The results obtained from this study are presented in **Tables 1** and **2**. The values are expressed in micrograms of copper per 100 milligram weight of sample. The maximum accumulation of copper was noted in the gills, whereas the minimum accumulation was found in intestines as compared to control. Intermediate levels of accumulation were found in hepatopancreas and small intestine. On the whole, the trend of degree of accumulation of copper in different organs was as follows: gills > muscles > hepatopancreas > intestine. During recovery span, except for the gills, all other organs recorded a decrease in accumulation, and showed recovery trend.

Discussion

Bioaccumulation is the ability of an organism to concentrate an element or a compound from the food and water chain to a level higher than that of its environment. It is the resultant process of many interactions within the compartments of the organisms.

The uptake of metals and their toxicity in aquatic fauna are influenced by many factors such as pH, hardness of water, alkalinity, temperature, etc. Metals exist in a variety of states, and their toxicity depends on their nature and chemical form (ionic or oxidized or reduced state) in combination with other organic substances and other metals. Researchers have reported that the absorption of heavy metals in the alimentary canal of aquatic animals depends upon their precise chemical form.⁴ A review of available literature on metal concentration in fish tissues reveals that the relationship between metal concentration, length, age and weight appears to be specific for metal and species.

In the present study, the following sequence for highest and lowest copper accumulation were observed respectively in the organs of freshwater crab *Barytelphusa gureini*: gills > muscles > hepatopancreas > intestine. Gills were found to accumulate more amounts of the toxicant

when compared to other organs. This may be because they receive copper from other organs through the circulation for subsequent excretion. This translocated copper may not be excreted out due to low concentrations which may be below the threshold level. It has been suggested that the physiological mechanism which regulates uptake and elimination of metals such as zinc, copper, etc., is only functional or functions at a higher rate above some threshold concentration of the metal.⁵ Researchers have pointed out that high concentrations of zinc in gills may be due to failure in excretion of the toxicant.⁶

It is well known that gonads, gills and digestive glands have higher zinc concentrations than other organs in many aquatic organisms. Gills which are in direct contact with water accumulate some amount of copper. The accumulation of copper in the gills may be due to adsorption on to the gill surfaces, and is dependent on the availability of proteins to which the copper may bind. The accumulation may be due to development of some defensive mechanism such as excessive mucus secre-

tion and clogging of gills. The penetration of copper across the gills may be the reason for low toxicity of this metal to *Barytelphusa gureini*.

Respiration is obviously the most vital of all functions, and serves as an index of all biochemical changes that occur due to the effect of toxicants on the metabolism of exposed animals. Any change in the oxygen consumption due to pollution stress creates a physiological imbalance in the organisms.⁷ It has been suggested that copper sulphate may induce alterations in gill structure, disintegration or rupture of respiratory epithelium, and coagulation of mucus film over the gill surface of *Barytelphusa gureini* which could be the reason for the observed lethargy and imbalance.

In the present study, next to the gills, muscles appear to accumulate copper in large amounts. Glycogen and the entire enzymatic machinery related to muscle contraction is found in the muscle, and hence, it acts as a vital metabolizing organ. The toxicant in the muscle may in-

Table 1 Bioaccumulation of Copper in Exposed Freshwater Crabs

Organs	Values	Control	Exposure Span (in days)				
			2	4	6	8	10
Gills	Exptl value	2.616	2.765	3.205	3.685	4.385	5.105
	SEM	± 0.036	± 0.027	± 0.036	± 0.056	± 0.062	± 0.070
	%V		+ 19.09	+ 37.18	+ 45.38	+ 76.08	+ 82.09
Muscles	Exptl value	1.980	2.267	2.365	2.486	2.685	2.714
	SEM	± 0.039	± 0.035	± 0.024	± 0.019	± 0.032	± 0.036
	%V		+ 12.12	+ 21.26	+ 28.33	+ 36.58	+ 62.90
Hepatopancreas	Exptl value	1.510	1.673	1.805	1.865	2.384	2.392
	SEM	± 0.039	± 0.032	± 0.027	± 0.024	± 0.026	± 0.029
	%V		+12.08	+ 21.50	+ 25.60	+ 59.60	+ 62.90
Small intestine	Exptl value	1.245	1.452	1.535	1.730	2.085	2.230
	SEM	± 0.025	± 0.041	± 0.036	± 0.036	± 0.052	± 0.061
	%V		+17.60	+ 21.50	+ 40.60	+ 65.90	+ 70.30

1. Values expressed as microgram of copper/100 milligram weight of sample
2. Each value is the mean of six observations
3. %V is coefficient of variations
4. Experimental values are statistically different from control with statistical significance at p<0.005 (not significant)

Table 2 Recovery of Copper from Exposed Freshwater Crabs

Organs	Values	Control	Recovery Span (in days)				
			2	4	6	8	10
Gills	Exptl value	2.616	4.860	4.295	3.958	3.616	3.125
	SEM	± 0.036	± 0.047	± 0.037	± 0.024	± 0.052	± 0.038
	%V		+ 79.5	+ 70.30	+ 67.01	+ 62.09	+56.06
Muscles	Exptl value	1.980	2.633	2.533	2.383	2.133	2.014
	SEM	± 0.039	± 0.023	± 0.027	± 0.033	± 0.046	± 0.052
	%V		+ 35.20	+ 29.89	+ 22.20	+ 19.32	+ 17.65
Hepatopancreas	Exptl value	1.510	2.143	2.010	1.885	1.783	1.622
	SEM	± 0.039	± 0.028	± 0.033	± 0.041	± 0.054	± 0.069
	%V		+ 43.80	+ 35.90	+ 27.56	+ 17.38	+ 15.90
Small intestine	Exptl value	1.245	1.985	1.719	1.535	1.490	1.320
	SEM	± 0.025	± 0.032	± 0.029	± 0.027	± 0.025	± 0.021
	%V		+ 61.90	+ 43.28	+ 26.30	+ 20.40	+ 18.30

1. Values expressed as microgram of copper/100 milligram weight of sample
2. Each value is the mean of six observations
3. %V is coefficient of variations
4. Experimental values are statistically different from control with statistical significance at p<0.005 (not significant)

terfere with the enzyme activities of muscle contraction. The high concentrations of copper in the muscle may also affect the process of glycogenolysis and ultimately hamper the generation of ATP and CP which are responsible for providing energy for contraction. Loss of balance, irregular movements, and lethargic conditions of the experimental animal may be associated with the depletion of energy budget.

Many researchers have reported high concentrations of cadmium, mercury and lead in the hepatopancreas of white leg prawn *Litopenaeus vannamei*.⁸ Similarly, a high degree of bioaccumulation of cadmium in the hepatopancreas has been observed as compared to muscle in the estuarine crab *Chasmognathus granulata*.⁹ Other investigators have reported higher zinc concentrations in digestive glands than the other organs in many aquatic invertebrates.⁴

In the present study, hepatopancreas showed less accumulation of copper. The hepatopancreas combines within

itself the functions of the pancreas, liver and small intestine of higher animals. It is the main metabolizing organ and may also contain all the detoxifying enzyme machinery. The toxicant in it may be changed into less toxic form, or may get detoxified resulting in less amounts of copper accumulation.

The present study also revealed that the intestine of *Barytelphusa gureini* showed a very low accumulation of copper as a toxicant as compared to other organs. No references could be located from the available literature to support this observation. It is the opinion of the authors that copper as a toxicant inhibits the absorption property of the intestine, and hence results in very low concentrations as compared to other organs.

Recovery span: When the experimental animals were placed in clean water after exposure to copper, the recovery of copper in almost all organs was rapid and significant. The concentration of copper in the organs depends on the level of exposure, nature of metal, and

ability of the animal to metabolize or excrete the compound. Metals may be excreted by one or more of the routes such as across the body surface, gills, gut wall and faecal matter.

It can be concluded that when the experimental animals from polluted water are transferred to clean water they have the capacity to recover from the pollutant's influence. In the present study, muscle tissues showed almost complete recovery. Hence the animal (freshwater crab) when consumed as food does not pose toxic hazard due to short-term copper exposure.

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