Pattern of accumulation of heavy metals (Copper and Zinc) in the estuarine hermit crab *Clibanarius longitarsus* (De Hann)

P. S. Lyla* & S. Ajmal Khan
Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai – 608 502, India
[*Email : lailanair3@gmail.com]

Received 15 April 2010; revised 4 May 2010

The 96 h LC50 value of copper and zinc was 8ppm and 12ppm respectively for *Clibanarius longitarsus*. Based on the LC50 value, three sublethal concentrations were chosen for individual exposure in each metal. Pattern of accumulation of heavy metals in the tissues of *C. longitarsus* noticed in the laboratory was similar to what was observed in the environment: hepatopancreas > ovary > muscle (exposed to both the metals–copper and zinc). Further, increased uptake of metals by tissues was recorded with increase in the test concentrations.

**Keywords**: *Clibanarius longitarsus*, Heavy metals, Hepatopancreas, Muscle, Ovary

**Introduction**

Unlike many contaminants, heavy metals are normal constituents of the marine environment. Marine organisms tend to accumulate heavy metals from the environment and are adapted to handle natural fluctuations in intake brought about by slight changes in their availability in water or food. Such a process of accumulation of pollutants by organisms is termed as bioconcentration or bioaccumulation. Bioaccumulation and the effect of heavy metal contaminants on marine organisms have been the focus of study by a large number of investigators since quite some time. Bioaccumulation in few crustaceans has been studied\(^{1-15}\). However such studies have not been attempted in hermit crabs compared to other crustaceans in particular crabs. Hermit crab *Clibanarius longitarsus* is a very good test organism and it can be used effectively in toxicity studies. Informations about bioaccumulation in this organism are a few. In the present study bioaccumulation of heavy metals in different tissues of this animal has been studied after exposure to 3 sublethal concentrations of heavy metals copper and zinc.

**Materials and Methods**

The hermit crab *C. longitarsus* was collected from Vellar estuary (Lat.11° 29’N; Long.79° 46’E,) during the study period from 2006 to 2007. Carapace length of the crabs used in the experiment was in the range of 15-20 mm. Crabs of only intermoult stage was chosen for the experiment. Water used for the study was collected from the same place, from where the animals were collected. Salinity of the experimental water was 27±1%. And temperature 29±1° C. Oxygen content was 4.4±0.2 ml/l. pH of the test water was 7.8±0.3. Healthy crabs were selected and acclimatized for a period of 7 days in the experimental tanks (fiberglass tank of dimension 30×30×50 cm) was having filtered estuarine water. Standards were prepared using analar grades of copper sulphate (CuSO\(_4\).5H\(_2\)O) and zinc sulphate (ZnSO\(_4\).7H\(_2\)O). All the standards were prepared in deionised water as recommended\(^{16}\).

Acute toxicity studies popularly known as “bioassay studies” were conducted to determine the potency of the metal pollutants. Acute toxicity studies were basic 96 hr static renewal type and conducted in conformity with the guidelines laid down\(^{16-19}\). The 96 hr LC50 values were found to be 8 ppm in the case of copper and 12 ppm in zinc.

Based on these 96 hr LC50 values, 3 sublethal concentrations were chosen for the metals copper and zinc (2, 1 and 0.5 ppm in copper and 3, 1.5 and 1 ppm in zinc). Experimental media were prepared with filtered estuarine water. Test media and toxicant were renewed every 24 hrs. Water was well aerated daily before preparing the medium. Ten animals each was exposed to the 3 sublethal concentrations of each metal for a period of 30 days and were experimentally matched with control animals of similar size. After exposure, crabs were taken from each concentration
and various tissues like hepatopancreas, muscle and ovary were removed and washed properly in double distilled water and kept in the oven at 110°C up to 24 hours and then digested with acid as suggested by Topping\textsuperscript{20}. Copper and zinc were estimated by the Atomic Absorption Spectrophotometer (Perkins Elmer Model 2280).

The concentration factor was calculated using the following formula suggested by Bryan\textsuperscript{21}:

$$\text{CF} = \frac{\text{Concentration of metals in tissues}}{\text{Concentration of metals in water}}$$

### Results

The level of copper and zinc in control animals and in those maintained in experimental concentrations is given in Table 1 along with the concentration factor (Fig. 1).

<table>
<thead>
<tr>
<th>Copper</th>
<th>Concentration in ppm</th>
<th>Hepatopancreas</th>
<th>C.F</th>
<th>Muscle</th>
<th>C.F</th>
<th>Ovary</th>
<th>C.F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>325.0</td>
<td>--</td>
<td>20.0</td>
<td>--</td>
<td>36.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>627.5</td>
<td>1255</td>
<td>27.0</td>
<td>54.00</td>
<td>72.0</td>
<td>144.0</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>740.0</td>
<td>740</td>
<td>40.3</td>
<td>40.30</td>
<td>94.0</td>
<td>94.0</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>1420.0</td>
<td>710</td>
<td>61.5</td>
<td>30.75</td>
<td>172.0</td>
<td>86.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Zinc</th>
<th>Concentration in ppm</th>
<th>Hepatopancreas</th>
<th>C.F</th>
<th>Muscle</th>
<th>C.F</th>
<th>Ovary</th>
<th>C.F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>106.0</td>
<td>--</td>
<td>36.0</td>
<td>--</td>
<td>87.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>158.0</td>
<td>158.0</td>
<td>51.5</td>
<td>51.50</td>
<td>117.0</td>
<td>117.0</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>162.0</td>
<td>108.0</td>
<td>68.5</td>
<td>45.70</td>
<td>139.0</td>
<td>92.7</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>308.0</td>
<td>102.6</td>
<td>72.5</td>
<td>24.20</td>
<td>162.0</td>
<td>54.0</td>
<td></td>
</tr>
</tbody>
</table>

### Discussion

There have been many studies on the bioaccumulation of metals by crustaceans. These studies conducted in the laboratories revealed the relationship between absorption of metals with both exposure concentration and time\textsuperscript{1,22}. Direct proportionality between whole body concentration and exposure has been reported in \textit{Palaemon elegans}\textsuperscript{2} and \textit{Pandalus montagai}\textsuperscript{23}. In the present study also a linear relation between absorption of metals by tissues and external medium was observed. The order of accumulation of heavy metals (among the 3 tissues taken presently) in \textit{Cancer pagurus}, \textit{Carcinus maenas}\textsuperscript{24}, and \textit{Homarus americanus}\textsuperscript{25} has been hepatopancreas > ovary > muscle. The previous study in Vellar estuary revealed maximum level of copper.
and zinc during monsoon and minimum during summer\textsuperscript{11} and that is why the present study has been undertaken with these metals. In the present study also similar order was found.

In the present study, bioaccumulation of copper was more in hepatopancreas. ANOVA done showed significant differences (\(F=134.55\ P<0.001\)) between the level of copper in hepatopancreas and other tissues (muscle and ovary). The 't' test also showed significant differences between hepatopancreas and muscle\textsuperscript{12} \(t=12.620\ P<0.001\) & hepatopancreas and ovary\textsuperscript{13} \(t=12.80\ P<0.001\) in the levels of copper. Similar trend in accumulation of copper and iron has also been reported in the hepatopancreatic cells of Procambarus clarki \textsuperscript{27} Narayanan et al.\textsuperscript{13} who studied bioaccumulation of this metal also made similar observations in the estuarine crab Thalaminata crenata. Such results are also available for many other crustaceans. As hepatopancreas serves as a digestive and storage organ, higher accumulation is taking place here. Earlier study\textsuperscript{28} in fresh water prawn Macrobrachium malcolmsonii with sub-lethal concentrations of zinc and cadmium had shown more accumulation in hepatopancreas followed by gills which is similar to the present study. Ovary functions as the nerve centre of activity during the reproductive period when synthesis and mobilization of resources will be at the peak. As the mobilization of resources will be done through blood, this organ will naturally receive and accumulate these heavy metals. Higher level of metals compared to muscle has been reported in the ovary of many organisms\textsuperscript{29}. Bioaccumulation of copper was the least in muscle. As it is a tissue receiving major portion of blood supply accumulation is taking place here although at a lower level. Miramand et al.\textsuperscript{30} and White & Rainbow\textsuperscript{31} also found the crustacean muscle to show a very low degree of heavy metal uptake relative to other tissues.

The order of accumulation was the same as that of copper and the zinc concentration was more in the hepatopancreas than ovary and muscle. ANOVA done showed significant differences (\(F=77.54\ P<0.001\)) between the level of zinc in hepatopancreas and other tissues (muscle and ovary). The 't' test also showed significant differences between hepatopancreas and muscle\textsuperscript{12} \(t=12.88\ P<0.001\) & hepatopancreas and ovary\textsuperscript{13} \(t=7.23\ P<0.001\) in the levels of zinc. Greater accumulation of zinc in the hepatopancreas is attributed mainly to the nature of function of the hepatopancreas. Al-Mohana & Nott\textsuperscript{32} who worked on Penaeus semisulcatus reported that the accumulated zinc in the hepatopancreas may be stored as phosphate granules. A storage protein such as zinc metallothionein as reported in Scylla serrata and zinc and copper binding protein as reported in Carcinus maenas may be synthesized here. As the excess metal ions are immobilized thus, there is a reduction in their toxicity\textsuperscript{33}. Reasons cited for the accumulation of copper in ovary and muscle hold good for zinc too. In the present study, the rate of accumulation was very high in the lowest test concentrations compared to higher test concentrations. White and Rainbow\textsuperscript{2} reported the presence of regulatory mechanism in the case of prawn Palaemon elegans which regulated body zinc concentration, when exposed to elevated zinc concentration at least upto certain limit. Amiard – Triquet et al., Ward & Parrish\textsuperscript{19}, Bryan et al.\textsuperscript{34}, and Wu & Chen\textsuperscript{35} also reported similar type of regulation of zinc in a number of decapod crustaceans. But presently there was no regulation in the lowest concentration, but such a mechanism may be responsible for inhibition at high concentrations. At high toxic concentrations, there is reduction in metal ion permeability which inhibits excess metal ion-accumulation\textsuperscript{36}.

Acknowledgement

Authors are thankful to Dr. T. Balasubramanian, Professor & Director, Centre of Advanced Study in Marine Biology for the encouragement and the authorities of Annamalai University for the facilities.

References


Usha Rani, G., Distribution of heavy metals in the Cochin estuary (Southwest coast of India) and their effect on mudcrab *Scylla serrata* (Forskal) (Decapoda: Crustacea) Ph. D. thesis, Annamalai University, India, 2003. 116 pp.


