6. ACUTE TOXICITY OF CCA AND ITS CONSTITUENTS COPPER, CHROMIUM AND ARSENIC IN VILLORITA CYPRINOIDES

6.1. Introduction

The Group of Experts on Scientific Aspects of Marine Environmental Protection (GESAMP) defines marine pollution as ‘the introduction by man, directly or indirectly, substances or energy into the marine environment (including estuaries) resulting in deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of seawater and reduction of amenities. The estuaries and coastal areas with maximum population density are more prone to the deleterious implications of pollution as it becomes the primary source for pollutant release. The heavy metals, released through the domestic sewage, industrial wastes volcanic eruption and weathering of rocks forms one of the major class of pollutants added into the estuaries and coastal waters. The metal pollutants are highly toxic to the aquatic organisms since in some form or the other these are soluble in seawater. The free ions, the most bioavailable forms of metals are readily taken up by the organisms and either accumulated in the body tissues or released. If get accumulated, both the essential metals in higher quantities and the non-essential metals in minute quantities are harmful to the normal functioning of the organisms.
Chapter VI

The impact of a chemical added into a particular environment can be studied by different methods. The ecological risk assessment and pollution-monitoring programme essentially employ toxicity studies as a tool to assess the immediate and direct effects of the particular toxicant. The aim of the toxicity test is to define the concentrations at which the toxicant is producing a selected deleterious response in a population (Ward & Parrish, 1982). The aquatic toxicity tests are conducted in a controlled condition with selected organisms especially marine or fresh water algae and macro invertebrates and fishes. The bivalve molluscs *viz.* mussels, clams have been well recognized as tools for a widely accepted biological pollution monitoring programme called ‘Mussel watch’ introduced world over in 1975 by Goldberg. These organisms are cosmopolitan in occurrence and are hardy enough to survive under laboratory conditions. These organisms are sessile, and can accumulate certain heavy metals without suffering mortality (Beaby & Eaves, 1983). The acute and chronic effects of a toxicant were determined by conducting a time dependent and time independent renewal or static acute toxicity tests or by conducting chronic toxicity studies. Although not environmentally unrealistic the results can be extrapolated to predict toxicant concentration that may be allowed in waters without adverse effects on living resources. *Mytilus, Perna, Crassostrea, Ostrea spp., Villorita spp.*, etc. are the common species of molluscs used in toxicity studies (Rainbow, 1995).

The present investigation was on *Villorita cyprinoides* (black clam), which is a benthic bivalve found in estuaries of Kerala. The species was
selected for the study because of the fact that these are commonly cultured and commercially important species in Kerala. These animals can easily be sampled, transported and maintained in the laboratory (Boening, 1999). Necessary background information on the species was available since studies were conducted under laboratory condition by Lakshmanan and Nambisan (1977), Abraham et al., (1986), Sathyanathan (1996) to assess the depuration and toxicity of various metals. Pillai et al., (1986) used Villorita as a tool to assess the environmental pollution at Vembanad Lake.

Chromated Copper Arsenate (CCA) is a waterborne preservative containing metals like copper, chromium and arsenic mixed in their oxide forms. The insecticidal properties of arsenic pentoxide and the fungicidal properties of chromium oxide make CCA one of the most efficient wood preservatives. There are about 208 preservative treatment plants in India, using about 2980 tonnes of preservatives equivalent to CCA (Gairola & Aggarwal, 2005). The marine plywood manufacturers are the major consumers of CCA. The wood when treated with this preservative fixes well into the wood. So the only possible source for direct toxicity is the run off from CCA treatment plants. The experiments are being conducted all over the world to find out the potential toxicity of this preservative. The run-off from such CCA treatment plants are known to contain high concentration of arsenic (III), chromium and copper (Cox, 1991). So the possibility of contamination of aquatic system by this source of heavy metals is indeed a matter of concern. Though studies regarding the acute toxicity of CCA the larvae of wood boring teredinids that
are target to the CCA were known, very little has been known about the direct acute toxicity of CCA on non-target organisms (Balaji et al., 2004). In the view of the known hazards and possible deleterious effects of copper, chromium and arsenic in the aquatic environment, an attempt is made to study the acute toxicity of CCA and its constituent metals copper, chromium and arsenic separately and their bioconcentration in black clam.

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6.2. Materials and Methods

The test was conducted in order to compare the toxicity of CCA with the toxicity of its constituent heavy metals copper, chromium, and arsenic (Ward and Parrish, 1982) and to determine the possibility of bioaccumulation of these metals in selected species of clam. The experiments were conducted using *V. cyprinoides* as the test organism. The test organisms were collected from Cochin backwaters, where the salinity during the period of collection ranged between 24 ± 2‰ and pH 7.6 ± 0.2. The clams were transported to the laboratory in plastic containers of 50 l capacity with minimum stress to the organism. In the laboratory, the collected test organisms were acclimatized in well-aerated tap water at 28 ± 2°C for 48 to 72 h prior to the experiment. The specimens from the same population were used for a single experiment. Size
of clams was measured using vernier calipers and sorted. The average length
of shells of clams used in the study ranges between 22 mm – 28 mm. The
experiment was conducted in 10 l trays with 5 l water. Water was kept aerated
for two days so as to minimize the effect of chlorine in it. The physico-
chemical parameters of water were checked before and after the experiment
are given in Table 6.1.

Analar grade of salt of each toxicant, CuSO$_4$.5H$_2$O (M.W.- 249.53),
CrCl$_3$.6H$_2$O (M.W.-266.48) and As$_2$O$_3$ (M.W.- 197.83) is used for the
experiment. Stock solutions of each of these were prepared by dissolving the
salts in distilled water not more than two days in advance of the test. Stock
solutions were serially diluted to get required concentrations in µg l$^{-1}$.

The present investigation was carried out using 7.5% solution of CCA
to treat rubber wood samples. The Central Institute of Fisheries Technology
has standardized a 7.5% solution of CCA for treating boat-building timbers
since it imparted sufficient protection under marine condition. The solution is
diluted through several steps to conduct range-finding studies in such a way
that the concentration of toxicant in each tank is 50% of the next higher
concentration. From the preliminary range finding studies the concentrations
for final toxicity studies were selected. In case of CCA, the concentrations
selected for the studies were 0.5 ppm, 0.625 ppm, 1 ppm, 1.25 ppm, 2.5 ppm,
5 ppm and 10 ppm. For each test a duplicate tank was run for reference. A
control tank was maintained in order to check the acceptability of the results
(Fig. 6.1.).
Ten clams were exposed to 5 l toxicant solution in each tray. During the period of experiment clams were not fed and water was not aerated. Every 24 h mortality was monitored and water was changed maintaining the concentration of the toxicant. The criterion for mortality was the valve gaping of 5 mm and the lack of response of the organism when gently prodded. After 96 h of exposure the live organisms were collected. The soft tissues of the clams were dissected out and shells were separated. Both the tissue and shell samples were dried at 80-82°C till to attain a constant dry-wt. The 0.2g of the dried samples was digested in HNO$_3$ - HClO$_4$ mixture in microwave acid digester. The concentration of copper, chromium and arsenic in the digested samples were analysed by ICP - AES (Perkin Elmer-Optima 2000 DV).

**6.2.1. LC$_{50}$ values and BCF values**

LC$_{50}$ values and their 95% upper and lower confidence limits were calculated using Trimmed Spearman - Karber estimate, which is a recent modification of Spearman-Karber estimate (1978). The software for this method obtained from Civil and Environmental Engineering, Old Dominion University (CEE/ODU) Civil/Environmental Model Library (CEML). The percent mortality at each toxicant concentration is determined and plotted in a graph.

Bioconcentration Factor is a measure of distribution of the heavy metal between biota and water. It is calculated using the equation:

$$B.C.F = \frac{C_{\text{biota}}}{C_{\text{water}}}$$

Where, $C_{\text{biota}}$ is the total metal concentration in biota (ppm)
6.3. Results and Discussion

Although the metal components of CCA are essential for the normal functioning of the organism, the results show that above a particular threshold level they can be acutely toxic. In all metals studied and in CCA, number of survivors decreased with increase in concentration.

The results indicate that copper is more toxic than all other metals studied. It was found that 0.5 ppm of CuSO$_4$ was found to be lethal to 50% of the population (Fig. 6.2.). Concentrations above 0.3 ppm were found to be causing much stress on the organism. Mucus secretion was significantly high in all these concentrations. The results of the study show that LC$_{50}$ value for chromium and arsenic is 3.6 and 3.72 (Fig. 6.3., Fig. 6.4.). In all concentrations of CCA taken for the study, the shells are found to be disintegrating. At higher concentrations of CCA the disintegration is found to be more. The dead organisms after 96 h was dissected and the tissue were found to be green in colour (Fig. 6.5.). In the case of CCA even 1 ppm solution showed 50% mortality and 10 ppm solution showed more than 50% death in the specified duration (Fig. 6.6.).

The analysis of tissue of clams in the control tank showed presence of 38 ppm of copper, 33 ppm of chromium and 23 ppm of arsenic. When concentration in the water increased the uptake and bioconcentration of copper in the tissue also increased. The tissue concentration of copper shows that the clams can concentrate and tolerate increased body burden of copper when
compared to the concentration of other metals studied here. At the LC$_{50}$ of 0.5 ppm the concentration of copper was 49.26 that was the highest value observed. The bioconcentration factor for copper was 77.40, 82.11 and 98.14 respectively for 0.3, 0.4 and 0.5 ppm of copper concentrations. But in the case of chromium the concentration of metal in the tissue sample was found decreasing as the concentration in the water increased. The range of concentrations studied near the LC$_{50}$ value of chromium showed that no significant increase in concentration. The bioconcentration studies showed that as the concentration increases the bioconcentration factor for chromium decreases. In the case of arsenic no such trend was observed while the concentration in the tissue increased to an amount of 263.05 ppm on exposure to 3 ppm. But at 2 ppm and 4 ppm the value was very low when compared to that at 3 ppm. The bioconcentration factors also showed the same trend. The data analysis showed that copper has the highest potential to get concentrated in the tissues than chromium and arsenic referring to the fact that the bioconcentration potential depends on the properties of the chemical.

The toxicity of a particular toxicant on particular organism varies significantly with changes in the conditions of the test. Salinity is one of the major factors that affect toxicity. The toxicity studies conducted in the same species of clam of 25 mm size, at a salinity of 1‰ showed that mortality was too rapid above 2 ppm and extensive mucus secretion was observed in copper concentrations above 0.5 ppm (Lakshmanan & Nambisan, 1977). Static toxicity studies conducted in the same species resulted in an LC$_{50}$ value of
1.21 µg l\(^{-1}\) (Abraham et al., 1986). This very low value when compared with the results of the study shows that the methodology used also affects the resultant toxicity values. The \(LC_{50}\) value of copper in green mussels for a period of 96h was reported as 0.175 ppm (Nambisan & Lakshmanan, 1986). The studies conducted by Meenakumari and Nair (1993) showed that in 48 h 50% mortality has occurred in green mussel population that is exposed to a copper concentration of 0.83 and reported that copper is found to be the most toxic metal studied.

The results of the study show that \(LC_{50}\) value for chromium and arsenic are 3.6 and 3.72. According to Brooks, (1997) arsenic and chromium are tolerated at moderately high levels by aquatic species. Chromium seems to be somewhat less toxic to aquatic organisms. The static 96h studies conducted in \(V.\ cyprinoids\) var \(cochinensis\) using chromium oxide resulted in an \(LC_{50}\) value of 11.5 µg l\(^{-1}\) (Abraham et al., 1986). Green mussels are found to be more efficient in tolerating toxicity of chromium since 48 h toxicity range was 7-12.8 ppm (Govindarajan et al., 1993), which was higher than the results obtained in this study in \(Villorita\ sp.\) In \(Mytilopsis\ sallei\) \(LC_{50}\) of chromium at a salinity of 10 ppt is 5.34 ± 0.22 ppm. Arsenic can exist in two different oxidation states, out of which Arsenic (III) was found to be more toxic than Arsenic (VI). \(LC_{50}\) range for arsenic (III) is given as 3000-7500 µg l\(^{-1}\) for molluscs. 48-h \(LC_{50}\) value for \(Perna\ viridis\) for arsenic was 2.39 ppm.
In blue mussels 100% mortality was observed for arsenite concentration of 16 ppm.

Studies have been conducted to assess the impact of CCA on bacteria fungi and borers that attack the wood, there has been very little attempt to study the acute toxicity of CCA on non-target organisms (Buchanan & Solomon, 1990). But the acute toxicity of this chemical to *Daphnia magna* and to a species of algae *Selenastrum capricornatum*, shows that metals acts jointly to cause toxicity since the toxicity of CCA was greater than that of its individuals metals (Cox, 1991). Experiments conducted by Keith and Warner (1990) shows that copper and chromium acts synergistically to cause toxicity. The study conducted on teredinid wood borers showed that arsenic of 1.5 ppm concentration was sufficient to cause 50% death in 72h and chromium was found to be least toxic since all animals active at 2 ppm even after 96h (Balaji *et al.*, 2004)

The analysis of the tissue of *V. cyprinoides* collected from the Cochin area of the Vembanad Lake ranged between 12-30 ppm (Pillai *et al.*, 1986). The depuration and bioconcentration studies conducted in *V. cyprinoides* showed that the organism can live with significantly higher content of copper in their body (Sathyanathan, 1996). The results showed that the clams concentrate fairly high content of copper. Studies conducted showed that B.C.F. for chromium in oysters and blue mussels ranged between 125 -192 (Train, 1979). Copper is found to have a higher bioconcentration potential than chromium (Won & Tack, 1994). The B.C.F values of arsenic were low.
except in the case of algae (Eisler, 1988a). Bioconcentration factor arsenic was 350 for *Crassostrea virginica* when it was exposed for 112 days in arsenic solution (Zaroogian & Hoffman, 1982).

### 6.4. Conclusion

Of the CCA components copper is found to be more toxic than chromium and arsenic. Copper can be acting along with arsenic and chromium to impart an added effect on its toxicity. All the three metals copper, chromium and arsenic of CCA have a tendency to get concentrated in the tissues of invertebrates like clams. The B.C.F value indicating the bioconcentration potential of a metal in an organism is higher for copper than the other two metals studied. In the case of CCA possibility of heavy metal pollution that it is imparted cannot be ignored. Combined toxicity studies are still to be done in order to conclude that whether these metals act in a synergistic way to cause toxicity in organisms. Since CCA is used after impregnating the wood with the chemical solution, the chemical components leached out from CCA is studied for its toxicity and bioconcentration characteristics are detailed in chapter VII.
Table 6.1. Hydrographical parameters of water used for the study

<table>
<thead>
<tr>
<th>Water parameters</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>28 ± 2°C</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>90 - 110 %</td>
</tr>
<tr>
<td>pH</td>
<td>8 ± 0.2</td>
</tr>
<tr>
<td>Salinity</td>
<td>0 ‰</td>
</tr>
</tbody>
</table>

Table 6.2. Bioconcentration factor (B.C.F.) for copper, chromium and arsenic in black clam tissue

<table>
<thead>
<tr>
<th>Metal</th>
<th>Metal concentration in tissue of clams in control tank (ppm)</th>
<th>Conc. of metal in water (ppm)</th>
<th>Conc. of metal in tissue (ppm)</th>
<th>Bioconcentration Factor (B.C.F.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>38</td>
<td>0.3</td>
<td>232.19</td>
<td>773.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4</td>
<td>328.44</td>
<td>821.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>492.68</td>
<td>985.36</td>
</tr>
<tr>
<td>Chromium</td>
<td>33</td>
<td>2</td>
<td>41.48</td>
<td>20.74</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>32.78</td>
<td>10.92</td>
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<td></td>
<td></td>
<td>4</td>
<td>15.89</td>
<td>3.97</td>
</tr>
<tr>
<td>Arsenic</td>
<td>23</td>
<td>2</td>
<td>91.40</td>
<td>45.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>263.05</td>
<td>87.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>61.23</td>
<td>15.30</td>
</tr>
</tbody>
</table>
Figure 6.1. a) Laboratory set up of toxicity studies b) clams exposed to CCA solution

Figure 6.5. *Villorita cyprinoides* exposed in (a) control tank and (b) CCA solution
Figure 6.2. The percentage mortality of black clams at different concentrations of copper

Figure 6.3. The percentage mortality of black clams at different concentrations of chromium
Figure 6.4. The percentage mortality of black clams at different concentrations of arsenic.

Figure 6.6. The percentage mortality of black clams at different concentrations of Chromated Copper Arsenate (CCA).