Chapter 3

ACUTE TOXICITY TEST

3.1 INTRODUCTION

Heavy metals are potentially harmful to most organisms at a level of exposure and absorption above a minimum threshold. Their presence in the environment has increased in general due to anthropogenic sources. Toxicity tests, such as acute lethality test, are useful for assessing chemical hazard to aquatic life (Krishnakumar et al., 1987).

Freshwater mussels are benthic filter feeding organisms. Freshwater mussels are exposed to metals that are dissolved in water, associated with suspended particles and deposited in bottom sediments. These freshwater mussels can bioaccumulate certain metals in high concentrations that greatly exceed those dissolved in water. In adult mussels, the most common site of metal uptake is the gills followed by the mantle and the kidney. The toxic effects of metals on freshwater mussels have been examined in a few acute toxicity tests but the sublethal effects of long-term exposure to low environmental concentrations are little understood.

Sublethal exposure to metals can alter growth, filtration, efficiency, enzyme activity and behavior. Sublethal effects are frequently observed at concentrations that are only half the lethal concentrations. However, few toxicity tests have used environmentally realistic exposure concentrations. Total concentration of Cd, Cu, Hg and Zn in many toxic surface waters are in the mg/l range, yet many toxicity studies
have exposed mussels to the concentrations of $\mu$g l$^{-1}$ or even the mg l$^{-1}$ range. An understanding about the processes by which metals affect freshwater mussels would provide insights on the ecotoxicological significance of metal concentration in the natural mussel populations and aid in the development of water quality criteria that adequately protect mussels (Teresa J. Naimo, 1995).

The relation between short-term and long-term ecotoxicity was different for each metal and could not be predicted from the results of the short-term experiments (Michiel H.S. Kraak, et al., 1992).

In assessing the safety level of any poisonous chemical for higher animals, the first task is to determine the acute toxic LC 50 value, a simple expression of the degree of toxicity that can be understood by toxicologists (Dubois and Geiling, 1959). The increasing awareness of aquatic pollution demands toxicity tests to assess the efficacy of the contaminants and to extrapolate their safe levels permissible in the environment. The median tolerance limit of any pollutant is meant as an elementary guide in the field of toxicology (Ward and Parrish, 1982). Without reference to the median tolerance limit, no information on sublethal effects can be deduced (Patin, 1982). Thermal acute toxicity tests represent an important method for establishing criteria to evaluate water quality and therein to protect the aquatic environments (National Academy of Sciences / National Academy of Engineering, Washington, 1972). Acute toxicity studies are generally employed to compare the sensitivities of different species to different potencies of the
chemicals and to derive, by using LC50 values, environmental concentration of chemicals which could be considered ‘safe’.

### 3.2 MATERIALS AND METHODS

#### 3.2.1 Collection of test organism

The freshwater mussel *L. marginalis* collected from the Sivankovil pond, Pondi at Thanjavur were brought to the laboratory in plastic buckets. The freshwater mussel with shell length 57±5mm was used in all experiments to avoid any possible error due to size differences. Healthy mussels were selected and acclimatized for a period of 3 days in fiber glass tank (30×30×50cm) containing pond water(Temperature 29±1°C, salinity 0.39±0.05%, pH 8.0±0.2, oxygen content 5.3±0.5ml-1)

#### 3.2.2 Preparation of Standard Stock Solution

Stock solution for copper, cadmium and synergistic metals (Bimetal mixer) were prepared by dissolving appropriate amount of metallic salt such as copper sulphate (CuSo4, 5H2O), cadmium chloride (CdCl2) containing 1g of the metal in 1 litre of deionised distilled water. Synergistic stock solution were prepared by 0.5g of copper and 0.5g of cadmium dissolved in 1 litre of deionised distilled water.

#### 3.2.3 Acute toxicity test

Acute bioassay studies were conducted to determine the potency of metal pollutants. The studies were of 96hr static renewal type and conducted in conformity with
Serial dilutions were prepared from the stock solution for each metal. Ten bivalves were exposed to 10 litres of test solution of heavy metals required to kill 50% of the bivalves was estimated in 96hr, following the method of Litchfield and Wilcoxon (1949) and Finney (1971). Median lethal concentration (LC50) was calculated with 95% confidence limits.

The test medium was changed once in every 24hr to avoid suffocation of the animals due to oxygen depletion pH, temperature and dissolved oxygen were tested every time when the water was changed.

3.2.4 Chronic toxicity test

Based on acute toxicity test (96hr LC50) sub lethal concentrations (10%, 20% and 30%) were derived for copper, cadmium and synergistic metals which were used as the experimental concentration of the heavy metals in the subsequent experiments.

Twenty five bivalves were exposed to each sublethal concentration of copper, cadmium and synergistic metal for a period of 10, 20 and 30 days and experimentally matched with control animals of similar size. Multiple sets of experiments were maintained for periodic analysis of metal exposure. Test media were renewed once in
every 24hrs and well aerated. A control batch was maintained simultaneously and six trails were run.

3.3 RESULTS

96 hr Lc50 values for *L. marginalis* were found to be 1.2 mg/l for copper, 1.8 mg/l for cadmium and 1.5 mg/l for synergistic metals. Sublethal concentrations 10%, 20% and 30% values for freshwater mussel were found to be 0.12 mg/l, 0.24mg/l and 0.36mg/l for copper, 0.18 mg/l, 0.36mg/l and 0.54mg/l for cadmium 0.15 mg/l, 0.30mg/l and 0.45mg/l for synergistic (Table 1).

3.4 DISCUSSION

Mussels except in control and lower concentrations of metals remained with their shells tightly closed. Some mussels exposed to higher concentrations of metals did not withdraw their foot when closing but left in between the valves after 48hrs exposure. Higher concentration of metals decreased byssus thread production and increased mucus secretion. Similar behavioural patterns were observed in green mussel *Perna viridis* exposed to selected heavy metals (Krishnakumar, *et al.*, 1987).

In the present study, of the 3 metals tested the order of toxicity to *L. marginalis* is cd > synergistic > cu. These patterns observed laboratory study confirm the patterns of the metal uptake by these bivalves in their natural habitats. These studies were in agreement with the studies made by Harrison and Quinn (1972) in freshwater mussels,

Bivalves exposed to a different sublethal concentrations showed differential ability of metal uptake. The linear relationship between the rate of absorption of metal by tissue and levels in the medium was also observed by Schulz – Baldes (1974), George and Coombs (1972) and Von westenhagen et al., (1978) in Mytilus edulis, Lakshmanan and Namboodar (1986) in Perna viridis and Krishnakumari and Vijayalakshmi (1992) in Saccostrea cucullata.

Calabrese et al., (1973) observed the 48 hours LC50 value of cadmium and lead in the oyster larvae. It was observed to be 3.8 mg/l and 1.6 mg/l respectively. It is said that larvae are more sensitive than their adults. In Chasmagnathus granulate 96 hours LC50 value of cadmium is higher than that of lead (Ferrer et al., 1999). Similar observation was noted in the present study that the cadmium concentration was higher than that of copper.

The behavioural changes, restlessness and excess secretion of mucus suggest that L. manginalis undergone chemical stress when exposed to heavy metals in the present study could be taken as an indicator of aquatic pollution.