CHAPTER 5

CHRONIC EXPOSURE OF ZINC IN MARINE ORGANISMS

5.1 INTRODUCTION

The problem of heavy metal pollution in coastal waters and its effects on aquatic organisms is drawing increasing attention. Information concerning chronic toxicities of zinc on aquatic organisms is still limited in the tropical regions (Hutomo et al. 1995). In this chapter the effect of chronic toxicity of zinc on chosen species is dealt in detail.

Chronic toxicity is generally considered to include non-lethal biological effects that occur over longer time periods - usually for 7 days or greater; often a significant part of a life cycle. In the present study, thirty day survival tests have been conducted as the standard method to evaluate chronic toxicity of zinc on chosen marine organisms. The results of chronic toxicity with appropriate sensitive species will be used to develop criteria to protect the marine organisms.

One static-non-renewal test was performed to evaluate chronic toxicity of zinc in phytoplankton (Coscinodiscus centralis) and four tests were performed by flow through method to evaluate the chronic toxicity of zinc in M. cephalus, P. monodon, P. viridis and T. jarbua.

In this study, the deviation of pH was less than 0.3; the concentration of DO in test solutions was higher than 5 mg/L. The salinity
was maintained at 33±1. The survival percentages of control group were 100% for all the marine organisms tested. During the test period, the temperature was maintained at 27±1°C, water quality parameters were measured every day by standard methods (APHA 1998).

5.2 MOLLUSC - *PERNA VIRIDIS*

Nominal concentrations of zinc for the chronic toxicity test, derived from the previous toxicity results, were 0.008, 0.016, 0.032, 0.064 and 0.125 mg/L. Control survival was found to be 100% in chronic test. Mussels exhibited classical concentration-dependent responses at the end of the 30 days (Figure 5.1). Among the tested concentrations, first mortality was observed after 19 days of exposure at higher concentration (0.125 mg/L) of zinc. The survival data of *P. viridis* demonstrated 25% of mortality in 0.125 mg/L of zinc concentration at the end of 19 days. The survival percentage of organisms after 30 days of exposure reduced to 25% at higher treatment of 0.125 mg/L (Figure 5.1). There was a significant difference (P<0.05) in survival of *P. viridis* at higher concentrations (0.032, 0.064 and 0.125 mg/L) when compared to control at the end of thirty days chronic exposure of zinc. There was no significant difference in the survival of *P. viridis* in 0.008 mg/L and 0.016 mg/L concentrations of zinc.

By considering the number of survivors at the end of experiment, NOEC, LOEC, and MATC were calculated to be 0.016, 0.032 and 0.023 mg/L respectively (Table 5.1). For biochemical endpoints, the LOEC_{Protein} was 0.008 mg/L, LOEC_{GSH}, LPO, GST, AChE were 0.016 mg/L and LOEC_{CAT} was 0.032 mg/L (Table 5.2).
Figure 5.1 Survival (%) of *P. viridis* in various concentrations of zinc

Table 5.1 Values of NOEC, LOEC, and MATC for marine organisms exposed to zinc

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Measured end point/duration</th>
<th>NOEC (mg/L)</th>
<th>LOEC (mg/L)</th>
<th>MATC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. viridis</em></td>
<td>Survival /30 d</td>
<td>0.016</td>
<td>0.032</td>
<td>0.023</td>
</tr>
<tr>
<td><em>P. monodon</em></td>
<td>Survival /30 d</td>
<td>0.007</td>
<td>0.015</td>
<td>0.010</td>
</tr>
<tr>
<td><em>M. cephalus</em></td>
<td>Survival /30 d</td>
<td>0.045</td>
<td>0.090</td>
<td>0.063</td>
</tr>
<tr>
<td><em>T. jarbua</em></td>
<td>Survival /30 d</td>
<td>0.025</td>
<td>0.050</td>
<td>0.035</td>
</tr>
<tr>
<td><em>C. centralis</em></td>
<td>Survival /5 d</td>
<td>0.30</td>
<td>0.48</td>
<td>0.38</td>
</tr>
</tbody>
</table>

5.3 CRUSTACEAN - *PENAEUS MONODON*

The nominal test concentrations were 0.007, 0.015, 0.030, 0.060 and 0.120 mg/L derived from previous toxicity results for five zinc exposed groups. Mortality of prawn PL increased with increase in concentrations at the end of chronic exposure test. There observed 100% survival in control
organisms throughout the period of test. The first 10% mortality was observed in 0.12 mg/L concentration of zinc after 20 days of zinc exposure. At the end of chronic test (30 days), there was 100% survival in the 0.007 mg/L concentration of zinc and no significant difference was observed from control. The percentage survival of *P. monodon* in the highest treatment (0.12 mg/L) was observed to be 30% (Figure 5.2). Very high significance (P<0.001) was observed in survival of *P. monodon* when compared to control from 0.015 mg/L to 0.12 mg/L concentrations of zinc.

![Figure 5.2 Survival (%) of *P. monodon* in various concentrations of zinc](image)

The highest zinc concentration that did not have an inhibiting effect (NOEC) on the survival was 0.007 mg/L. The lowest concentration that affected the survival of organism (LOEC) was 0.015 mg/L. The MATC was calculated to be 0.010 mg/L (Table 5.1). The LOEC values based on biochemical endpoints were LOEC$_{protein}$: 0.030 mg/L, LOEC$_{GSH,GST,CAT}$: 0.015 mg/L, LOEC$_{LPO,AChE}$: 0.007 mg/L (Table 5.2).
Table 5.2  LOEC values (mg/L) for biochemical biomarkers exposed chronically (30 d) to zinc

<table>
<thead>
<tr>
<th>LOEC</th>
<th>P. viridis</th>
<th>P. monodon</th>
<th>M. cephalus</th>
<th>T. jabrua</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOEC&lt;sub&gt;Protein&lt;/sub&gt;</td>
<td>0.008(↓)</td>
<td>0.030(↓)</td>
<td>0.090(↓)</td>
<td>0.12(↓)</td>
</tr>
<tr>
<td>LOEC&lt;sub&gt;GSH&lt;/sub&gt;</td>
<td>0.016(↓)</td>
<td>0.015(↑)</td>
<td>0.045(↑)</td>
<td>0.050(↑)</td>
</tr>
<tr>
<td>LOEC&lt;sub&gt;LPO&lt;/sub&gt;</td>
<td>0.016(↑)</td>
<td>0.007(↑)</td>
<td>0.045(↑)</td>
<td>0.025(↑)</td>
</tr>
<tr>
<td>LOEC&lt;sub&gt;GST&lt;/sub&gt;</td>
<td>0.016(↑)</td>
<td>0.015(↓)</td>
<td>0.045(↓)</td>
<td>0.050(↓)</td>
</tr>
<tr>
<td>LOEC&lt;sub&gt;AcH&lt;/sub&gt;</td>
<td>0.016(↑)</td>
<td>0.007(↑)</td>
<td>0.090(↑)</td>
<td>0.050(↓)</td>
</tr>
<tr>
<td>LOEC&lt;sub&gt;CAT&lt;/sub&gt;</td>
<td>0.032(↑)</td>
<td>0.015(↓)</td>
<td>0.090(↑)</td>
<td>0.100(↓)</td>
</tr>
</tbody>
</table>

(↓) the activity was decreased, (↑) the activity was increased

5.4  CHORDATES - MUGIL CEPHALUS

The nominal concentrations of zinc in test medium were 0.022, 0.045, 0.090, 0.180 and 0.360 mg/L; these concentrations were derived from previous toxicity results. The results of thirty day chronic exposure with M. cephalus showed that survival percentage decreased gradually with increasing zinc exposure concentrations (Figure 5.3). Control survival was 100% till the end of toxicity test. First mortality was observed after 25 days of exposure in 0.180 mg/L (15% mortality) and 0.360 mg/L (30% mortality) concentrations of zinc. At the end of chronic toxicity test (30 days), survival percentage of M. cephalus was observed to be 50% (Figure 5.3) in highest treatment (0.360 mg/L). In concentrations 0.022 and 0.045 mg/L, survival was similar to control and did not show statistical significance while rest of the concentrations showed significant difference from control at P<0.001.
Figure 5.3  Survival (%) of *M. cephalus* in various concentrations of zinc

The calculated NOEC, LOEC and MATC based on survival endpoints were 0.045, 0.090 and 0.063 mg/L respectively (Table 5.1). From the results of biochemical endpoints, LOEC$_{Protein, AChE, CAT}$ was found to be 0.090 mg/L and LOEC$_{GSH, LPO, GST}$ were 0.045 mg/L (Table 5.2).

5.5  CHORDATES - *TERAPON JARBUA*

The nominal concentration of zinc in test chambers were 0.012, 0.025, 0.050, 0.100 and 0.200 mg/L (derived from previous toxicity test results). None of the control organisms showed mortality throughout the test period. *Terapon jarbua* exhibited a concentration dependant survival response. The five percent mortality was reported on the 16$^{th}$ day at higher concentration (0.20 mg/L), from 17$^{th}$ to 28$^{th}$ day the mortality was found to increase gradually 10% to 50%. The survival of *T. jarbua* did not differ statistically from control in test concentrations 0.012 and 0.025 mg/L,
while in 0.050, 0.100 and 0.20 mg/L of zinc there were very high significance (P<0.001). At the end of 30 days, survival of *T. jarbua* was found to be 35% at the highest zinc concentration of 0.200 mg/L (Figure 5.4).

**Figure 5.4 Survival (%) of *T. jarbua* in various concentrations of zinc**

The highest zinc concentration that did not have an inhibiting effect (NOEC) on the survival of *T. Jarbua* was 0.025 mg/L. The lowest concentration that affected the survival of organism (LOEC) was 0.050 mg/L. The MATC was calculated to be 0.035 mg/L (Table 5.1). The LOEC values based on biochemical endpoints were LOEC$_{\text{protein}}$ : 0.012 mg/L, LOEC$_{\text{GSH,GST,ACHe}}$ : 0.050 mg/L, LOEC$_{\text{LPO}}$ : 0.025 mg/L, LOEC$_{\text{CAT}}$ : 0.100 mg/L (Table 5.2).

### 5.6 PHYTOPLANKTON - *COSCINODISCUS CENTRALIS*

The test chamber had nominal zinc concentrations of 0.30, 0.48, 0.77, 1.23, 1.97, 3.15 and 5.03 mg/L (exposure conditions were similar to
those for acute test). Control survival was found to be 100%. During the 120 hours short term chronic exposure test performed on *C. centralis*, a gradual decrease in the percentage survival was observed upon increase in concentration of zinc. Maximum growth inhibition was observed in 5.03 mg/L of zinc while 100% survival was found in 0.008 and 0.3 mg/L concentrations of zinc. The mean percentage of survivors from two chronic tests were found to be 95%, 88%, 71%, 60% and 35% in concentrations 0.48, 0.77, 1.23, 1.97 and 3.15 mg/L respectively. There was no survival of *C. centralis* in 5.03 mg/L of zinc. The Figure 5.5 indicates the nominal concentration between 0.48–5.03 mg/L, a definite reduction was found in survival.

![Figure 5.5 Survival (%) of *C. centralis* in various concentrations of zinc](image)

The highest zinc concentration that did not have an inhibiting effect (NOEC) on the growth of *C. centralis* was 0.30 mg/L. All the other concentrations (0.48-5.03mg/L) affected the growth. The lowest concentration
that retards the growth (LOEC) of the organism was 0.48 mg/L and MATC was calculated to be 0.38 mg/L respectively (Table 5.1).

5.7 DISCUSSION

The presence of zinc had a significant impact on the survival of marine organisms. The chronic toxicity tests revealed that survival of the organisms decreases with increase in exposure of zinc concentration. High concentrations of zinc are likely to be toxic, as zinc is known to impair ion regulation. Several researchers have suggested that survival in chronic toxicity tests is considered as the best index of toxicity because of its sensitivity, followed by impacts on reproduction, biochemical changes and growth parameters (Versteeg et al 2006, Villarroel et al 2003). The percentage animal survival markedly declined toward the end of the experiment (25–30 days) in this study. Organisms exposed to higher concentrations performed better in chronic tests with zinc than organisms exposed to lower concentrations of 0.007 to 0.030 mg/L: survival was higher. The fact that zinc deprived organisms do not recover immediately to a level similar to the results of non-deprived organisms when exposed to zinc which suggests that exposure over generations is required to observe long-term effects of zinc toxicity. This suggests that bioaccumulation of metals is generally higher at lower exposure concentrations (Muyssen and Janssen 2002).

In the present study, geometric mean of the no-observed-effect concentration and the first-observed-effect concentration (MATC) in 30-day tests ranging from 0.010 to 0.063 mg/L and 5 day test of C. centralis was observed to be 0.38 mg/L. Among the five organisms examined, the most sensitive organism was found to be Prawn PL (P. monodon) when compared to juvenile stages of other organisms (Fry of M. cephalus, T. jarbua, mussel (3-4 cm)), this is because earlier life stages of aquatic organisms are generally
more sensitive to metal toxicity than older stages or adults. Similar condition was reported by Thongra-ar et al (2003), who compared mercury toxicity in *Lates calcarifer* seabass larvae with mercury toxicity on other juvenile organisms.

The order of sensitivity in chronic exposure was

*P. monodon* > *P. viridis* > *T. jarbua* > *M. cephalus* > *C. centralis*.

It would be expected that planktonic species were more sensitive to metal toxicity than other animals on long term exposure. However, the opposite result was observed in that *C. centralis* which was found to be tolerant to zinc than other organisms. This is possibly due to the use of a static non-renewal test resulting in a large reduction of zinc in the test solutions in which the algae could tolerate over 96 h exposure (Thongra-ar et al 2003).

Chronic studies on tropical marine organisms are seldom reported and data on temperate species are also limited; the most sensitive taxonomic group tested appeared to be the crustaceans, which corresponds to the current findings (Chongprasith et al 1999). Chronic toxicity tests conducted with the saltwater invertebrate, *Americamysis bahia*, formerly classified as *Mysidopsis bahia* resulted in survival of 10% at 10.6 µg/L, 84% at the next lower test concentration of 6.4 µg/L and 95% in the controls. No unacceptable effects were observed at 6.4 µg/L or any lower concentration. The chronic toxicity limits, therefore, are 6.4 and 10.6 µg/L, with a chronic toxicity value of 8.237 µg/L. The 96 h LC$_{50}$ of 15.5 µg/L resulted in an acute-chronic ratio (ACR) of 1.882 (USEPA, 1987).

Lussier et al (1985) conducted a chronic test for 24 hours in old Mysid shrimp (*M. Bahia*) and found NOEC and LOEC as 120 and 231 µg/L for survival. The reported values for invertebrates and fish were high when
compared to present study. Wainiya (1980) reported that the chronic toxicity test in developmental stages of gametes in Temnopleurus toreumaticus (sea urchin) exposed to zinc (salinity – 33) was 260 and 226 µg/L of NOEC and LOEC limits respectively.

According to Xu and Pascoe (1993), zinc which entered the tissues of organisms, could also disrupt nerve functions such as those controlling food digestion, which could lead to a decrease in growth rate, reproduction or metal tolerance. Sawasdee and Köhler (2009) studied the embryo toxicity of zinc in ramshorn snail M. Cornuarietis, and reported that at sub-lethal concentrations of 0.2–2.0 mg/L, exposure resulted in a delayed development of the snail embryos, reflected by delayed formation of eyes and tentacles, a reduced heart rate, and a delay in hatching. Gomot (1997) suggested that zinc and other heavy metals may affect the physiological functions by modifying the locomotor and respiratory activities and modulate the effects of neurotransmitters.

In the fish ectoparasite, G. turnbulli, concentrations between 30 and 120 mg/L of Zn has decreased the survival and reproduction (Gheorghiu et al 2006). Jorge and Moreira (2005) has reported an abnormal shape larvae of P. perna for LOEC of 250 µg/L Zn. According to Kraak et al (1994), a LOEC of 382 µg/L Zn affected the filtration rate of the zebra mussel Dreissena polymorpha and they observed the mortality just after exposure to high zinc concentrations (2758 µg/L).

Alexandre et al (2012) investigated the effect of chronic exposure to zinc in spat of the Pacific Oyster (Crassostrea gigas), from metamorphosis up to 10 weeks. A dose response relationship was derived from growth data; yielding a median effective concentration (EC$_{50}$) of
7.55 µM. Mortality was 100% after 20 days of exposure which supports the present study.

Sub-lethal zinc chronic exposure of 0.08 mg/L had detrimental effects on *Artemia parthenogenetica* fecundity and chronic toxicity was considered as limiting step for colonization of Artemia sp. The comparison of survival end points from this study with selected chronic toxicity results for related species is given in the following table (Table 5.3).

**Table 5.3  Comparison of NOEC, LOEC and MATC values (mg/L) for zinc in marine organisms with those reported by other studies**

<table>
<thead>
<tr>
<th>Species, endpoint, Test type</th>
<th>Exposure Duration</th>
<th>NOEC µg/L</th>
<th>LOEC µg/L</th>
<th>MATC µg/L</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainbow trout, JGS, S</td>
<td>30d</td>
<td>52</td>
<td>104</td>
<td>74</td>
<td>Brinkman and Hansen (2004)</td>
</tr>
<tr>
<td>Cutthroat trout, JGS, S</td>
<td>30d</td>
<td>95</td>
<td>190</td>
<td>134</td>
<td>Brinkman and Hansen (2004)</td>
</tr>
<tr>
<td>Rainbow trout, JGS, S</td>
<td>30d</td>
<td>79</td>
<td>166</td>
<td>114</td>
<td>De Schamphelaere and Janssen (2004)</td>
</tr>
<tr>
<td>Mussel, embryo larval test</td>
<td>48h</td>
<td>&lt;25</td>
<td>25</td>
<td>NC</td>
<td>Jorge and Moreira (2005)</td>
</tr>
<tr>
<td>Sea Urchin, Sperm cell, F</td>
<td>20min</td>
<td>&lt;5</td>
<td>11</td>
<td>0.38 (EC50)</td>
<td>Thongra-ar et al (2003)</td>
</tr>
</tbody>
</table>

ELS – Early life stage, JGS – Juvenile growth stage, S-survival, F-fertilization, NC – Not calculable
From Table 5.3, the chronic zinc toxicity values in the literature are comparable to the present study. Masters et al (1991) noted chronic toxicity values, i.e. the geometric mean of the no-observed-effect concentration and the first-observed-effect concentration, in seven-day tests ranging from 40 to 140 mg/L of zinc and Gillespie et al (1999) found a 7-day LC$_{50}$ of 100 mg/L zinc.

Biochemical markers can provide evidence of exposure to contaminants, and their detection in organisms can provide information about metal bioavailability. A number of biomarkers have been developed and applied successfully to various aquatic sensitive species (Galloway et al 2004, Hyne and Maher 2003; Livingstone 2001) but they have been rarely integrated in conventional chronic toxicity assays and/or linked to individual population-level effects.

In the present study, exposure of four marine organisms to sub-lethal concentrations of zinc had caused both an increase and decrease in enzyme activity especially in antioxidant enzymes and AChE activity. Although these results have been obtained using different species, it illustrates the uncertainty about the actual dose of toxicant that reaches the target site. It is therefore difficult to assess if the observed enzyme inhibition is due to direct toxicant-enzyme interactions (by acting on the active site or by changing the conformation of the enzyme) rather than due to inhibition of de novo synthesis of the enzyme. It has been reported that the contaminant stress in living organisms often results in the production of reactive oxygen species (ROS) (Sevcikova et al 2011).

The overproduction of ROS such as H$_2$O$_2$ and superoxide radical (O$_2$$^-\cdot$) cause oxidative stress (Livingstone 2001). The toxic effects of ROS can be counteracted by cellular antioxidant enzymes such as superoxide dismutase
(SOD), catalase (CAT) and glutathione-S-transferases (GST). Therefore, changes of these enzymatic activities should indirectly indicate the toxic effects of metals on living organisms (Li et al. 2008). The current study indicated the same trend for enzymes (GST, CAT, and GSH) evolved in oxidative stress.

De Coen and Janssen (1997) examined the effect of sub-lethal concentrations (0.3 mg/L) of HgCl$_2$ on the activity of alkaline phosphatase, acid phosphatase, amylase, trypsin and pepsin of the teleost, Channa punctatus, after 7, 15 and 30 days. Even though, an overall inhibition in enzyme activity was observed during the first week (probably due to the direct action of mercury), after 15 and 30 days, an increase in enzyme activity was noted.

One of the main advantages of biomarkers is their potential as early warning signals for chronic toxicity (De Coen and Janssen 1997). The protein content on the four marine organisms found to decrease upon zinc exposure while increase in LPO activity was observed in all the four organisms on exposure to zinc. This biomarker is a reflection of the physiological state of the entire organism and a measure of its energy budget, which can be affected by chemically induced allocation of energy (Jemec et al. 2008).

Jemec et al. (2007) reported that enzyme activities were expressed per animal and not per protein amount, because significant changes of the latter were found in daphnids exposed to pesticide and insecticide. This suggests that increasing concentrations of these chemicals affected not only the investigated enzymes, but proteins in general. Consequently, enzyme activities expressed per protein content differ from that expressed per animal, implying that cautious interpretation of enzyme activities is needed in toxicity
experiments. Similar observations were made by Printes and Callaghan (2003).

The sensitivity of biochemical biomarkers to chronic chemical exposure depends on the mode of action of a chemical. Thus, the 30 d LOEC values for zinc exposed animals were as follows:

**P. viridis**: LOEC\textsubscript{Protein} < LOEC\textsubscript{GSH} = LOEC\textsubscript{LPO} = LOEC\textsubscript{GST} = LOEC\textsubscript{AChE} < LOEC\textsubscript{CAT}.

**P. monodon**: LOEC\textsubscript{LPO} = LOEC\textsubscript{AChE} < LOEC\textsubscript{GSH} = LOEC\textsubscript{GST} = LOEC\textsubscript{CAT} < LOEC\textsubscript{Protein}.

**M. cephalus**: LOEC\textsubscript{GSH} = LOEC\textsubscript{GST} = LOEC\textsubscript{LPO} < LOEC\textsubscript{Protein} = LOEC\textsubscript{AChE} = LOEC\textsubscript{CAT}.

**T. jarbua**: LOEC\textsubscript{Protein} < LOEC\textsubscript{LPO} < LOEC\textsubscript{GSH} = LOEC\textsubscript{GST} = LOEC\textsubscript{AChE} < LOEC\textsubscript{CAT}.

Thus the same biochemical biomarker can be most sensitive to one chemical and least sensitive to the other, as for instance LPO activity. This implies the need to evaluate a suite of biomarkers to fully understand the integrated toxic effect of a contaminant to organism as proposed by Brown et al (2004). It can be concluded that for all four marine organisms tested, 30 d “LOEC biomarkers” based on disturbance (inhibition and/or increase) of enzyme activity, from the six enzymes studied, was a good indicator of chronic toxicity levels.

### 5.8 SUMMARY AND CONCLUSION

To summarize, these results indicate that the crustacean *P. monodon* is most sensitive species among the chosen five organisms relatively
to other crustacean species in the USEPA saltwater zinc criteria dataset. The reason for *P. monodon* instead of phytoplankton could be due to the duration of exposure and method of toxicity testing. A suitable exposure response was achieved with the concentrations tested and survival in control exposures was satisfactory. The results provide survival information for a wide range of organisms starting from plankton to chordates. The present study attempts to overcome the difficulties associated with the conduct of chronic exposure tests of zinc sensitive salt water test species using desirable flow-through method. The results of this study fill critical gaps in our existing knowledge of zinc toxicity saltwater species and should provide useful additions to toxicity databases used for development of regulatory water quality criteria. The study also provides data required to develop a more appropriate and a protective zinc chronic criterion based on saltwater species rather than fresh water species.

Based on the present study, it is concluded that further research is needed to optimize the dietary constituents, feeding rate and biomarkers in chronic toxicity tests. Biomarkers measure events along the entire metabolic pathway during exposure to a metal, and since the toxic effect on organisms is secondary to change inside cells, biomarkers hold promise for higher sensitivity and earlier detection of toxicants than the traditional assays measuring acute toxicity. Biomarkers can act as early warning signals of imminent, irreversible, permanent damage to an organism (Depledge 1993). Use of biomarkers in water quality monitoring provides more realistic assessment of impacts and exposure of marine organisms to specific contaminant in water. Enzyme activities are the common biomarkers and their effects on organisms are dealt in detail in next chapter.