Environmental Safety Level of Lead (Pb) Pertaining to Toxic Effects on Grey Mullet (Mugil cephalus) and Tiger Perch (Terapon jarbua)

G. Hariharan,1 R. Purvaja,2 R. Ramesh2

1Institute for Ocean Management, Anna University Chennai, Chennai 600025, Tamil Nadu, India
2National Centre for Sustainable Coastal Management, Anna University Campus, Chennai 600025, Tamil Nadu, India

ABSTRACT: Acute and chronic bioassay toxicity test of Lead (Pb) in Grey Mullet (Mugil cephalus), and Tiger perch (Terapon jarbua) was conducted. LC50 values (Lethal Concentration) from acute tests and chronic values were calculated by the geometric mean of the No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC) in a study period of 30 days. This research was conducted to evaluate the quantitative relationship between toxicity test statistics and correlation between toxicant and the organisms exposed. Three test average LC50 was analyzed for 24, 48, 72, and 96 h and the 96 h average LC50 of Mugil cephalus and Terapon jarbua is 2.57 ± 0.47 and 2.99 ± 0.23 mg/L of Pb, respectively. Significant correlation is observed with the increased time duration and exposure concentration. The NOEC and LOEC values were calculated based on survival of test organisms for Mugil cephalus and Terapon jarbua and the values are 0.014 and 0.029 and 0.011 and 0.022 mg/L, respectively. The chronic value is found to be 0.011 mg/L for Mugil cephalus and 0.021 mg/L for Terapon jarbua. The intensity of biochemical and histological alterations increased gradually with increased Pb concentration and the exposure time. Toxicity testing is the primary step to determine the water quality safe limit on marine organisms. The outcome of the study indicates that the sensitivity of juvenile organisms to Pb, persistence of toxic effects and biomarkers as a tool capable of revealing the toxic effects of heavy metals on the environment and aquatic biota. © 2014 Wiley Periodicals, Inc. Environ Toxicol 00: 000–000, 2014.

Keywords: acute and chronic toxicity; biomarker; histology; NOEC, LOEC; water quality criteria

INTRODUCTION

The fate of heavy metals and its pollution in aquatic ecosystems are currently a great concern, as beyond the tolerable limits they become toxic. Metals generally enter the aquatic environment through the atmospheric deposition, erosion of geological matrix or due to anthropogenic activities caused by discharge of industrial effluents, domestic sewage, and disposal of mining wastes. Heavy metals are nonbiodegradable and hence, their entry into the aquatic environment persists for a long time and become a source of pollution.
creating a destruction in the natural ecosystem, causing risks to human beings and other living organisms (Miretzky et al., 2004; Nair et al., 2006). The metal contaminants in aquatic systems usually remain either in soluble or suspension form and finally tend to settle down to the bottom layer or taken up by the organisms (Das, 2007). Accumulation of heavy metals by aquatic biota can result in tissue burdens that produce adverse effects not only in the exposed organisms, but also in human beings (Laxmi Priya et al., 2011). Therefore, it is an essential to study the detrimental effects of heavy metals so as to formulate the strategies for safeguarding aquatic organisms. Surveys of coastal aquatic communities have indicated that the quantity and species composition of aquatic organisms are affected in the contaminated areas. However, it is not known whether the impact was related to heavy metal contamination or other disturbance factors. The data available on the toxicity of heavy metals on aquatic organisms is inadequate.

Pb is one among the major toxic heavy metals contaminating the aquatic ecosystem. Source of Pb is commonly available in nature and hence, mining and smelting of it is easier. Pb usage significantly increased during the industrial revolution resulted in anthropogenic emissions of it in urban centers, which played a major role in its global distribution. The ability of Pb to exist in different oxidation states, entail them in various processes leads to oxidative stress in aquatic organisms. Biological accumulation of Pb is evident in tissues of fish, including skin, scales, gills, eyes, liver, kidneys, and muscles (AFS, 2008). Lower levels of Pb exposure to fishes demonstrate range of effects, such as muscular and neurological degeneration and destruction, growth inhibition, mortality, and reproductive problems (Rabbitto et al., 2005).

Toxicity tests using aquatic organisms have proven to be powerful tools and also play an important role in the development of proposals for environmental management and protection, particularly the toxicity tests provides statistical data to estimate the toxic effects of chemicals (Hunt et al., 2002; Le et al., 2005; Zaosheng et al., 2007). Toxicity tests with the embryo/larval and early juvenile life stages of fish are mainly the most sensitive life stages can be used to estimate the safety level concentrations for fish in their entire life cycle and primarily are useful in establishing the water quality criteria (Duquesne et al., 2004; USEPA, 2008). The present study was conducted to determine the acute and chronic toxicity of the heavy metal under continuous flow through system on the fingerlings of Mugil cephalus and Terapon jarbua. These species were selected for bioassays because they can easily be grown under laboratory conditions. It almost fulfills the requirements of derivation of seawater quality criteria and is economically available and omnipresent in the entire Indian coast throughout the year. Biomarkers are indicative of pollutant exposure and the effects are ranging from changes in cellular, biochemical, molecular, or physiological level measured in cells, body fluids, tissues, or organs within an organism. Biomarkers reflect the general stress or exposure to specific classes of environmental contaminants (Walker et al., 2006; Radwan et al., 2010; Tut et al., 2010). Several studies by different authors have investigated the effects of prolonged Pb exposure on aquatic fishes. The outcome of the studies illustrated that chronic exposure to elevated Pb concentrations induce a wide range of effects including biochemical, histological, and bioaccumulation aspects. The key objective of the study was to identify the relationship between the toxicant and organisms. In addition, it was shown to be a sensitive and the results obtained from this study will be useful to derive the safety limits for setting up coastal water quality standard for marine aquatic life protection.

MATERIALS AND METHODS

Acute and Chronic Toxicity Bioassay Tests

M. cephalus and T. jarbua (3.0–4.5 cm in size) were collected from the coastal areas of Chennai, India. They were immediately transported to the laboratory and acclimatized to laboratory condition. The acute and chronic toxicity tests were conducted in accordance with the USEPA, (2002a–c). The test concentrations for toxicity tests were selected from the range finding tests. Definitive acute and chronic toxicity tests were conducted for continuous flow through system by using the Ismatec peristaltic pump. The life-stage toxicity tests for fish were conducted as described by Stephan et al. (1985). During acute and chronic toxicity tests, physico-chemical parameters (such as salinity, pH, water temperature, and dissolved oxygen) were also analyzed twice a day. The test chambers were under continuous observation and the dead organisms were removed immediately from the test chambers. The mortality was noticed at a 24 h interval. Test animals were not fed during the acute test. Maximum-allowable control mortality was 10% for a 96 h and 20% for 30 days period of testing in chronic assay (USEPA, 2002a, 2002b).

Measured Concentration and Observation of Test Chambers

Main stock toxicant of 0.1% Pb (i.e., 1000 ppm) was prepared by dissolving 1.85 g of lead (II) acetate (MERCK, Germany) in 1000 ml with ultrapure deionized water (Millipore-Milli-Q). For acute and chronic toxicity tests, the nominal and measured concentrations of toxicant were compared to evaluate the stability of exposed concentration in test chamber and also to determine the accuracy of the analytical method. The nominal test concentrations were measured in all test chambers at the 24, 48, 72, and 96 h intervals for acute toxicity tests whereas for chronic toxicity, measurement was made at an interval of 10 days. The mean dissolved concentration and percentage of recovery between the results of nominal and measured concentrations are
summarized in Tables I and II. Finally, the results of acute and chronic toxicity tests were calculated based on the measured concentration. The accuracy of measurement was determined by analysis of certified reference materials for every batch of 20 samples and it was always found to be within 10–20%, similar to accuracy of matrix spikes and precision of duplicate measurements taken at the same intervals.

**Biomarker Analysis**

At the end of the chronic toxicity test, the samples of surviving test animals were pooled and made in duplicates. The analysis of oxidative stress parameters (lipid peroxides) and antioxidant enzyme activities (catalase and reduced glutathione and glutathione-S-transferases) were measured as described by Lowry (1951), Beers and Seizer (1952), Habig et al. (1974), Jollow et al. (1974), and Okhawa et al. (1979). After chronic exposure of the organisms to Pb, each test animal was taken from control and test tanks for dissection to determine the histological alterations in internal organs, such as gills, intestine, and liver. For this study, automatic tissue processing method was used as reported by Kim et al. (2006).

**Bioaccumulation Study**

At the end of the chronic exposure, surviving test organisms were removed using a Teflon scalpel and the tissue was washed with distilled water and oven dried at 95°C. The tissue was ground to fine powder and digested (Grasshoff et al., 1999) and the metal accumulation was assayed using Atomic Absorption Spectrophotometer (AAS—PerkinElmer AA Analyst 800).

### TABLE I. Acute toxicity test: nominal, measured concentration and percentage of recovery exposed to Pb

<table>
<thead>
<tr>
<th>Species Name</th>
<th>Nominal Conc.</th>
<th>Test 1</th>
<th>Percentage of Recovery</th>
<th>Test 2</th>
<th>Percentage of Recovery</th>
<th>Test 3</th>
<th>Percentage of Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mugil cephalus</td>
<td>Control</td>
<td>BDL</td>
<td>NC</td>
<td>BDL</td>
<td>NC</td>
<td>BDL</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.88</td>
<td>88.0</td>
<td>0.93</td>
<td>93.0</td>
<td>0.89</td>
<td>89.0</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>1.37</td>
<td>91.3</td>
<td>1.44</td>
<td>96.0</td>
<td>1.40</td>
<td>93.3</td>
</tr>
<tr>
<td></td>
<td>2.25</td>
<td>2.02</td>
<td>89.8</td>
<td>2.06</td>
<td>91.6</td>
<td>2.13</td>
<td>94.7</td>
</tr>
<tr>
<td></td>
<td>3.38</td>
<td>3.11</td>
<td>92.0</td>
<td>3.18</td>
<td>94.1</td>
<td>3.16</td>
<td>93.5</td>
</tr>
<tr>
<td></td>
<td>5.10</td>
<td>4.56</td>
<td>89.4</td>
<td>4.66</td>
<td>91.4</td>
<td>4.82</td>
<td>94.5</td>
</tr>
<tr>
<td>Terapon jarbua</td>
<td>Control</td>
<td>BDL</td>
<td>NC</td>
<td>BDL</td>
<td>NC</td>
<td>BDL</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.38</td>
<td>92.0</td>
<td>1.36</td>
<td>90.7</td>
<td>1.39</td>
<td>92.7</td>
</tr>
<tr>
<td></td>
<td>2.25</td>
<td>2.14</td>
<td>95.1</td>
<td>2.1</td>
<td>93.3</td>
<td>2.11</td>
<td>93.8</td>
</tr>
<tr>
<td></td>
<td>3.38</td>
<td>2.98</td>
<td>88.2</td>
<td>3.19</td>
<td>94.4</td>
<td>3.21</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>5.1</td>
<td>4.96</td>
<td>97.3</td>
<td>4.88</td>
<td>95.7</td>
<td>4.89</td>
<td>95.9</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>6.46</td>
<td>83.9</td>
<td>7.28</td>
<td>94.5</td>
<td>6.51</td>
<td>84.5</td>
</tr>
</tbody>
</table>

BDL—below deduction level; NC—not calculable.

### TABLE II. Chronic toxicity test: nominal, measured concentration, and percentage of recovery exposed to Pb

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Nominal Conc.</th>
<th>1st day</th>
<th>10th day</th>
<th>20th day</th>
<th>30th day</th>
<th>Average Measured Conc.</th>
<th>Percentage of Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mugil cephalus</td>
<td>Control</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>0.008</td>
<td>0.008</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007 ± 0.001</td>
<td>90.6</td>
</tr>
<tr>
<td></td>
<td>0.017</td>
<td>0.016</td>
<td>0.015</td>
<td>0.016</td>
<td>0.016</td>
<td>0.016 ± 0.001</td>
<td>92.6</td>
</tr>
<tr>
<td></td>
<td>0.035</td>
<td>0.033</td>
<td>0.034</td>
<td>0.034</td>
<td>0.034</td>
<td>0.034 ± 0.001</td>
<td>96.4</td>
</tr>
<tr>
<td></td>
<td>0.070</td>
<td>0.064</td>
<td>0.065</td>
<td>0.066</td>
<td>0.066</td>
<td>0.065 ± 0.001</td>
<td>93.2</td>
</tr>
<tr>
<td></td>
<td>0.140</td>
<td>0.137</td>
<td>0.136</td>
<td>0.134</td>
<td>0.136</td>
<td>0.136 ± 0.001</td>
<td>97.0</td>
</tr>
<tr>
<td>Terapon jarbua</td>
<td>Control</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>0.007</td>
<td>0.009</td>
<td>0.006</td>
<td>0.009</td>
<td>0.005</td>
<td>0.007 ± 0.002</td>
<td>103.6</td>
</tr>
<tr>
<td></td>
<td>0.015</td>
<td>0.014</td>
<td>0.018</td>
<td>0.013</td>
<td>0.016</td>
<td>0.015 ± 0.002</td>
<td>101.7</td>
</tr>
<tr>
<td></td>
<td>0.030</td>
<td>0.031</td>
<td>0.030</td>
<td>0.027</td>
<td>0.026</td>
<td>0.029 ± 0.002</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>0.060</td>
<td>0.060</td>
<td>0.060</td>
<td>0.058</td>
<td>0.060</td>
<td>0.060 ± 0.001</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td>0.120</td>
<td>0.119</td>
<td>0.118</td>
<td>0.119</td>
<td>0.117</td>
<td>0.118 ± 0.001</td>
<td>98.5</td>
</tr>
</tbody>
</table>

BDL—below deduction level; NC—not calculable
Statistical Analysis

Acute and chronic toxicity test results were calculated based on the dissolved concentration of Pb. The LC50 at 95% confidence limits was calculated using Probit Analysis (Finney, 1971). In the chronic toxicity test, NOEC and LOEC values were calculated based on the survival of the test organisms (Dunnett, 1964). Enzyme values were given as mean ± SD and analyzed by Graphpad Prism 5 software. An one-way ANOVA in conjunction with Dunnett’s test was used to determine whether the treatments were significantly different from the control group (P ≤ 0.05).

RESULTS

Acute and Chronic Toxicity Test

In toxicity analysis, physico-chemical parameters plays a vital role in determining the toxicity of chemicals. Hence, parameters, such as temperature, dissolved oxygen and pH were analyzed in the present study in all test chambers twice a day. Table III summarizes the physico-chemical characteristics of the test chambers during both the exposures. There was not a significant variation in physico-chemical variables in the test chamber throughout the study period and were well within the acceptable limits.

Test results for acute and chronic toxic exposure of Pb on M. cephalus and T. jarbua are summarized in Tables IV and V. At the end of the study period for acute and chronic exposure assay no mortality was observed in the control groups. Mortality was absent at the time of exposure to different concentrations of Pb, but it amplified with prolonged exposure of time and with increased concentrations. Significant correlation is observed with increasing time and mortality rate. The specimens show various changes in behavioral activity with the increasing concentrations and longer exposure time. In all the test exposures, close observation of the fish movements, shows that all the fishes are hyperactive and they are trying to escape from the tank, especially during the first few hours. During acute toxicity organisms stayed in a vertical position for a few minutes with the anterior side and trying to gulp the air and the tail was pointed in a downward direction. Soon they settled in the bottom of the tank and after a while their bellies turned upward and eventually the fish died.

The LC50 values and their 95% confidence limits are summarized in Table IV based on the measured concentration. The 96 h LC50 of Pb for M. cephalus and T. jarbua are 2.57 ± 0.47 and 2.99 ± 0.23 mg/L, respectively. For the three acute toxicity tests conducted, the average LC50 was analyzed for 24, 48, 72, and 96 h, respectively. Significant correlation is observed with increasing time and mortality rate and the r² values of M. cephalus and T. jarbua are 0.77

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acute Test</th>
<th>Chronic Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>23.3 ± 0.6</td>
<td>23.5 ± 0.9</td>
</tr>
<tr>
<td>D.O. (mg L⁻¹)</td>
<td>6.7 ± 0.5</td>
<td>6.5 ± 0.6</td>
</tr>
<tr>
<td>pH</td>
<td>7.7 ± 0.3</td>
<td>7.8 ± 0.5</td>
</tr>
<tr>
<td>Salinity</td>
<td>33.9 ± 1.1</td>
<td>33.5 ± 1.4</td>
</tr>
</tbody>
</table>

| TABLE III. Physicochemical characteristics of the test chambers during toxicity tests |
|-----------------------------------------------|----------|----------|
| Parameters | Acute Test | Chronic Test |
| Temperature (°C) | 23.3 ± 0.6 | 23.5 ± 0.9 |
| D.O. (mg L⁻¹) | 6.7 ± 0.5 | 6.5 ± 0.6 |
| pH | 7.7 ± 0.3 | 7.8 ± 0.5 |
| Salinity | 33.9 ± 1.1 | 33.5 ± 1.4 |

| TABLE IV. Lethal concentrations (LC50) of Pb depending on exposure time (24–96 h) for M. cephalus and T. jarbua with 95% confidence intervals |
|----------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Species Name   | 24 h LC50 | 48 h LC50 | 72 h LC50 | 96 h LC50 | 24 h LC50 | 48 h LC50 | 72 h LC50 | 96 h LC50 | 24 h LC50 | 48 h LC50 | 72 h LC50 | 96 h LC50 |
| M. cephalus    |          |          |          |          |          |          |          |          |          |          |          |          |
| Test 1         | NC | 11.43 | 5.47–27109.8 | 3.81–68.52 | 2.56–6.94 | NC | 11.72 | 5.61 | 3.61–31.15 | 2.32–5.07 | NC | 10.81 | 5.22 | 3.27–6.17 | 2.05–4.21 | NC | 11.32 ± 0.46 | 5.72 ± 0.57 | 3.57–6.57 | 2.14–4.20 | NC | 11.32 | 5.46–40306 | 3.60–42.14 | 2.25–5.33 |
| Test 2         | NC | 11.72 | 5.43–91677.6 | 3.61–31.15 | 2.32–5.07 | NC | 10.81 | 5.22 | 3.27–6.17 | 2.05–4.21 | NC | 11.32 ± 0.46 | 5.72 ± 0.57 | 3.57–6.57 | 2.14–4.20 | NC | 11.32 | 5.46–40306 | 3.60–42.14 | 2.25–5.33 |
| Test 3         | NC | 11.72 | 5.43–91677.6 | 3.61–31.15 | 2.32–5.07 | NC | 10.81 | 5.22 | 3.27–6.17 | 2.05–4.21 | NC | 11.32 ± 0.46 | 5.72 ± 0.57 | 3.57–6.57 | 2.14–4.20 | NC | 11.32 | 5.46–40306 | 3.60–42.14 | 2.25–5.33 |
| T. jarbua      |          |          |          |          |          |          |          |          |          |          |          |          |          |

NC—not calculable.
and 0.98, respectively. The chronic toxicity tests indicated that the survival of *M. cephalus* and *T. jarbua* decreased with the increase in exposure concentrations. The percentage of survival in the control and first concentrations of *M. cephalus* (0.007 mg/L) and *T. jarbua* (0.007 mg/L) is 100%. The survival rate considerably decreases with the increase in Pb concentrations. The survival rate of organisms is lower during exposure to higher concentrations of Pb such as 0.136 and 0.118 mg/L for *M. cephalus* (25%) and *T. jarbua* (40%), respectively, in the 30 days exposure period. The NOEC and LOEC values calculated based on survival of test organisms are 0.014 and 0.029 mg/L for *M. cephalus* and 0.011 and 0.022 mg/L for *T. jarbua*. Chronic toxicity value calculated based on the geomean of the NOEC and LOEC is 0.011 for *M. cephalus* and 0.021 mg/L for *T. jarbua*.

### Biochemical Changes

In the experiment conducted with the *M. cephalus* and *T. jarbua* the organisms exposed to Pb for a period of 30 days indicated a significant (*P* < 0.05) changes in the enzyme activities of the whole body tissue and gill samples when compared to the control groups (Figs. 1–4). The enzyme activities had little effect when exposed to lowest concentration (first two concentrations) but the effect increased significantly as the concentration is higher while comparing the whole body tissues and gill samples of species with the control. When the organisms were exposed to higher concentrations (3rd, 4th, and 5th), maximum effect with prominent deviations are observed. Activities such as GSH and LPO are almost similar (Figs. 1–4). GSH and LPO are minimally affected by the lowest concentration (first two concentrations) but they increased significantly at higher concentration when compared with control groups with the specie’s whole body tissues and gill samples. Substantial decrease in CAT activity is noticed in whole body tissue and gill samples of *M. cephalus* when it is exposed to increasing concentration of Pb. In contrast, the whole body tissue samples of *T. jarbua* showed an increased CAT activity and the peak in the activity is observed with the higher concentration (0.118 mg/L). The decreased trend in the activity of GST in all the organisms during chronic exposure of Pb is given in Figures 1–4. Overall, when comparing the activity of GST in all the groups, the control and first concentrations showed no significant difference. However, when the concentration increased, the activity of GST showed a significant (*P* < 0.05) divergence which varies based on the exposure concentration and the organism exposed. In all the organisms, there are significant (*P* < 0.05) differences noticed between control and LOEC of all organism’s gills and whole body tissue samples (Figs. 1–4). On the contrary, the control and NOEC of samples show moderate variations (Figs. 1–4).

### Histological Alteration

#### Gill

Figures 5 and 6 show the histological changes in the gills of juvenile fishes exposed to Pb. Gills of control animals showed a normal morphological structure: A row of distinct and regular secondary lamellae running perpendicular to the upper and lower surfaces of each primary lamella (control). The epithelial cells covered both types of lamellae; finally the mucous were thinly distributed in the primary lamellar epithelium and less frequently along the secondary lamellae. Considerable changes are observed in both the species except for the first concentration. Secondary lamellae of the lowest concentration showed slight damage whereas no damage occurred in the primary lamellae. Fusion of secondary lamellae (black color circle) and increased inter lamellar spaces are observed in organisms exposed to higher concentrations (0.016 and 0.034 mg/L of *M. cephalus* and *T. jarbua*). In general, the gill alterations are highly prominent and the primary and secondary lamellae are severely damaged compared to the control.

### TABLE V. Percentage of survival, LOEC, NOEC, and chronic values of Pb exposed to *M. cephalus* and *T. jarbua* (mg/L)

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Nominal Conc.</th>
<th>Measured Conc.</th>
<th>Percentage of Survival</th>
<th>NOEC (mg/L)</th>
<th>LOEC (mg/L)</th>
<th>Chronic Value (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. cephalus</em></td>
<td>Control</td>
<td>BDL</td>
<td>100</td>
<td>0.007</td>
<td>0.016</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>0.008</td>
<td>0.007</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.017</td>
<td>0.016</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.035</td>
<td>0.034</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.070</td>
<td>0.065</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.140</td>
<td>0.136</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. jarbua</em></td>
<td>Control</td>
<td>BDL</td>
<td>100</td>
<td>0.015</td>
<td>0.029</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>0.007</td>
<td>0.007</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.015</td>
<td>0.015</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.030</td>
<td>0.029</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.060</td>
<td>0.060</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.120</td>
<td>0.118</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BDL—below deduction level.
Fig. 1. Biochemical changes in the gill samples of *M. cephalus* after 30th day of exposure to Pb; values are mean ± standard deviations, *n* = 4, symbols (*) represent statistical differences between control and treated groups; *P* ≤ 0.05; *significant; ***,**** highly significant.
Fig. 2. Biochemical changes in the whole body tissues samples of *M. cephalus* after 30th day of exposure to Pb; values are mean ± standard deviations, *n = 4*, symbols (*) represent statistical differences between control and treated groups; *P < 0.05; *significant; **,**,** highly significant.
Fig. 3. Biochemical changes in the gill samples of *T. jarbua* after 30th day of exposure to Pb; values are mean ± standard deviations, *n* = 4, symbols (*) represent statistical differences between control and treated groups; *P* ≤ 0.05; *significantly; **highly significant.

Environmental Toxicology DOI 10.1002/tox
Fig. 4. Biochemical changes in the whole body tissues samples of *T. jarbua* after 30th day of exposure to Pb; values are mean ± standard deviations, *n* = 4, symbols (*) represent statistical differences between control and treated groups; *P* ≤ 0.05; *significant; **, ***highly significant.
Liver histology from control and exposed organisms are illustrated in Figures 7 and 8. In the control group, the liver exhibits a normal architecture and there are no histological abnormalities with hepatocytes presenting a homogenous size, clear central vein, and homogenous (control—Figs. 7, 8). However, in the organisms exposed to higher concentrations, the liver shows vacuolar degeneration in the hepatocytes as well as necrosis and aggregations of inflammatory cells between the hepatocytes. Complete structural disruption is observed in the organisms exposed to 0.065 and 0.136 mg/L of Pb in *M. cephalus* and *T. jarbua*, respectively.

Intestine

Figures 9 and 10 show the section of intestine of control and Pb exposed *M. cephalus* and *T. jarbua*. Intestinal sections of control as well as first concentration of Pb showed normal architecture of folded epithelium in the form of lumen which is taller and bigger in the anterior and fused with each other. Mucosa layer is composed of columnar epithelium. The lumen is lined with epithelia consisting of absorptive and mucous...
secretory cells. In contrast, a section through the intestine of fish exposed to higher concentrations shows atrophy in the muscle with severe degenerative and necrotic changes in the intestinal mucosa and submucosa. Inflammatory cells and hemorrhage are seen in the submucosa and mucosa.

**DISCUSSION**

Toxicity testing is a relatively simple laboratory bioassay that measures the biological response of marine organisms, particularly at their most sensitive early life stages (Duquesne et al., 2004). In India, the studies on the toxicity effect of Pb on the juveniles of *M. cephalus* and *T. jarbua* were found to be inadequate. Assessment of the impact of the exposure scenario on the expression of toxicity has become more prevalent in recent years. Generally, the aim of a toxicity test is to determine the negative effects of a chemical. In this study, the set of experiments demonstrated that the significant differences are observed between control and increasing exposure concentration. The 96 h LC50 values of *M. cephalus* and *T. jarbua* exposed to different concentrations of Pb is found to be 2.57 ± 0.47 and 2.99 ± 0.23 mg/L, respectively. The 96 h LC50 of Pb value observed in the

**Fig. 6.** Histological changes in gill sections of *Terapon jarbua* after 30 days chronic exposure to Pb (control and five treated organisms). (A) Primary lamellae, (B) secondary lamellae. Secondary lamellar fusion (yellow colour ring), inter spaces increases of primary lamellae (double headed arrow) degeneration primary lamellae (red colour ring), epithelial lifting (thin yellow colour arrow). Scale bar: 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
The present study is comparable with other relative species reported in the earlier studies (Krishnakumari et al., 1983; Ramakrishnan et al., 2012; PAN—Database: http://www.pesticideinfo.org/). Minor differences in LC$_{50}$ values with the earlier studies may be due to different environmental conditions, physico-chemical properties of the test. Based on acute and chronic results from the present study, it is seen that Pb is the most toxic compound specially in the early life stages of *M. cephalus* and *T. jarbua*. Pb in the internal organs completely disrupted the cells and consequently interrupting the physiological functions (Sheir et al., 2010).

In present chronic exposure assay, the control group of organisms showed better survival rates during the end of the experiment. There is no mortality found in the NOEC for all the exposed species. The chronic exposure study revealed that the percentage of survival declined remarkably in the end of the experiment along with the reduction in food uptake (25–30 days). Several researchers (Hunt et al., 2002; Valenti et al., 2005) have shown that survival in chronic tests is the best indicator for toxicity because it is less variable than growth and physiological parameters. However, Kristin and Kaushik (1987) also suggested that survival is only a

---

**Fig. 7.** Histological changes in liver sections of *M. cephalus* after 30 days chronic exposure to Pb (control and five treated organisms). Hepatocytes (HC); central vein (CV); hepatopacreas necrosis (HN); hepatic cell proliferation (PHC). Scale bar: 20 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
good indicator when the organisms are exposed to toxicants and when they survived throughout the entire life cycle. The major factor contributing to the mortality rate might be due to the toxic effect of pollutant on the osmoregulatory mechanism of the test animal (Gravato et al., 2005; Giarratano et al., 2011). The declined survival rate might also be due to the long-term exposure and it is evidenced from the bioaccumulation of heavy metals which higher at lower exposure concentrations (McGeer et al., 2003). Such a decreasing trend might be due to the damage of the respiratory epithelium and respiratory enzymes as suggested by Vijayavel and Balasubramanian (2006), Vijayavel et al. (2007), and Tawari-Fufeyin et al. (2008).

Many studies have shown that behavioral responses are not necessarily correlated to toxic concentrations. Lower concentrations might induce increased swimming activity, while higher concentrations induce a decreased activity as shown in the present study (Pandey et al., 2008; Magalhaes et al., 2012). Little et al. (1989) and Fingerman et al. (1997) observed that swimming ability is heightened when fishes are exposed to the organophosphorus lower exposure concentration, while higher concentrations reduced their swimming ability. This increase in swimming activity typically characterized an escape response, where the organism strived to avoid the area contaminated by the chemical (Smith and Bailey, 1988). Toxicity test are generally focused only on the mortality/survival of exposed organisms. On the whole, survival is the common endpoint used to study the toxicity of environmental pollutants to aquatic organisms. Since the chronic exposure to Pb, decreased the survival rate.
considerably with increased exposure, it is a good evidence that the Pb inhibits survival rate in aquatic organisms.

Mortality/survival data is the primary requirement during the derivation of water quality safety limit (Hunt et al., 2002). In toxicity tests with aquatic organisms, the “safe” concentration is commonly expressed as LOEC/NOEC. Both expressions are in between the highest concentration with no significant effect and the lowest concentration with a significant effect. Predominantly, this integrative strategy serves as practical means for evaluating the effects of survival studies in aquatic species whose NOEC and LOEC are poorly understood, mainly in Indian coastal areas. Literature on the toxicity of Pb to marine organisms is limited and therefore, the present research will fulfill the requirement of safety limit derivation of Indian coast.

Biomarkers reflect general stress or exposure to specific classes of environmental contaminants. Therefore biomarkers are considered as a useful tool and widely

Fig. 9. Histological changes in intestine sections of *Mugil cephalus* after 30 days chronic exposure to Pb (control and five treated organisms). (L1): Normal intestinal lumen, (L2): Damaged intestinal lumen, intestinal mucosal lifting (thin arrow), necrosis of lumen (black colour ring); Tunica submucosa (TS). Scale bar: 20 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
incorporated into environmental monitoring programs (Walker et al., 2006; Tu et al., 2010). In the present study, the relationships among the biochemical responses, accumulation of Pb and histological changes of exposed organisms are well correlated with one another. The response of biomarkers in aquatic organisms might be varied depending on species and types of pollutant. In this study however, the baseline levels of biochemical activities in whole body tissues and gills of two fishes are comparable between control and treated groups. It revealed a varied response in antioxidants and protein content in gills and whole body tissues of organisms when exposed to Pb. The amount of total protein decreased with increased exposure concentration and it indicated that the organisms are in high stress condition. Verlecar et al. (2006) and Yadav et al. (2009) concluded that the metal toxicity could possibly damage the protein, as well as DNA and lipids.

The adverse effect of many chemicals on animals relates to their capacity to undergo reactions for producing ROS and lipid peroxidation. Heavy metals can interact with cell membrane structures and alter normal physiology by stimulating LPO (Ahmad et al., 2006; Radwan et al., 2010). In the present study, the increase in LPO could be attributed to the fact that epithelial membrane is the first site of contact of trace metals at the gill surface. The peroxidative damage to gill membrane might result from oxidative deterioration of polyunsaturated fatty acids thus disturbing the solute and water transport and also affecting osmoregulatory functions.

Fig. 10. Histological changes in intestine sections of Terapon jarbua after 30 days chronic exposure to Pb (control and five treated organisms). (L1): Normal intestinal lumen, (L 2): Damaged intestinal lumen, intestinal mucosal lifting (thin arrow), necrosis of lumen (black colour ring). Scale bar 20 μm & control: 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
of gills (Pandey et al., 2008). Similar observations are seen in the present investigation and the accumulation of Pb is higher in *T. jarbua* and *M. cephalus* during 30 days of exposure (Fig. 11). The accumulation of heavy metal concentrations in fish tissues are observed to be higher than the surrounding environmental concentration and ultimately these metals can produce ROS resulting in LPO and antioxidant enzyme alterations (Soares et al., 2008). The increased level of LPO observed in the present investigation could be attributed to accumulation of intermediate products during chronic exposure. The apparent increase in LPO might be due to the accumulation of the heavy metals in the organs and the results also indicated the significant concentration Pb in the treated organisms. Overall bioaccumulation illustrated the higher amount of Pb accumulation in lower exposure concentrations when compared to higher exposed concentrations. The accumulation of Pb is found to be several folds higher in exposed organisms in all concentrations.

The antioxidant enzyme CAT is an extremely important component of intracellular and antioxidant defences of organisms (Jamil, 2001). It reduces the H$_2$O$_2$ to water and oxygen to prevent oxidative stress and for maintaining cell homeostasis. In the present study, CAT activity is reduced in organisms exposed to Pb when compared to control animals. Ezemonye and Enuneku (2011) confirmed that heavy metals can alter antioxidant levels and induce oxidative stress in living systems. In higher exposed concentrations, CAT activity significantly decreased and this decreased CAT activity point out to protect the cells against H$_2$O$_2$. It has been reported that the enhanced superoxide dismutase and catalase activities in the hepatocytes of the common carp (*Cyprinus carpio* L.) could be induced by heavy metals (Vinodhini and Narayana, 2009).

In this study, the higher exposed concentration of Pb induced elevation in the GSH level in *M. cephalus* and *T. jarbua*. Van der Oost et al. (2003) described that GSH is involved in many detoxification processes by the conjugation of electrophilic compounds catalyzed by GST and the catalytic conversion of organo hydroperoxides to alcohols by GPx in which GSH acts as cofactor. In the present study, increased level of GSH might be due to GST activity for neutralizing enhanced GSH (Pisanelli et al., 2009). Thomas and Juedes (1992) demonstrated that heavy metals, such as Cd$^{2+}$, Hg$^{2+}$, and Pb$^{2+}$ increased concentration of GSH in fish tissues and also suggested that ‘in vivo’ metal treatment could also interfere with GSH metabolism. In the present investigation, histology of the internal organs of both species of fishes exposed to Pb showed degenerative, necrotic, and proliferative changes. Based on the histological observations, gill was found to be considerably damaged when compared to other organs, the study shows that gill and hepatopancreas are the target organ for toxic action of heavy metals. The gill lamellae are a major route for the uptake of soluble xenobiotics (Aline et al., 2007). Generally the toxic effect of heavy metals on exposed organisms are most commonly found in gills with the maximum extent of damages (Oliva et al., 2009; Hesni et al., 2011; Vasanthi et al., 2012).

The present histological observations indicated that the prevalence of toxicant increased the interspaces of primary lamellae. The common lesions observed included hyperplasia and fusion of the secondary lamellae (Olurin et al., 2006; Aline et al., 2007). The results are in agreement with those observed in other fish species under the influence of different pollutants (Kakuta and Murachi, 1997; Olurin et al., 2006; Triebskorn et al., 2008). These histological changes might be due to toxicant intake or an adaptive response to prevent the entry of the toxicant thorough the gill surface. The histological results, such as proliferation of the epithelial cells, partial fusion of some secondary lamellae, and epithelial lifting can be described as defense mechanisms. In general, these resulted in increasing the distance between the external

---

**Fig. 11.** Bioaccumulation of Pb in *M. cephalus* and *T. jarbua* on exposure to different concentrations. Accumulation in the whole tissue of *M. cephalus* and *T. jarbua* after 30th day of exposure to Pb; values are mean ± standard deviations, n = 2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
environment and the blood and thus served as a barrier to the entry of contaminants (Poleksic and Tutundzic, 1994; Fernandes and Mazon 2003; Fatma, 2009).

Liver is one of the critical target organ that accumulates substantial amount of heavy metals during chronic exposures (Sobha, 2007). The severity of the hepatic alterations observed in this study increased with increased Pb concentration. These changes might be due to direct toxic effect of the toxicant on hepatocytes as the hepatopancreas are the site of detoxification of all types of toxins and chemicals (Soufy et al., 2007). The present study confirmed that the organisms subjected to higher concentration of Pb showed serious disruption of liver, i.e., significant histological changes are observed in organisms exposed to higher concentrations of Pb. The liver is the organ most associated with the detoxification and biotransformation of foreign compounds that enter into the body. However, its regulatory mechanism can be impaired by accumulated toxicants which could result in structural damage (Camargo and Martinez, 2006). Teh et al. (1997) studied liver lesions in fish as evidence for the exposure to multiple environmental stressors, specific lesions in the liver, and hepatopancreas have also been mentioned in many literatures (Schwaiger et al., 1992; Oliveira Ribeiro et al., 1996). In addition, the hepatopancreas being a target and center for metabolism, might concentrate heavy metals (Montaser et al., 2010).

In aquatic organisms, the intestine is considered as a vital organ for detoxification of xenobiotics in the marine environment (Giari et al., 2008). Many authors have confirmed that intestine as the main organ of metal accumulation and thus, an ideal tool for biomarker studies (Mohamed, 2009; Vasan-thi et al., 2012). The present study confirmed the intestinal section of control M. cephalus and T. jarbua showed normal arrangement of blind ending tubules and epithelium, whereas the Pb exposed organisms showed high level of disruption and the severity increased with increased concentration of Pb. The most common lesions in intestine observed in this study are necrosis in the intestinal mucosa and sub-mucosa, atrophy, aggregations of inflammatory cells in the mucosa with edema between them. According to Bhatnagar et al. (2007), the irritation and destruction of the mucosal membrane of the intestine hampered the absorption. The histological alterations in the intestine of the studied fish are in agreement with those observed by many investigators regarding the effects of different toxicants on fish intestine (Hanna et al., 2005; Cengiz and Unlu, 2006; Soufy et al., 2007; Mohamed, 2009).

CONCLUSION

The pattern of metal toxicity in exposed organisms has elicited significant response at NOEC and LOEC with biomarker studies. Exposure to Pb tends to rapidly induce production of enzymes as well as cause histological changes. Significant relationships between biomarkers and metal exposure at NOEC and LOEC were demonstrated in the laboratory experiments for the enzymes. The correlation results of control with NOEC values were found to be the value of nonstimulation of biochemical response. The present work with selected organisms illustrated the application of this approach to natural and environmental survivorship which might enter the aquatic environment in non negligible concentrations. And the present chronic toxicity test results (NOEC, LOEC, and chronic value) confirmed the threshold level of M. cephalus and T. jarbua that survived in environmental condition (i.e., providing protection of 95–100% of the test species in Chennai coastal ecosystem in India). The assessment of acute and chronic toxicity is the first step to determine the water quality safe limit on marine organisms, which will be evaluated in future and this kind of studies are yet to be developed for Chennai coast. As sensitivities can vary between species, laboratory, and field populations, an extensive research could be conducted to evaluate if the results observed in species are representative of effects observed in other species.

REFERENCES


Cengiz E, Unlu E. 2006. Sublethal effects of commercial delta-methrin on the structure of the gill liver and gut tissues of...


