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Toxic Effects of Lead on Biochemical and Histological Alterations in Green Mussel (Perna viridis) Induced by Environmentally Relevant Concentrations

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Acute and chronic toxicity tests were conducted on green mussel (*Perna viridis*) to determine the adverse effects of lead (Pb). Exposure of organisms to acute toxicity test for 96 h and lethal concentration (LC50) was the endpoint of the test. Acute toxicity for 96-h LC50 and 95% confidence intervals of *P. viridis* was 2.62 ± 0.12 (2.62–3.24) mg/L Pb. Chronic toxicity tests revealed that survival of exposed organisms decreased with elevated exposure concentrations. No-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) were calculated based on survival of test organisms. Results of this study demonstrated an increase in toxicity in test organisms with rise in exposure time and concentration. In this study, histology and biochemical enzymes, namely, catalase, reduced glutathione, glutathione S-transferase, and lipid peroxides, were correlated with chronic value and survival endpoints of *P. viridis* after chronic exposure to Pb. Biochemical and histological responses to different concentrations of Pb were assessed and significant differences were observed between control and increasing exposure concentrations. Biomarker studies in internal organs confirmed that the observed changes are due to adverse effects of Pb. This assessment of toxicity was the first step to determining the seawater quality criteria for marine organisms.

Increased industrialization has led to contamination of coastal ecosystem by heavy metals in high concentrations, which is now a global problem. Pollutants affect marine ecosystems that ultimately reach the oceans (Shahidul and Tanaka, 2004). Among the coastal pollutants, heavy metals are one of the serious problems and their impacts are of utmost concern (Tosti and Gallo, 2012; Cooper et al., 2009; Akcali and Kucuksezgin, 2011). Overall assessment of metal pollution in the coastal regions of Chennai, India, revealed that accumulation of heavy metals has increased in all compartments, namely, sediment, biota, and water. Specifically, a rising trend was noted during the past few decades (Shanmugam et al., 2007; Batvari et al., 2008; Laxmi Priya et al., 2011; Seshan et al., 2011). Anthropogenic pressure on the resources of the seas is exponentially affecting the health of many organisms, leading to changes in the structure of the food web and resulting in bioaccumulation and biomagnification in marine organisms. Among the metal pollutants, Pb is a nonessential metal common in nature and considered toxic to humans and aquatic life (Mager et al., 2010).

Biomarkers can be characterized as functional measures of exposure to stressors, which are usually expressed at the suborganism level of biological organization (Torres et al., 2008). Heavy metals accumulated in tissues of organisms may catalyze reactions that generate reactive oxygen species (ROS), which subsequently lead to environmental oxidative stress. It is well
known that heavy metal contaminants induce oxidative stress in marine animals by generating ROS such as cellular antioxidant defenses (Gravato et al., 2005; Verlecar et al., 2006). Further, for biomonitoring purposes antioxidant and oxidative parameters are regarded as potential biomarkers for exposure to metal pollution (Oliva et al., 2009). Consequently, several studies on the gross histology of marine organisms exposed to heavy metals suggested that increased exposure concentration produced significant effects and lower concentration also exerted impacts during chronic exposure (Keating et al., 2007; Adil et al., 2011). Mussels and other marine bivalves are widely used as sentinel organisms in “mussel watch” programs and for indicating levels of pollutants in the coastal marine environment due to their ability to bioaccumulate organic or toxic elements (Almeida et al., 2007; Pereira et al., 2011). Although studies using both lab-exposed and field-transplanted mussels suggest that pollutants exert adverse effects on mussels (Gagne et al., 2013), the biological effects of chemical contaminants on indigenous mussels or other bivalves are not generally examined. The green mussel, *Perna viridis*, widely distributed in India, forms a reliable and cheap source of animal protein and constitutes an important cultivable species (Murugan et al., 2008). The aim of this study was to investigate selected enzymes and histology as a tool to be used to detect continuous exposure and effects of Pb in green mussel *P. viridis* following acute and chronic exposure.

**MATERIALS AND METHODS**

**Experimental Organisms**

The green mussels, *P. viridis* (3.0–4.5 cm), were collected from the rocky shore regions of Kovalam, Chennai, India, immediately transported to the lab and acclimatized to room temperature in a 300-L fiberglass-reinforced plastic (FRP) tank. During acclimatization and chronic toxicity testing of *P. viridis*, *Chlorella* sp. was provided as feed.

**Acute and Chronic Toxicity Bioassay Tests**

The main stock solution of 0.1% (1g/L) of Pb was prepared by dissolving 1.83 g of lead(II) acetate (Merck, Germany) in 1L of ultrapure deionized water (Millipore-Milli-Q). The bioassay tests for acute and chronic toxicity tests were conducted under a continuous flow-through system and requirements for the life stage of fish were selected as described by Sprague (1973), Stephan et al. (1985), and the U.S. Environmental Protection Agency (U.S. EPA, 2002a, 2000b). The organisms were exposed in the definitive test with Pb 0, 2.25, 3.38, 5.1, 7.7 or 11.55 mg/L. The chronic toxicity test was assessed for 30 d of exposure. Chronic concentrations were selected from the 2-wk range-finding test of control, 0.014, 0.029, 0.058, 0.116, and 0.232 mg/L for *P. viridis*. Lead was measured at 24-h intervals for acute toxicity tests and 10-d intervals for chronic toxicity tests. Dissolved Pb was analyzed using atomic absorption spectrometry (AAS; PerkinElmer AA analyst 800) as described by Grasshoff et al. (1999). During acute and chronic toxicity tests, physicochemical variables (salinity, pH, water temperature, and dissolved oxygen [DO]) were analyzed twice a day by using a precalibrated TKK-DOA water-quality monitoring probe WQC–24. During the chronic toxicity test, organisms were fed twice a day and the uneaten feeds were removed.

**Biomarker Enzyme Analysis**

Biomarker enzyme analysis was carried out in whole-body tissue and gill samples of *P. viridis* after termination of the chronic exposure period. Tissues were homogenized in a buffer containing sucrose (0.25 M), Tris (10 mM), and ethylenediamine tetraacetic acid (EDTA, 1 mM) adjusted to pH 7.4. The homogenates were then centrifuged at 4500 × g for 15 min at 0–4°C. The resulting supernatant was used for the estimation of various biochemical assays. Total proteins were determined by using the method of Lowry et al. (1951), and absorbance was measured at 750 nm. Lipid peroxidation (LPO) levels were estimated as described by
Okhawa et al. (1979), in which the absorbance was measured at 532 nm and expressed in nanomoles malondialdehyde (MDA) formed per milligram protein. Catalase (CAT) was measured as the decay of hydrogen peroxide levels at 240 nm (Beers and Seizer, 1952) and expressed in micromoles hydrogen peroxide consumed per minute per milligram protein. The level of reduced glutathione (GSH) was expressed in micromoles GSH per milligram protein (Jollow et al., 1974). Glutathione S-transferase (GST) activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate according to the protocol of Habig et al. (1974). The reaction rate was recorded at 340 nm, and enzyme activity was expressed in nanomoles of GSH and CDNB conjugate formed per minute per milligram protein.

Histology

Tissue samples such as adductor muscles and gills were dissected and fixed in 10% buffered formalin and then dehydrated in graded series of ethanol, and finally immersed in xylene and embedded in paraffin wax (58–60°C) using an automatic processor. Paraffin blocks were trimmed to suitable size and sections of tissues were cut using a microtome at 5–6 µm thickness. After deparaffinization, sections were rehydrated, stained with hematoxylin and eosin, mounted with Cristal/Mount, and subsequently subjected to pathological assessment (Kim et al., 2006).

Statistical Analysis

Results from both acute and chronic toxicity tests were calculated based on the dissolved concentration of Pb. The 96-h LC50 and 95% confidence limits were calculated using probit analysis (Finney, 1971). In the chronic toxicity test, NOEC and LOEC were calculated based on survival of test organisms on d 30 of chronic toxicity test (Dunnett, 1964). Enzyme values were expressed as mean ± SD and analyzed by Graphpad Prism 5 software. One-way analysis of variance (ANOVA) in conjunction with Dunnett’s test was used to determine whether the treatments were significantly different from the control group (p ≤ .05).

RESULTS

Acute and Chronic Toxicity of P. viridis Exposed to Pb

Toxicity tests were conducted using a continuous flow-through bioassay test method to determine acute and chronic lethal concentration values of Pb and mortality was noted at every 24-h interval. Lab seawater quality was consistent throughout the tests, and physicochemical properties were monitored every 12 h. Mean values of temperature, DO, pH, salinity, and Pb were in the range of 24.4 ± 0.7°C, 6.3 ± 0.6 mg/L, 7.8 ± 0.5, 30.7 ± 0.5, and 1.7 µg/L, respectively. The nominal test concentrations were measured in all test chambers at 24-h and 10-d intervals for both acute and chronic toxicity tests. For all toxicity tests, the nominal and measured concentrations of toxicant were compared to evaluate the stability of exposed concentration in the test chamber and to determine the accuracy of analytical method. The mean dissolved concentration and percent recovery between nominal and measured concentrations are summarized in Table 1. Results of acute and chronic toxicity tests were calculated based on the measured Pb concentration.

The LC50 values and their 95% confidence limits are summarized in Table 2. Perna viridis exposed to different concentrations of Pb showed an average value of 2.62 ± 0.12 mg/L in 96-h LC50. Mortality was absent from the initial time of exposure to different concentrations of Pb, whereas it amplified with longer exposure time with increased metal levels. The survival percentage of exposed organisms at the end of chronic exposure, results of NOEC, LOEC, and chronic value are provided in Table 3. The chronic toxicity tests revealed that survival of P. viridis decreased with increased exposure levels (Table 3). Minimum survival of organisms was observed at the highest concentration of Pb (0.11 mg/L) with 45% after 30-d exposure. The values of NOCE (0.016 mg/L)
**TABLE 1.** Nominal and Measured Pb Concentrations (mg/L) in Acute and Chronic Toxicity Test Solutions and Relative Percentage of Recovery

<table>
<thead>
<tr>
<th>Test</th>
<th>Nominal concentration</th>
<th>Test 1 (mg/L)</th>
<th>Percent of recovery</th>
<th>Test 2 (mg/L)</th>
<th>Percent of recovery</th>
<th>Test 3 (mg/L)</th>
<th>Percent of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</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>0.93 ± 0.11</td>
<td>93.0</td>
<td>0.90 ± 0.09</td>
<td>90</td>
<td>0.95 ± 0.06</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>1.41 ± 0.08</td>
<td>94.0</td>
<td>1.40 ± 0.15</td>
<td>93.3</td>
<td>1.41 ± 0.07</td>
<td>94.0</td>
</tr>
<tr>
<td></td>
<td>2.25</td>
<td>2.05 ± 0.16</td>
<td>91.1</td>
<td>2.04 ± 0.11</td>
<td>90.7</td>
<td>2.10 ± 0.13</td>
<td>93.3</td>
</tr>
<tr>
<td></td>
<td>3.38</td>
<td>3.17 ± 0.18</td>
<td>93.8</td>
<td>3.06 ± 0.10</td>
<td>90.5</td>
<td>3.20 ± 0.10</td>
<td>94.7</td>
</tr>
<tr>
<td></td>
<td>5.10</td>
<td>4.61 ± 0.11</td>
<td>90.4</td>
<td>4.49 ± 0.23</td>
<td>88.0</td>
<td>4.69 ± 0.19</td>
<td>92.0</td>
</tr>
<tr>
<td>Chronic</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>0.006</td>
<td>0.006</td>
<td>0.01</td>
<td>0.007</td>
<td>0.008</td>
<td>0.008</td>
<td>129.2</td>
</tr>
<tr>
<td></td>
<td>0.012</td>
<td>0.018</td>
<td>0.014</td>
<td>0.017</td>
<td>0.014</td>
<td>0.016</td>
<td>131.3</td>
</tr>
<tr>
<td></td>
<td>0.025</td>
<td>0.030</td>
<td>0.025</td>
<td>0.024</td>
<td>0.026</td>
<td>0.026</td>
<td>105.0</td>
</tr>
<tr>
<td></td>
<td>0.050</td>
<td>0.056</td>
<td>0.053</td>
<td>0.057</td>
<td>0.051</td>
<td>0.054</td>
<td>108.5</td>
</tr>
<tr>
<td></td>
<td>0.100</td>
<td>0.108</td>
<td>0.106</td>
<td>0.111</td>
<td>0.109</td>
<td>0.109</td>
<td>108.5</td>
</tr>
</tbody>
</table>

Note. BDL, below detection level; NC, not calculable.

**TABLE 2.** Lethal Concentration (LC50) of Pb Depending on Exposure Time (24–96 h) for *P. viridis* and 95% Confidence Intervals (mg/L)

<table>
<thead>
<tr>
<th>Toxicity test</th>
<th>24-h LC50 (mg/L)</th>
<th>48-h LC50 (mg/L)</th>
<th>72-h LC50 (mg/L)</th>
<th>96-h LC50 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 1</td>
<td>NC</td>
<td>13.99</td>
<td>6.94</td>
<td>2.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.18–267.2</td>
<td>4.77–41.65</td>
<td>2.29–3.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.76</td>
<td>4.96</td>
<td>2.56</td>
</tr>
<tr>
<td>Test 2</td>
<td>NC</td>
<td>8.74–111.07</td>
<td>3.86–9.60</td>
<td>2.16–3.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.29</td>
<td>6.86</td>
<td>2.54</td>
</tr>
<tr>
<td>Test 3</td>
<td>NC</td>
<td>10.17–144.87</td>
<td>4.43–35.24</td>
<td>2.14–3.09</td>
</tr>
<tr>
<td>Average LC50</td>
<td></td>
<td>13.68 ± 0.81</td>
<td>6.25 ± 1.12</td>
<td>2.62 ± 0.12</td>
</tr>
</tbody>
</table>

Note. NC, not calculable.

**TABLE 3.** Percentage of Survival, LOEC, NOEC, and Chronic Values of Pb Exposed to *P. viridis* (mg/L)

<table>
<thead>
<tr>
<th>Toxicity test</th>
<th>Concentration (mg/L)</th>
<th>Percent of survival</th>
<th>NOEC (mg/L)</th>
<th>LOEC (mg/L)</th>
<th>Chronic value (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic</td>
<td>Control</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.008</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.016</td>
<td>100</td>
<td>0.016</td>
<td>0.026</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>0.026</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.054</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.109</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

and LOCE (0.026 mg/L) were observed. The chronic value was calculated based on the geometric mean of the NOEC and LOEC as 0.02 mg/L.

**Bioaccumulation**

In this study, the organisms showed significant increase in Pb concentration in their body after chronic exposure (Figure 1). Accumulation of Pb was found to be severalfold higher in exposed organisms at all concentrations. Overall bioaccumulation results indicated a higher amount of Pb accumulation at lower exposure concentrations compared to higher exposure concentrations.
Biochemical

Induction of antioxidant and oxidative parameters were observed at different exposure Pb levels. Of the target parameters, LPO, CAT, GSH, and GST levels were significantly increased following exposure to Pb (Figures 2 and 3). Significant reduction of protein content was observed in whole-body tissue and gill samples of Pb-treated organisms. In general, gill samples showed marked decreased activity of CAT compared with whole body. LPO levels of all treated groups showed increasing trend. Control and treated organisms were significantly different, as well as organisms exposed to higher concentrations (0.058, 0.116, and 0.232 mg/L of Pb). The significant reduction of LPO levels was noted in gills and whole body in groups that were exposed to higher concentrations. Exposed organisms displayed decreased levels of GSH during chronic Pb exposure. Activity of GST in control and lower metal concentrations showed no significant differences, whereas activity at higher concentrations varied significantly.

RESULTS OF HISTOLOGICAL OBSERVATIONS ON ORGANISMS EXPOSED TO LEAD

Histological Observation of *P. viridis* Gill

A healthy untreated gill showed two pairs of demibranchs that were suspended from the ctenidial axis, which was fused along the dorsal margin of the mantle. Each arm of a demibranch was made up of ascending and descending lamellae. The neighboring gill filaments were attached to one another through interlocking clumps of cilia providing gills with a sheet-like consistency, and the lamellae were joined to each other by interlamellar junctions (Figure 4). Similarly, no significant changes were observed in the lowest exposed concentration (0.008 mg/L), whereas organisms exposed to 0.054 mg/L displayed elongated gill filaments with a swollen lumen (black color ring). The shape disruption was mainly due to necrosis. Severe loss of epithelium was observed. In spite of severe denudation, small tufts of cilia were seen on the lateral sides (yellow color arrow). At higher concentrations (0.109 mg/L), the exposed organisms showed total loss of gill architectures and damaged interlamellar junctions.

Histological Observation of *P. viridis* Adductor Muscle

The section through adductor muscle of control organisms displayed thick connective tissues around the muscle bundle and thin connective tissue around the muscle fiber (Figure 5). Similar structure of adductor muscle was observed in organisms exposed to lower concentrations of Pb (0.008 mg/L) (Figure 5). However, adductor muscle of individuals exposed to higher concentration demonstrated loss of fibrous structure, necrosis in connective tissue (thin arrow), loss of muscular integrity, and connective tissue disruption (arrow). The individuals exposed to Pb at 0.059 and 0.109 mg/L displayed greater alterations, such as extensive myodegeneration (blue color ring), connective tissue damage, inflammatory responses in the central connective tissues, vacuolization between the muscle bundles, and finally splitting of muscle fibers (Figure 4).

DISCUSSION

In this study, acute and chronic toxic effects of Pb on histological and biochemical responses...
were studied in green mussel, *P. viridis*. The 96-h LC50 of Pb in *P. viridis* was 2.62 ± 0.13 mg/L. Tan and Lim (1984) and Yap et al. (2004) reported 96-h LC50 of Pb values as 4.46 (168 h) mg/L and 4.12 mg/L, respectively, in *P. viridis*. The percent mortality during 24–48 h did not markedly alter, while increasing exposure time (48, 72, and 96 h) produced varied response. The toxicity results confirmed that LC50 values were decreased with rising exposure concentration, in addition to prolonging duration. Significant correlation was observed between increasing time (24, 48, 72, and 96 h) and LC50 values. Data on chronic toxicity of Pb to
marine organisms are limited. Control organisms showed better survival at the end of the experiment, whereas in the treated group percent survival markedly declined toward the end of the experiment. Data indicate that Pb inhibits the survival rate in aquatic organisms.

The chronic value, NOEC, LOEC, and percent survival results were confirmed with the threshold level of Pb for *P. viridis* that survived in other environmental conditions.

At the end of the chronic exposure, percent survival declined and food uptake activity
was also reduced. During chronic exposure, respiration behavior such as shell opening and filtration activity changed considerably. An increase in Pb concentration resulted in a negative impact on the survivorship and behavioral variations (Viera et al., 2009). Cheung and Cheung (1995) studied respiratory impairment in bivalves resulting from exposure to heavy metals and concluded that oxygen consumption generally decreases when bivalves are acutely exposed to heavy metals. Similar results were obtained using the same test species (Vijayavel et al., 2007; Ivanina and Sokolova, 2009). Survival is a common endpoint used to study toxicity of environmental pollutants to aquatic organisms providing a screening method for toxic evaluation.

Data obtained from this study will be useful to
determine the concentrations of single contaminants that may produce ecologically significant effects, and may also be used to establish acceptable environmental standards.

Assessment of biochemical and histological alterations is an important method adopted to assess the impacts of pollution. In general, organs such as skin and gills were in direct contact with the surrounding environment, and the hepatopancreas, kidney, and intestine are the major target organs for toxicants (Oliva et al., 2009). In this study, the organisms showed significant increase in Pb concentration in their body after chronic exposure. Accumulation of Pb was found to be severalfold higher in exposed organisms at all concentrations. Overall bioaccumulation results indicated higher amounts of Pb accumulation at lower exposure concentrations compared to higher exposure concentrations.

FIGURE 5. Histological changes in adductor muscle sections of *Perna viridis* after 30 d of chronic exposure to Pb (control and five treated organisms). Loss of fibrous structure and necrosis of connective tissue (thin arrow), loss of muscular integrity and connective tissue disruption (thick arrow), vacuolization between the muscle bundles (blue color ring). Scale bar, 20 µm.
Bioaccumulation provides knowledge of how enriched organisms are, in particular, elements, with respect to the surrounding environment (Kaoud and El-Dahshan, 2010). The results of this study indicated that *P. viridis* were capable of accumulating higher levels of Pb. The present bioaccumulation pattern was in agreement that metals occur in natural marine ecosystems at low concentrations but are capable of exerting biological effects (Oliva et al., 2009). Generally, bivalves are capable of concentrating trace metals from solutions, food, and particulate matter in the marine environment, and enrichment over ambient seawater level is common (Dumalaganan and Gonzales, 2010). Higher extent of bioaccumulation of heavy metals in aquatic organisms is comparable with the environmental concentrations that were reported by Yap et al. (2003, 2004, 2006) and Liu and Kueh (2005).

Biochemical response to different concentrations of Pb was assessed and significant differences were observed between controls and those with increasing exposure concentration. In this investigation, the maximum reduction in protein content was observed in *P. viridis* exposed to higher concentrations of Pb. The reduction in protein content may be attributed to loss in appetite and/or breakdown of protein. Higher metal concentration directly affects growth and health, and finally decreases the percent survival (Leonard et al., 2011; Ezemonye and Enuneku, 2011). The present findings are in agreement with the fact that protein content negatively correlated with increasing concentration of Pb exposure. Verlecar et al. (2006) found that metal toxicity might possibly damage protein, as well as DNA and lipids. The biological effects of ROS are generally similar in aerobic organisms, and these ROS oxidize biologically important molecules. Therefore, a continuous rate of ROS production, molecular oxidation, and antioxidant consumption takes place even in healthy aerobic cells and tissues. An imbalance among these reactions leads to a condition called oxidative stress (Halliwell and Gutteridge. 1999). Oxidative stress usually typifies the toxicity induced by xenobiotics (Gravato et al., 2005; Jose et al., 2007).

There was an increase in LPO levels observed in all treated Pb groups. Maximal LPO levels were found in gills of *P. viridis*, and increase in LPO levels might be attributed to accumulation of intermediate products during chronic exposure (Regoli et al., 2006). The rise in LPO may be attributed to accumulation of Pb in organs of *P. viridis*, as metals are capable of becoming involved in processes leading to oxidative stress in molluscs (Radwan et al., 2010; Dabas et al., 2012). The antioxidant CAT was an extremely important component of intracellular and antioxidant defenses of the organisms (Pandy et al., 2008). In this study, CAT activity was found to be decreased. Similarly, organisms exposed to lower Pb concentrations showed no significant effects on CAT activity. However, groups exposed to higher Pb concentration displayed significant reduction in CAT activity. This decreased CAT activity protects cells against H$_2$O$_2$. Vinodhini and Narayanan (2009) reported that hepatocytes of the common carp (*Cyprinus carpio* L.) are induced by heavy metals. The decrease in the level of CAT activity might be attributed to nondefense response against antioxidant activities to cope with this increased oxidative stress and protect cells from damage (Chandran et al., 2005).

During the course of Pb exposure in *P. viridis*, GSH content decreased and GST activity increased. This observed change may have occurred either through direct action of the metal on the enzyme or indirectly via the production of ROS that interacts directly with the enzyme, depletion of its substrate (GSH), and/or downregulation of GST through different mechanisms (Roling and Baldwin, 2006). In agreement with these findings, Verlecar et al. (2008) also reported increased GST activity in *P. viridis* exposed to Hg. Canesia et al. (1999) and Vieira et al. (2009) showed that the elevation in GST activity induced by Cu and Hg might reflect enhanced utilization of glutathione peroxidase (GPx) in conjugation reactions involved in the metabolism of lipid hydroperoxides and carbonyl compounds formed by the Cu-induced peroxidation of cellular membranes. Jena et al. (2009) suggested that depletion of
GSH was due to greater utilization of GSH in toxic environments to combat prooxidants such as metals or petroleum hydrocarbons. Ezemonye and Enuneku (2011) reported that the decrease in GSH level may be the consequence of enhanced GSH utilization to conjugate heavy metals and counteract ROS and lipid peroxidation products. The accumulation of Pb was 1000-fold higher than the exposure concentration, and heavy metal accumulation in cells result in decreased availability of reduced GSH, due to both GSH binding and oxidation (Canesia et al., 1999). GST, a biotransformation enzyme, plays a critical role in the detoxification of xenobiotic compounds by catalyzing the addition of GSH to xenobiotic substrates. The resulting GST conjugates tripeptide GSH with electrophilic and other xenobiotics. The observed increase in GST activity for \textit{P. viridis} might be due to activation of the natural antioxidant defense system by xenobiotics, suggesting that a detoxification process versus pro-oxidation forces, mediated by this enzyme, was induced (Elia et al., 2007).

The gills of \textit{P. viridis} showed degenerative, necrotic, and proliferative changes of gill filaments in the exposed groups. Because gills were the main site for gas exchange and other important functions such as ionic and osmotic regulation and acid–base equilibrium, changes in gill structure involve respiratory anomalies and electrolytic balance disruption (Hesni et al., 2011; Vasanthi et al., 2012). Heavy-metal-mediated changes in marine bivalve soft tissues, especially those associated with gill filament morphology, were studied by Gregory et al. (1999). Organisms exposed to higher concentrations of Pb (0.054 and 0.109 mg/L) revealed total loss of gill architecture, interlamellar junction, complete desquamation of epithelial cells with loss of cilia, dilation of gill tip, and clubbing of cells. The identified disruption in the gill filaments confirmed that the already-mentioned damages were due to adverse effects of heavy metals (Gregory et al., 1999; Vasanthi et al., 2012). A similar pattern of Cu-mediated histological changes in \textit{Crassostrea madrasensis} was reported by Ittoop et al. (2006). These changes are in agreement with the changes observed after sublethal Cd exposure to the freshwater mussel \textit{Anodonta woodiana} (Fitriawan et al., 2011). Data showed the synergistic adverse effect of Pb on \textit{P. viridis} gills. Adductor muscle of \textit{P. viridis} of control groups displayed normal structural integrity, whereas groups exposed to Pb showed prominent negative effects such as loss of fibrous structure organization, necrosis of connective tissue, loss of muscular integrity, and connective tissue disruption. Maharajan et al. (2011) studied the adductor tissue necrosis of connective tissue in the muscle tissue of \textit{Panulirus homarus} that was exposed to Cu, and suggested that this may be one of the reasons for the presence of a large number of hemocytes. Sheir et al. (2010) revealed Hg-induced injury to the posterior adductor muscle characterized by loss of muscle fiber bundle integrity and some inflammation. \textit{Perna viridis} exposed to 0.052 and 0.100 mg/L had greater alterations compared to control organisms. The observed injury of the adductor muscle clearly confirms that the adductor muscles are responsible for opening and closing the shell, and any damage to this muscle may compromise feeding behavior or ventilation, and may also increase the risk of predation if the shell cannot be tightly closed. These pathological changes were previously reported as a consequence of metal toxicity in \textit{P. viridis} (Vasanthi et al., 2012; Sheir et al., 2010).

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