Accumulation of metals in the viscera and muscles of the green-lipped mussel *Perna viridis* from Ta-Peng Bay Lagoon

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Abstract

In this preliminary biomonitoring study, accumulated metal concentrations (Cu, Pb, Zn, Ni, Cr, Cd, As, Hg) have been measured in the mussel *Perna viridis* collected from Ta-Peng Bay Lagoon, respectively in December 2005, March 2006, June 2006 and September 2006. All samples were analysed by AAS, and statistical analyses were performed with SPSS 10.0 for windows. The results clearly indicated that viscera surpass muscles much in the concentrations of metal elements, and the concentrations of Cu and Zn were considerably high than others, Zn especially. We also can find seasonal variations of difference in the concentrations of metals in viscera and muscles. In autumn, Ni had more high concentration than others; Cu and Zn had the highest concentration in spring. Follow the season series, cd concentrations (in viscera) were increased, but the concentrations of muscles were poor variation. Results of correlation analyses among these heavy metals concentrations, there is a significant relation (p < 0.01) for concentration of Cu, Pb, Zn, Ni and As in tissues; relation (p < 0.05) for concentration of Cd in tissues. It signified that the concentration of increase or decrease of viscera and muscles were influenced each other. Only Cd element had a relation (p < 0.05) with season, it mean that the Cd concentration (in viscera) was increased follow the season series. As a whole, the concentration variations of muscles of green-lipped mussels were less than viscera.

Keywords: heavy metal, bivalve, mussel, *Perna viridis*, Ta-Peng Bay Lagoon

1. Introduction

Mussel Watch programs have been used to assess coastal environmental pollution since they were proposed in the 1970s. Recently new directions for monitoring marine pollution and implications in estimation of metal bioavailability in "Mussel Watch programs" have been recommended. Heavy metal levels in mussel (*Perna viridis*) had reported from Malaysia [1] and Hong Kong [2]. Within the Asia-Pacific region, the green-lipped mussel has also been established as a biomonitoring agent for heavy metals such as Hong Kong, Thailand and India.
They are great and possess economy value, and the green-lipped has more sensitive accumulation of heavy metals in Cu, Pb, Zn and Cd [1]. In biomonitoring programs using marine bivalves as sentinel monitors of metal pollution, it is important to appropriately interpret the observed metal tissue concentrations such that the real contamination history can be accurately reflected by the monitoring data [5]. In natural environment, marine organisms are exposed to a mixture of heavy metals at the same time. Thus, it is very likely that exposure to one heavy metal may generate influences on the bioaccumulation of other heavy metals. Various metal-accumulating bivalve species show a high presence and abundance in coast sites and play an important role as biomonitors for trace metal pollution in global monitoring programs. Ta-Peng Bay Lagoon is a shallow productive coastal lagoon, located at the south-southwestern coast of Taiwan. It receives discharges sewage from near fisheries, domestic and agricultural wastewater. We analysis the metal concentrations of viscera and muscles of the green-lipped mussel *Perna viridis*. The results reported here will provide valuable information on heavy metal pollution in Ta-Peng Bay Lagoon.

2. Materials and Methods

2.1. Sampling study site

The study site, Ta-Peng Bay Lagoon is located at the south-southwestern Taiwan, covering an area approximately 444.6105 of hectare, and an average depth of 2.19m. Only an outlet way is in southwest of Ta-Peng Bay Lagoon. The silt often silted up in exit way, due to the ocean current and tidal current. The domestic, agricultural wastewater and fisheries sewage inflow the lagoon, cause both the seawater quality and the ecology of lagoon went down. According to the preliminary investigation, species are well mixed everywhere in Ta-Peng Bay Lagoon, but not quantities.

In this study, there were four different bivalves got from Ta-Peng Bay Lagoon, respectively *Scapharca satowi*, *Katelysia hiantina*, *Perna viridis* and *Anomalocardia squamosa*.

To compare the total metal content at the different samples, the metal pollution index (MPI) was used, obtained with the equation [6]

\[ \text{MPI} = (C_{f1} \times C_{f2} \times \cdots \times C_{fn})^{1/n} \]

Where \( C_{fn} = \) concentration of the metal \( n \) in the sample.
2.2 Sampling and sample pretreatment

The sampling was carried out four times, respectively at December 2005, March 2006, June 2006 and September 2006. The specimens of mussels were selected for a standard shell size (70mm ± 9mm), and transported to the laboratory with ice freezing then stored in -20°C. All the soft tissues and byssus of mussels were carefully removed by shelling the bivalves with a plastic knife, and then thoroughly rinsed with double distilled water to remove extraneous impurities. Whole tissues were dried at 105°C until a constant dry weight, and ground to a fine powder in a mortar before analysis. Approximately 1.0±0.01g(dry weight) of soft tissues was weighed. The mussels were identified according to “Being friend with molluses”, a reference book [7].

2.3. Chemical analysis

Each sample was added with 10 ml of concentrated nitric acid (65%), and placed in a conical flask to predigest overnight at inside air temperature, and fully digested at high temperature (95°C) for at least 5 h. After cooling, 2ml of 30% hydrogen peroxide was added and keep on digestion at 95°C till the solution was clear. After cooling, the extract filtered with Watchman 40 filter paper and the washing DDW used for conical flask rinsing were transferred into volumetric flask, added DDW until the total volume to 50 ml. The method is according to Environmental Analysis Laboratory EPA, Executive Yuan, R.O.C. (NIEA C303.02T).

The samples were analysed for six elements by an air-acetylene flame / Graphite Atomizer atomic absorption spectrophotometer (AAS) HITACHI Model Z-5000.
2.4. Statistical analysis

Multivariate analysis methods such as one-way analysis of variance (ANOVA) and correlation analysis (bivariate correlations) have been used to extract information from the chemical analysis in order to find the relationships among these heavy metals in organisms [8, 9]. The correlation analysis was used by Pearson correlation. Correlation is significant at 0.05 level (two-tailed). One-way analysis of variance (ANOVA) was utilized to investigate the effects of season and species on variations in metal concentrations in the organisms studied. All statistical analyses were performed with SPSS 10.0 for windows.

3. Result and Discussion

3.1 Heavy metals in the tissues

The mussels have different amount of heavy metals in their viscera and muscles. The means of measured metal concentrations are listed at Table 1. The results clearly indicated that viscera surpass muscles much in the concentrations of metal elements, and the concentrations of Cu and Zn were considerably high than others, Zn especially. This can compare with previously researches that it is common to find higher concentrations of Zn and Cu in soft tissue of bivalves [1, 2, 6, 9].

We can find seasonal variations of difference in the concentrations of metals in viscera and muscles. In autumn, Ni and had more high concentration than others; Cu and Zn had the highest concentration in spring. Follow the season series, Cd concentrations (in viscera) were increased, but the concentrations of muscles were smoothly variated. Some elements also had short-term obvious variation in period of this studied time, such as Pb (viscera) from spring to summer, Cr (viscera) from winter to spring, Cd (viscera) and As (viscera) from summer to autumn and Hg (viscera and muscles) from spring to autumn. As a whole, the concentration variations of muscles of green-lipped mussels were less than viscera.

<table>
<thead>
<tr>
<th>Element</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>5.57</td>
<td>6.73</td>
<td>11.53</td>
<td>5.19</td>
<td>19.92</td>
<td>27.71</td>
<td>21.94</td>
<td>12.68</td>
</tr>
<tr>
<td>Pb</td>
<td>1.80</td>
<td>0.05</td>
<td>0.52</td>
<td>0.34</td>
<td>9.15</td>
<td>8.49</td>
<td>1.66</td>
<td>3.36</td>
</tr>
<tr>
<td>Zn</td>
<td>68.60</td>
<td>54.43</td>
<td>50.93</td>
<td>49.14</td>
<td>75.75</td>
<td>88.65</td>
<td>68.11</td>
<td>69.90</td>
</tr>
<tr>
<td>Ni</td>
<td>0.15</td>
<td>0.16</td>
<td>0.44</td>
<td>0.24</td>
<td>1.20</td>
<td>0.70</td>
<td>1.30</td>
<td>0.76</td>
</tr>
<tr>
<td>Cr</td>
<td>2.94</td>
<td>1.53</td>
<td>2.59</td>
<td>0.81</td>
<td>19.88</td>
<td>7.00</td>
<td>1.62</td>
<td>2.97</td>
</tr>
<tr>
<td>Cd</td>
<td>0.06</td>
<td>0.07</td>
<td>0.08</td>
<td>0.06</td>
<td>0.04</td>
<td>0.18</td>
<td>0.26</td>
<td>1.03</td>
</tr>
<tr>
<td>As</td>
<td>2.06</td>
<td>1.60</td>
<td>0.62</td>
<td>1.06</td>
<td>2.27</td>
<td>3.25</td>
<td>1.23</td>
<td>4.31</td>
</tr>
<tr>
<td>Hg</td>
<td>ND</td>
<td>ND</td>
<td>0.14</td>
<td>0.03</td>
<td>ND</td>
<td>ND</td>
<td>0.4</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table 1 Metal concentrations (μg/g dry weight), mean values and Metal Pollution Index (MPI) in the selected mussels.
Fig. 2 The variation of metal concentration (μg/g, dry weight) in the studied seasons
3.2. The Metal Pollution Index

We counted the Metal Pollution Index (MPI) with the following equation, and got the statistical chart.

\[ MPI = (C_{f1} \times C_{f2} \times \cdots \times C_{fn})^{1/n} \]

Where \( C_{fn} \) = concentration of the metal \( n \) in the sample.

Because of the ND value, the counts of Hg concentration were eliminated from the MPI equation.

![Fig. 3 The variation in the MPI for different season and tissue](image)

The chart clearly indicated the viscera were considerably higher than muscles in any season. It showed the most of metal concentrations were accumulated at viscera. In the season series, the viscera had the highest MPI in spring, but the lowest value in muscles also in spring. For human consumption, we still suggest that not to surfeit when we eat mussels.

3.3 Relations between metals in tissues and seasons

Results of correlation analyses (bivariate correlations with Pearson correlations coefficients) among these heavy metals concentrations were listed in Table 2. There is a significant relation \((p < 0.01)\) for concentration of Cu, Pb, Zn, Ni and As in tissues; relation \((p < 0.05)\) for concentration of Cd in tissues. It signified that the concentration of increase or decrease of viscera and muscles were influenced each other. Cu and Zn, Ni; Pb and Ni, Cr; Zn and As, Ni and Cr, Cd and As all had a significant relation \((p < 0.01)\); Cu and Pb, Pb and Zn, Zn and Ni also had relation \((p < 0.05)\). This can be explained that the concentration of increase or decrease were influenced each other. We also can find that Cd and season had a relation \((p < 0.05)\). From the Fig.2 we can know that the Cd concentration (in viscera) was increased follow the season series.

Table 2. Correlation coefficients between metal concentration in tissues and seasons; \(n=24\)
<table>
<thead>
<tr>
<th>Season</th>
<th>Tissue</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
<th>Ni</th>
<th>Cr</th>
<th>Cd</th>
<th>As</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>1</td>
<td>-0.14</td>
<td>-0.38</td>
<td>-0.37</td>
<td>-0.11</td>
<td>-0.32</td>
<td>0.49*</td>
<td>0.01</td>
<td>0.28</td>
</tr>
<tr>
<td>Tissue</td>
<td>0</td>
<td>1</td>
<td>0.72**</td>
<td>0.61**</td>
<td>0.66**</td>
<td>0.74**</td>
<td>0.25</td>
<td>0.45*</td>
<td>0.55*</td>
</tr>
<tr>
<td>Cu</td>
<td>-0.14</td>
<td>0.72**</td>
<td>1</td>
<td>0.47*</td>
<td>0.66**</td>
<td>0.63**</td>
<td>0.17</td>
<td>0.15</td>
<td>0.23</td>
</tr>
<tr>
<td>Pb</td>
<td>-0.38</td>
<td>0.61**</td>
<td>0.47*</td>
<td>1</td>
<td>0.47*</td>
<td>0.57**</td>
<td>0.54**</td>
<td>0.05</td>
<td>0.38</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.37</td>
<td>0.66**</td>
<td>0.66**</td>
<td>0.47*</td>
<td>1</td>
<td>0.43*</td>
<td>0.06</td>
<td>0.23</td>
<td>0.59**</td>
</tr>
<tr>
<td>Ni</td>
<td>-0.11</td>
<td>0.74**</td>
<td>0.63**</td>
<td>0.57**</td>
<td>0.43*</td>
<td>1</td>
<td>0.53**</td>
<td>0.19</td>
<td>0.07</td>
</tr>
<tr>
<td>Cr</td>
<td>-0.32</td>
<td>0.25</td>
<td>0.17</td>
<td>0.54**</td>
<td>0.06</td>
<td>0.53**</td>
<td>1</td>
<td>-0.11</td>
<td>-0.20</td>
</tr>
<tr>
<td>Cd</td>
<td>0.49*</td>
<td>0.45*</td>
<td>0.15</td>
<td>0.05</td>
<td>0.23</td>
<td>0.19</td>
<td>-0.11</td>
<td>1</td>
<td>0.64**</td>
</tr>
<tr>
<td>As</td>
<td>0.01</td>
<td>0.55**</td>
<td>0.23</td>
<td>0.38</td>
<td>0.59**</td>
<td>0.07</td>
<td>-0.20</td>
<td>0.64**</td>
<td>1</td>
</tr>
<tr>
<td>Hg</td>
<td>0.28</td>
<td>0.23</td>
<td>-0.02</td>
<td>-0.23</td>
<td>-0.14</td>
<td>0.22</td>
<td>-0.11</td>
<td>-0.11</td>
<td>-0.23</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

### 4. Conclusion

Organism, which is able to show spatial and temporal changes in metal concentrations, is a suitable candidate to be used in biomonitoring surveys [10]. In this preliminary biomonitoring study, we found the viscrea of mussels had considerable high ability to accumulate the metals, and had the great seasonal variation. Mussels of the genus *Mytilus* are popular biomonitor and have been used extensively in Mussel Watch programmes as in the US and France [10]. We suggest that the mussels *Perna viridis* also can play a biomonitor in Ta-Peng Bay Laggon even the coast of Taiwan.

### 5. Reference

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