Chelation technique for the removal of heavy metals (As, Pb, Cd and Ni) from green mussel, *Perna viridis*

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Present research was carried out to study the efficiency of chelation method to remove heavy metals like arsenic (As), lead (Pb), cadmium (Cd) and nickel (Ni) from *P. viridis*. Chelation method was studied using three types of chelating agents, namely trisodium citrate, disodium oxalate and sodium acetate. Metals concentrations were analysed using ICP-MS technique. Results showed that the sodium acetate gave the highest percentage removal of heavy metals (As 59.50%, Pb 88.57%, Cd 68.01% and Ni 79.67%) followed by disodium oxalate (As 46.89%, Pb 85.46%, Cd 60.41% and Ni 47.80%) and trisodium citrate (As 38.13%, Pb 68.90%, Cd 70.49% and Ni 36.92%). The findings showed that sodium acetate was able to chelate and remove all the studied heavy metals to levels below the permissible limit set forth by Malaysian Food Regulations (1985) and EU Commission Regulation (2006).

[Keyword: Heavy metal, *Perna viridis*, Chelating agent, ICP-MS]

**Introduction**

Asian green mussel, *Perna viridis* is a commercially important bivalve. Bivalves (F. Mytilidae) are filter feeders feeding on phytoplankton, small zooplankton and other organic materials. They are usually seen in habitats with water salinity in the range of 18–33ppt and temperature in the range of 11–32ºC. *Perna viridis* generally spawn twice in a year, between early spring and late autumn. In Malaysia, *P. viridis* are widely culture at Penang, Perak, Selangor, Negeri Sembilan, Malacca and Johor. However, the brackish water in between Johor Baharu, Malaysia and Malaysia-Singapore Second Link Bridge area have become the most productive farming location of *P. viridis* to almost 95 percent of the mussel production of Malaysia.

Kahle and Zauke (2002) had reported that some aquatic organisms having the ability to concentrate contaminants in their tissue and organ systems to more than a million times, compared to their concentration in their habitat. Mussel as a filter feeder is accumulates huge amounts of toxic pollutants mainly heavy metals from its habitat. Effluents arising from human activities, infrastructure developments, agricultural activities, tourism and allied activities are the major source of heavy metal contamination of mussel growing areas. Further the contaminated sediments accumulated over the years could become a secondary source of heavy metal contamination to the overlying waters.

Heavy metals could be classified as potentially toxic (e.g arsenic, cadmium, lead, mercury), probably essential (e.g nickel, vanadium, cobalt) and essential (e.g copper, zinc, iron, manganese). Toxic elements could be harmful even in low concentration when ingested over a long time period. The essential metals could also produce toxic effect when their intake is excessive. Many investigations and surveys were conducted for biomonitoring the concentration of heavy metals in *P. viridis* as well as to confirm the health status of the surrounding environment.

The concentrations of Cd, Pb, As and Ni in *P. viridis* tissues that had been reported were 13.89 µg/g, 40.07 µg/g, 43.6 µg/g and 16.5 0 µg/g.

Depuration process had been carried out to naturally purify bacterial, viral, PAHs, organochlorine pesticides and transition metal (Cu and Zn) contamination from the bivalves. However, no commercially viable industrial technology was currently developed to remove toxic elements which are cumulated through long-term ingestion of relatively small quantities in *P. viridis*. Purpose of the study is to develop method that could safely remove heavy metals (As, Pb, Cd and Ni) from contaminated *P. viridis* to comply with the permissible levels set by the Malaysian Food Regulations (1985) and EU Commission Regulation (2006).
Commission Regulation of EU (2006) for human consumption. Chelation technique that had been reported is conducted on solid waste, soil, sediment and wastewater treatment plants. This study examines the potential the chelation method for removal of selected heavy metals in *P. viridis* utilizing chelating agents such as trisodium citrate, disodium oxalate and sodium acetate.

**Materials and Methods**

Analysis of As, Pb, Cd and Ni in *P. viridis* samples were conducted using inductively coupled plasma mass spectrometry, ICP-MS (Perkin Elmer ELAN 6100). All reagents used in the study were analytical grade and were used without any purification. All the solutions were prepared using deionized water obtained from the NANO pure water system. Samples were digested using HNO₃ (Analytical grade, Merck 65%). All the plastic and glassware were cleaned by soaking in diluted HNO₃ (10%) and rinsed with distilled water prior to use. The element standard solutions used for calibration were produced by diluting a stock solution of 29 element standard 3 (10 mg/L), supplied by Merck, Germany. Chelating agents used were trisodium citrate dihydrate, C₆H₅Na₃O₇.2H₂O (QRëC™), sodium oxalate, Na₂C₂O₄ (Bendosen), sodium acetate trihydrate, CH₃COONa.3H₂O (QRëC™).

The *P. viridis* were collected from Sungai Melayu located at Johor Strait (Johor Baharu, Malaysia) as shown in Fig. 1. The *P. viridis* with shell length of 5.0-7.5 cm were used in the research. All *P. viridis* were stored at -10°C until prepared for the analysis.

Treatment of *P. viridis* for the heavy metals removal was conducted using the three types of chelating agents. *P. viridis* were put in sack and were soaked in the beaker that contains the chelating agents with stirring for 1 hour. *P. viridis* was rinsed with deionized water and digested before analyzed using ICP-MS. *P. viridis* treatment was optimized using chelating agent (200 to 1000 µL/L), for 1, 3 and 5 hours of treatment time, at different treatment temperature (29.50±0.50°C, 32.50±0.50°C and 37.50±0.50°C) and at different pH (acidic: pH4-5, neutral: pH6-7 and basic: pH8-9).

All prepared samples were digested using 65% of HNO₃ (APHA 30 30 E). Digestion was done through partial reflux until clear solutions were obtained. After the digestion process, the samples were allowed to cool and filtered through a Whatman Grade 2 filter Paper and then diluted to 50 mL with deionized water. The prepared samples were then analysed for As, Pb, Cd and Ni using ICP-MS. The concentration are presented in µg/g. To check for contamination and performance of the instrument, procedural blank and standard were analysed once for every ten samples.

**Results**

The concentrations of heavy metals (µg/g) in the *P. viridis* from Johor Strait are presented in Table 1. The concentration of trisodium silicate varied from 200 to 1000 mg/L to elucidate the optimum concentration of chelating agent. The efficiency of trisodium silicate at varying concentration in the removal of heavy metals concentration in *P. viridis* is presented in Fig. 2. From the results it is evident that the levels of heavy metals studied were successfully reduced by trisodium citrate treatment (As: 43.32%, Pb: 73.21%, Cd: 74.54%, Ni: 42.12%) and the concentration of 500 mg/L was found most effective with highest percentage of reduction of heavy metals.

![Fig. 1—A red circle on the map showing sampling point of Sungai Melayu area located at Johor Strait (Johor Baharu, Malaysia)](image_url)

**Table 1—Permissible limits of heavy metals in bivalve tissue as per EU and Malaysian standards and the observed concentration in *P. viridis* collected from samples.**

<table>
<thead>
<tr>
<th></th>
<th>As (µg/g)</th>
<th>Pb (µg/g)</th>
<th>Cd (µg/g)</th>
<th>Ni (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permissible limit for</td>
<td>European Union</td>
<td>1.00</td>
<td>1.50</td>
<td>1.00</td>
</tr>
<tr>
<td>Malaysia</td>
<td>1.00</td>
<td>2.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Observed levels on December 2011</td>
<td>1.58±0.23</td>
<td>9.16±1.68</td>
<td>1.30±0.50</td>
<td>2.84±0.57</td>
</tr>
</tbody>
</table>
Further investigating was done in which the treatment time was varied to one, three and five hours using the optimum dosage of trisodium citrate at 500 mg/L. Results showed that the percentage of heavy metal removal increased as the time of treatment increases (Fig. 3). Five hours of treatment showed highest percentage of heavy metal removal (As: 58.41%, Pb: 74.73%, Cd: 77.44%, Ni: 51.67%).

Effect of temperature on the efficiency of trisodium citrate as chelating agent was also studied and results are presented in Fig. 4. It is observed from the results that percentage reduction of heavy metal concentration in the mussel tissue increases from 29.50±0.50°C to 32.50±0.50°C and decreased at 37.50±0.50°C. Highest reduction of (As: 42.12%, Pb: 74.86%, Cd: 70.88%, Ni: 51.98%) was observed at 32.50±0.50°C.

The efficiency of trisodium citrate as a chelating agent of heavy metals in *P. viridis* was tested at varying pH conditions. The results are presented in Fig. 5. Study indicated that trisodium citrate as a chelating agent was most efficient at neutral pH (As: 38.86%, Pb: 79.27%, Cd: 74.84%, Ni: 51.51%) at a given concentration, temperature and treatment time.

The result of the comparative evaluation of three chelating agents is presented in Table 2. The results

![Fig. 2](image1.png)

**Fig. 2**—Efficiency of trisodium citrate at varying concentrations towards removal of heavy metals from *P. viridis* at treatment time: 1h, pH: 6-7, and temperature: 29.50±0.50°C.

![Fig. 3](image2.png)

**Fig. 3**—Effect of time treatment on heavy metal removal in *P. viridis* at trisodium citrate: 500 mg/L, pH: 6-7, and temperature: 29.50±0.50°C.

![Fig. 4](image3.png)

**Fig. 4**—Effect of temperature on heavy metal removal in *P. viridis* using trisodium citrate at concentration of 500 mg/L, treatment time: 5 h, and pH: 6-7.

![Fig. 5](image4.png)

**Fig. 5**—Effect of pH on heavy metals removal in *P. viridis* at concentrations of trisodium citrate: 500 mg/L, treatment time: 5 h, and treatment temperature: 32.50±0.50°C.

The proposed mechanism of chelation reaction towards heavy metals is illustrated in Reaction 2 for the case of trisodium citrate. M⁺ = Heavy metals contaminated in the *P. viridis* (Reaction 2)
Table 2—The percentage removal of heavy metals in *P. viridis* using chelation technique for various chelating agents:

<table>
<thead>
<tr>
<th>Chelating agents</th>
<th>As (µg/g)</th>
<th>Pb (µg/g)</th>
<th>Cd (µg/g)</th>
<th>Ni (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average initial conc. for 6 replicates</td>
<td>1.58±0.23</td>
<td>9.16±1.67</td>
<td>1.30±0.50</td>
<td>2.84±0.57</td>
</tr>
<tr>
<td>Trisodium citrate</td>
<td>0.98±0.69</td>
<td>2.85±1.56</td>
<td>0.38±0.12</td>
<td>1.79±1.09</td>
</tr>
<tr>
<td>Disodium oxalate</td>
<td>0.84±0.06</td>
<td>1.33±0.42</td>
<td>0.52±0.23</td>
<td>1.48±0.60</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>0.64±0.11</td>
<td>1.05±0.60</td>
<td>0.42±0.19</td>
<td>0.58±0.07</td>
</tr>
</tbody>
</table>

Permissible limit; Malaysia: As (1.00 µg/g), Pb (2.00 µg/g), Cd (1.00 µg/g), Ni (1.00 µg/g).

The obtained results from ICP-MS showed that the initial *P. viridis* samples contain As, Pb, Cd and Ni at concentration above the permissible limit. In the optimization treatment condition of trisodium citrate, 500 mg/L concentration of dosing, 32.50±0.50°C of treatment temperature and at neutral pH, pH6-7 were initially selected as it gave the highest percentage removal of heavy metals in *P. viridis*. Fig. 6. Heavy metals successfully removed above 50% for all studied heavy metals after 5 hours of treatment compared to 1 hour and 3 hours of treatment but still not achieve the permissible limit for Pb and Ni. Thus, 1 hour of treatment time was applied for *P. viridis* treatment with other chelating agents (e.g. disodium oxalate and sodium acetate) as Pb and Ni content in *P. viridis* after treatment are not much different with respect to difference treatment times (1, 3 or 5 hours).

The analysis suggests that there is a trend on heavy metals removal by trisodium citrate which differed by the dosing as shown in Fig. 2. The removal of the heavy metals increased and reached optimum at dosing concentration of 500 mg/L. Exceeding this optimum dosing concentration, the percentage removal of heavy metals decreased accordingly. This event could be explained by the Le Chatelier’s principle whereby the increased in concentration of the trisodium citrate will enhance the reversible reaction towards the formation of starting reactant, trisodium citrate and thus decrease the citrate ion production to chelate the heavy metals. Meanwhile, Fig. 3 shows that the percentage removal of heavy metals is proportional to time. It is most probably indicates that the longer period of treatment time will give enough time for chelating agents to chelate with the heavy metals and excreted out from the *P. viridis* flesh. The increased with temperature up to 32.50±0.50°C as shown in Fig. 4 may cause an increase in the number of mucus glands and the amount of mucus secreted per gland of *P. viridis*. This could help to excrete the heavy metals from the flesh thus increased the percentage of heavy metals removal. Whereby, heavy metals removal decreased at 37.50±0.50°C as might due to the high mucus production which covered up the flesh surface and prevented the chelating agent to excrete heavy metals from *P. viridis*. Finally, the highest heavy metals removal occurred at neutral pH condition. It was probably the neutral pH enhances the irreversible reaction which increased the production of citrate ion to chelate with the heavy metals ion in *P. viridis*.

It is obvious that from the present investigation of chelation treatment using all the three studied chelating agents manage to decrease the selected heavy metals content in the *P. viridis* flesh after the treatment. Among the three studied chelating agents, sodium acetate gave the highest percentage removal of heavy metals in *P. viridis* followed by disodium oxalate and trisodium citrate. This trend could be explained in terms of molecular structure. Branching structure of the molecule was assumed to make the trisodium citrate and disodium oxalate more bulky and less polar, thus decrease the capability of penetrating through the *P. viridis* flesh. Hence, allow the heavy metals removal to occur only at the surface of the *P. viridis* flesh. Furthermore, the higher dissociation degree of sodium acetate in water allows more instantaneous formation of acetate ions which will increase the penetration, chelation and excretion of chelated heavy metals from the *P. viridis* flesh as compare to disodium oxalate and trisodium citrate.
The proposed mechanism of chelation reaction towards heavy metals is illustrated in Reaction 2 for the case of trisodium citrate.

\[ M^+ = \text{Heavy metals contaminated in the } P. \text{ viridis} \] (Reaction 2)

When the chelating agents are dissolved in water, they will dissociate into citrate and sodium ions. The sodium ion attains high susceptibility towards hydroxyl ion in water to form sodium hydroxide. The formerly oxygen atom bonded to sodium atom in citrate ion has accepted one electron from sodium atom, to attain the octet configuration thus leading to the formation of three lone pair electrons. This event allows these types of oxygen atoms to chelate with heavy metal ions through dative bonding formation.

Conclusion

The chelation method is found to be a potential technique for the removal of studied heavy metals in \( P. \text{ viridis} \). Present investigation illustrates the efficacy of the studied chelating agents in the order of sodium acetate followed by > disodium oxalate and > trisodium citrate. Interestingly, sodium acetate is able to chelate the studied heavy metals in \( P. \text{ viridis} \) to levels permissible for human set forth by EU and Malaysian food regulations.

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References: