Metal concentrations in *Mytilus galloprovincialis* from southern Dardanelles, Turkey

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**ABSTRACT**

Bioavailability of Pb, Cu, Cd, Ni, Zn and Al in the soft tissues of indicator mussels *Mytilus galloprovincialis*, sampled at the southern Dardanelles coastal area, has been investigated. Comparison of the results with the food codex values reveal threshold levels for lead, copper and cadmium, sometimes over passing the healthy consumption limits. From the multivariate statistical analysis of data it is found that metal content in these mussels are correlated (p<0.05) directly to the polluting source distances such as mining and other industrial discharges in the order of Pb>Cd>Cu.

**INTRODUCTION**

All minor and trace elements which are susceptible to be found in the world ocean are introduced into the aquatic environment, either naturally or by industrial activities. Due to their very special filtration and elimination mechanisms mussels have been recognised as biological indicators of the environmental metal contamination since decades. Also due to their sampling and stocking adequacy, without loss of the element content, they are still being used as bio-indicators of metal contamination in sea and fresh water. As a result of the seasonal, ecological and anthropogenic pressures, mussels sometimes may even change physiologically for adaptation to this type of pollution. Since bioavailability of these substances in the food web is a possible risk for human health, it is important to assess accurately their levels in the bio-indicator organisms. The most commonly studied metals are Pb, Cd and Cu, known to lead to illnesses when over accumulated in human body because of their toxic and synergic effects. For example, the allowable background lead concentration in seawater should not exceed 1.27 μg l⁻¹ if the mussel is not to reach the human food consumption. The study area consisted of the southern coastal line of the Strait of Dardanelles, situated between the Marmara and Aegean basins in western Anatolia. This area together with Bosphorus constitutes the heavily charged Turkish Strait System, serving as a biological corridor for the passage of different species of fish to the adjacent seas. In addition to pollution and oil spills from the intensive ship transport, other anthropogenic industrial and municipality discharges contribute to the dispersion of pollution in a two-layered strait system. The surface current can be considered responsible for transporting pollution downstream from the Black Sea through both sides of the Aegean Sea, and finally to the Mediterranean Sea.
Thus, aim of this work was to distinguish metal pollution via the determination of the metal content accumulated in sessile organisms such as mussels. Mussels are chosen for metal analysis rather than water, since they are not affected by the strong currents in the strait system. Research work for metal determinations in sea food exists for the adjacent seas\cite{6,17} and the eastern Black Sea coast\cite{2}, but not for this area in the Dardanelles.

The Mediterranean blue mussel *Mytilus galloprovincialis*, a commonly consumed bivalve species, is used for monitoring Pb, Cd, Cu, Zn, Al and Ni.

**EXPERIMENTAL**

**Sampling**

Mussels were collected from the southern Dardanelles coastal line (Figure 1). Besides the heavy load present in the straits, the area is polluted by several industries. Sampling area was selected in such a way to cover an impact zone of about 65 kilometres from a principally polluting Pb, Zn extraction mine (indicated as MINE). Station numbering started as 1, at the closest discharge point and continued downstream towards the city of Canakkale, which is additionally polluted by other local industries depositing their wastes directly into the sea. At Station 10, located at the Gallipoli Peninsula, mussels are cultivated in cages for local consumption and exportation. In the sampling strategy these points together with their respective distances from the mine were taken into account. Further, end of summer was chosen as the more appropriate sampling season in order to account summer recruitment of molluscs, providing sufficient soft tissue with other adequate characteristics\cite{15}. Mussels were picked directly by hand when the sea was at the lowest level containers filled with seawater and brought to the laboratory. They were kept alive for 48 hours in their original seawater, in order to eliminate guts from the digestive system\cite{4}. Then, soft tissues of at least 10 mussels, picked from each station, were dissected and stored in the deep freezer at –20°C until analysis.

**Digestion**

About 5 gram of defrosted sample from each station was put in a 25ml tube with a 5:1 mixture of nitric acid and peroxide. All samples after digestion in a microwave oven were filtered through 125µm paper.

**ICP-AES determinations**

The ICP multi-element standard solutions (Merck VIII, 100mg l\(^{-1}\)) were used for the preparation of three different metal concentration solutions (0.1, 1 and 10mg l\(^{-1}\)) for the calibration of the ICP-AES instrument (Model type *Varian*, Liberty AX sequential). After that the Pb, Cd, Cu, Zn, Al and Ni concentrations were determined by atomic absorption spectroscopy. Data were processed and analysed statistically by the multivariate principal component method PCA\cite{9,11} in order to establish correlations between the element content in the mussel soft tissue and the distance to the polluting sources.

![Figure 1 : Dardanelle's map indicating sampling stations (1-10) and their respective distances from the lead mine](image1.png)

![Figure 2 : Two principal component projections (PCA) for metal concentrations as a function of distance from polluting point sources](image2.png)
Statistical analysis

Statgraph[16] was used for the multivariate statistical analysis of metal concentration levels as a function of distance from polluting point sources. In order to discriminate between the most important components of data the well known principal component (PCA) and factor analysis methods were efficient. A further clustering analysis regrouped stations with similarities for comparison purposes.

RESULTS AND DISCUSSION

Metal levels

Metal concentrations in soft tissues, expressed in µg g⁻¹, are presented per station in TABLE 1. We notice that at Station 1 located 18 km from the Mining industry, the lead content in mussels was extremely high and decreases gradually with the distance from the polluting source. Similarly, we observe that, concentrations of Cd are mostly higher than the acceptable codex[19] value of 1 µg g⁻¹ at all stations except 5, 7 and 9. On the other hand, Cu is 3 times higher at the downstream stations 4-9, than the upstream ones (1-3). The other metals present a similar behaviour but they are below the acceptable consumption limits. On the other hand, concentration level of Ni at Station 9 (1.2 µg g⁻¹), is very close to the upper limit of 1.4 µg g⁻¹. Note that this value is lower than the measured water values[13] at the creek mouth (Station 2), about 10 km downstream.

The above metal concentrations can be compared with respect to the Food Codex law of TFC[19] and the CE standards[5]. We observe that due to the presence of some toxic element concentrations in the mussel soft tissues, such as Pb, Cd, Cu they cannot always be considered as edible. We notice especially that at Station 1, located at 18 km distance from the lead and zinc Mining, the Pb content of mussels picked at the closest discharge point was 80 µg g⁻¹. It is evident that this value is much greater than the allowable food codex value of 1.5 µg g⁻¹. Even at the presence of strong currents (averaging around 2m/sec), at the first downstream stations (1 to 3) Pb values still exceeded the allowable limit. Note that at the cross station 10 situated at the opposite side of the Dardanelles Strait, lead concentrations were below the critical limit.

Considering our sampling method and concentration determinations as non-biased, we proceeded to statistical analysis of the data in order to establish correlations between metal concentrations in mussels and the respective distance of the sampling stations from the pollution source. From the multivariate statistical analysis, basically, two principal components were found to explain the variance-covariance structure of our data. The graphical visualisation in figure 2 shows that amongst the most important components (Pb, Cd) an inverse correlation with the contamination distance exists. The second component explains a non-point, industrially polluted zone (Cu, Ni, Al) by the Sarcią Creek (Station 2) discharges to the Strait. To a lesser extent a third component could be responsible for the Zn pollution.

Factor loadings with estimated communalities, for three factors, had eigenvalues greater than or equal to one and accounted for 78% variability of the original element data. Finally, employing the clustering method, the final distance metric was obtained for the nearest agglomeration distance as 20 km for Pb contamination. The latter corresponds to Station 1, showing the highest

**TABLE 1 : Heavy metal levels in mussel soft tissues at the Dardanelles coastal zone. “Distance” indicates the distance of the sampling stations from the MINE**

<table>
<thead>
<tr>
<th>Station</th>
<th>Distance km</th>
<th>Al (±0.001)</th>
<th>Cd (±0.005)</th>
<th>Cu (±0.001)</th>
<th>Ni (±0.003)</th>
<th>Pb (±0.006)</th>
<th>Zn (±0.005)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.84</td>
<td>52.168</td>
<td>3.234</td>
<td>1.207</td>
<td>0.630</td>
<td>82.515</td>
<td>28.331</td>
</tr>
<tr>
<td>2</td>
<td>49.57</td>
<td>59.785</td>
<td>1.353</td>
<td>1.268</td>
<td>0.338</td>
<td>7.534</td>
<td>9.626</td>
</tr>
<tr>
<td>3</td>
<td>49.62</td>
<td>59.311</td>
<td>3.442</td>
<td>1.293</td>
<td>0.662</td>
<td>15.133</td>
<td>10.174</td>
</tr>
<tr>
<td>4</td>
<td>60.72</td>
<td>62.276</td>
<td>1.925</td>
<td>2.547</td>
<td>0.211</td>
<td>29.346</td>
<td>18.631</td>
</tr>
<tr>
<td>5</td>
<td>62.60</td>
<td>59.656</td>
<td>0.176</td>
<td>1.294</td>
<td>0.304</td>
<td>0.596</td>
<td>8.978</td>
</tr>
<tr>
<td>6</td>
<td>62.87</td>
<td>60.021</td>
<td>3.754</td>
<td>2.412</td>
<td>0.467</td>
<td>16.097</td>
<td>34.122</td>
</tr>
<tr>
<td>7</td>
<td>63.57</td>
<td>57.989</td>
<td>0.511</td>
<td>1.282</td>
<td>0.604</td>
<td>8.042</td>
<td>19.429</td>
</tr>
<tr>
<td>8</td>
<td>63.91</td>
<td>64.884</td>
<td>2.241</td>
<td>2.752</td>
<td>0.750</td>
<td>8.003</td>
<td>32.382</td>
</tr>
<tr>
<td>9</td>
<td>64.45</td>
<td>62.504</td>
<td>0.124</td>
<td>2.729</td>
<td>1.234</td>
<td>14.727</td>
<td>15.755</td>
</tr>
<tr>
<td>10</td>
<td>37.89</td>
<td>59.884</td>
<td>5.796</td>
<td>1.376</td>
<td>0.162</td>
<td>2.159</td>
<td>14.286</td>
</tr>
</tbody>
</table>

**TABLE 2 : Factor scores for each row of concentrations of the 10 sampling stations ordered with their respective distances from the lead mine (column 1)**

<table>
<thead>
<tr>
<th>Station</th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-7.143</td>
<td>1.953</td>
<td>-2.258</td>
</tr>
<tr>
<td>2</td>
<td>0.324</td>
<td>-1.031</td>
<td>-3.759</td>
</tr>
<tr>
<td>3</td>
<td>-0.004</td>
<td>-1.480</td>
<td>-0.563</td>
</tr>
<tr>
<td>4</td>
<td>-1.283</td>
<td>-1.902</td>
<td>-0.255</td>
</tr>
<tr>
<td>5</td>
<td>1.575</td>
<td>1.037</td>
<td>-0.029</td>
</tr>
<tr>
<td>6</td>
<td>0.405</td>
<td>-3.506</td>
<td>0.991</td>
</tr>
<tr>
<td>7</td>
<td>0.632</td>
<td>2.700</td>
<td>-0.151</td>
</tr>
<tr>
<td>8</td>
<td>-0.476</td>
<td>-2.715</td>
<td>1.569</td>
</tr>
<tr>
<td>9</td>
<td>2.635</td>
<td>3.564</td>
<td>1.573</td>
</tr>
<tr>
<td>10</td>
<td>3.335</td>
<td>1.379</td>
<td>2.883</td>
</tr>
</tbody>
</table>
score of contamination level, amongst the sampling stations in TABLE 2.

Equation estimating the common factors between the standardised concentrations and common factors was obtained for the first rotated factor by the following equation:

\[ 0.928 \times Al - 0.107 \times Cd + 0.027 \times Ni - 0.812 \times Pb - 0.168 \times Zn \]  (1)

In eq. (1) direct correlation with distance is indicated by a positive sign, an inverse one by a negative sign. Finally, results were fitted to linear models by regression analysis, (ANOVA) in order to describe a significant relationship between Pb and distance, explaining 49 % of variability, Cu with 31 % (p = 0.05), and Cd 3.6 % (p = 0.10).

**CONCLUSIONS**

This work showed that metal availability in the Mediterranean mussel *Mytilus galloprovincialis*, originating from the southern Dardanelles coastal zone, is mostly affected by industrial discharges. For the first time it is reported that certain element content in the soft tissues of mussels measured in the summer of 2005 have very high values, with an importance in the order of Pb>Cd>Cu. From multivariate statistical analysis, it is found that a direct correlation (p<0.05) exists between these toxic element concentrations in *Mytilus galloprovincialis*, and the distance from pollution source (covering 20-60km along the south-western coastal area of the Dardanelles). Thus, our results classify this most commonly consumed bivalve species in the area sometimes as unsafe, according to food regulations. This result might have been underestimated in some older reports employing the traditional hot plate digestion, where a considerable amount is lost due to evaporation. Therefore, we conclude that a potential risk of seafood intoxication exists if these bivalves are consumed for a certain time. We also suggest that similar unbiased analysis can be applied to monitoring seasonal metal content, in all bio indicators.

**ACKNOWLEDGEMENTS**

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**REFERENCES**

[19] TFC (Turkish Food Codex Law Number 24885), Published Officially on 23.09.2002.