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Application of Cluster Analysis in Interpretation of Toxic Metals Accumulation in Biomonitoring Marine Organisms*

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Abstract. Levels of heavy metal (cadmium, zinc, copper, lead, nickel, iron) concentrations in tissues of snails and mollusks collected from the Varna Gulf, Black Sea were determined using atomic absorption spectroscopy. In principle, no elevated concentrations of toxic metals were detected as compared to the critical values offered by the environmental agency in Bulgaria. Nevertheless, the monitoring results offer an opportunity to assess in a reliable way the sea water quality using the marine organisms as biomonitors. The cluster analysis applied revealed that the accumulation of heavy metals indicated clustering into several groups, where the metals found in the shell were significantly different from the other soft tissues. The multivariate analysis indicates the ability of snails and mollusks to accumulate heavy metals, hence fulfilling the criteria as good biomonitors.

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1 Introduction

Marine organisms (mollusks and snails) have been successfully considered as species suitable for bioindicators of a marine environment quality. They are widely distributed and accumulate easily heavy metals [1-9]. Since these benthic organisms could be found in many costal aquatic ecosystems, their tendency of accumulation of heavy metals is of significant interest. Very often special attention is paid to the heavy metal accumulation in the soft tissues of the organisms due to their higher ability to retain the toxic species. Chemometric studies on monitoring data from benthic organisms have also indicated their bioindicator status [10,11].

It is the goal of the present study to offer an analytical procedure for qualitative determination of heavy metals in the soft tissues of mollusks and snails collected

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at different sampling sites along the coastal line of the Gulf of Varna, Black Sea.

Cluster analysis was applied in this study to observe the differences of metal distribution in the different tissues of mollusks and snails. The use of multivariate statistical techniques, such as cluster analysis (CA) is useful in the interpretation of complex data matrices to better understand the heavy metals and ecological status of the systems studied, allowing the identification of possible factors/ sources that might influence heavy metals and can offer a valuable tool for reliable management as well as rapid solution to pollution problems [12-16]. Besides, the use of multivariate analysis, statistically, could assist in determining the potential biomonitor accurately, by referring to cluster groups of their different parts. Moreover, multivariate methods are recommended for the use in monitoring studies since they can help reduce the costs of carrying out further environmental surveys [17].

2 Experimental

Mollusks and snails were sampled in three sampling sites which cover the entire Gulf of Varna. The samples collected were properly described by size (3.5 to 5.5 cm in length). The sampling depth was 6 m. The influence of the anthropogenic sea impact is strongly reduced due to the significant depth of the local seabed (more than 20 m). About 60 species were sampled in each sampling location for any of the sampling events. The samples were then treated in the laboratory: opening by a plastic knife, removing of the salt water, dissection of the soft tissue, freeze drying and storage in polymeric containers at low temperature.

The dissolution of the organic matter followed the instructions described in [9]. Approximately 0.5 g of the dried soft tissue was treated after weighting by 5 cm^3 concentrated nitric acid and the mixture was kept at room temperature for 24 hours. Then stirring for 3 hours follows. It was the last step before decomposition procedure using a microwave system (MEGA, USA). The solution obtained was treated by 1 cm^3 hydrogen peroxide (30%v/v) and filtered by Whatman filters. The final volume was adjusted to 50 $cm^3$by adding deionized water.

For the analysis of snails, 30–40 individual of snails with almost similar sized were randomly taken from the main sample and thawed at room temperature (26–29°C) on a clean tissue paper. The soft tissues were then separated from the shell by crunching the shell carefully.The soft tissues were then dissected and pooled into six different components namely ceacum, foot, muscle, operculum, remainder, and tentacle besides the shell. The soft tissues and the shell were dried for 72 hours at 60°C in an oven to constant dry weights [18,19].

About 0.5 grams of tissues was digested in 10 ml of concentrated nitric acid (AnalaR Grade; 69%). They were placed in a hot block digester first at low temperature (40°C) for 1 hour and were then fully digested at high temperature (140°C) for at least 3 hours. The digested samples were then diluted to a volume of 40 ml with double distilled water (DDW). The sample was then filtered through Whatman No.1 filter paper (Dia: 110 mm; Schleicher & Schuell, Whatman International Ltd Maidstone England).

The chemical analysis was performed by the use of atomic absorption spectrometry (AAS) (Perkin Elmer 5100 PC instrument). The calibration was performed by the standard addition method for all toxic metals. In order to determine the uncertainty of the measurements replicates of the samples were used and the relative standard deviation (RSD in %) was approximately 5–10%. Accuracy was estimated by measurements on a standard reference materials TORT 1 and DOLT-3.

For the statistical analysis, the distributions of heavy metals in the different parts were determined by using cluster analysis. All data were log10 (X + 1) transformed prior to the statistical analysis. STATISTICA 7.0 software was used to conduct the cluster analysis.

3 Results and Discussion

In general, it was found that the tentacle of the gastropods was highly accumulative of Cu and Zn as shown by the gastropods from all the sites, where they ranged from 112–178 μg/g dw and 117–161 μg/g dw, respectively. Meanwhile, the operculum was mostly accumulative of Fe (638–2921 μg/g dw). On the other hand, the shell was highly accumulative of Cd (4.41–5.37 μg/g dw), Pb (53.2–63.8 μg/g dw) and Ni (24.4–27.9 μg/g dw).

Distributions of heavy metals in the different parts of the snails are better be explained by cluster analysis as shown in Figure 1. Generally, the accumulations of Cu, Cd, Zn, Pb and Fe by the shell were significantly different from the other tissues as they were solely clustered into one group. This could be due to the fact that some trace metals are incorporated into the shells of the snails through substitution of the calcium ion in the crystalline phase of the shell or are associated with the organic matrix of the shell [20,21] instead of induction of metallothionein as being found in the soft tissues. However, for the accumulation of Ni, the shell and tentacle were significantly different from the other soft tissues. Besides, most of the soft tissues were found clustered into two distinct groups (by ignoring the shell). For the accumulation of Cu, the caecum, muscle and operculum were clustered as one group while another group consisted of the remainder, tentacle and foot. As for Cd, the first group consisted of the caecum and operculum while the second group consisted of the remainder, foot, muscle and tentacle. The accumulation of Zn by the operculum was significantly different from the remainder as it was solely clustered, while the caecum, remainder, muscle, foot and tentacle were clustered as one group.

For Pb, the caecum, remainder, muscle and foot were clustered into one group

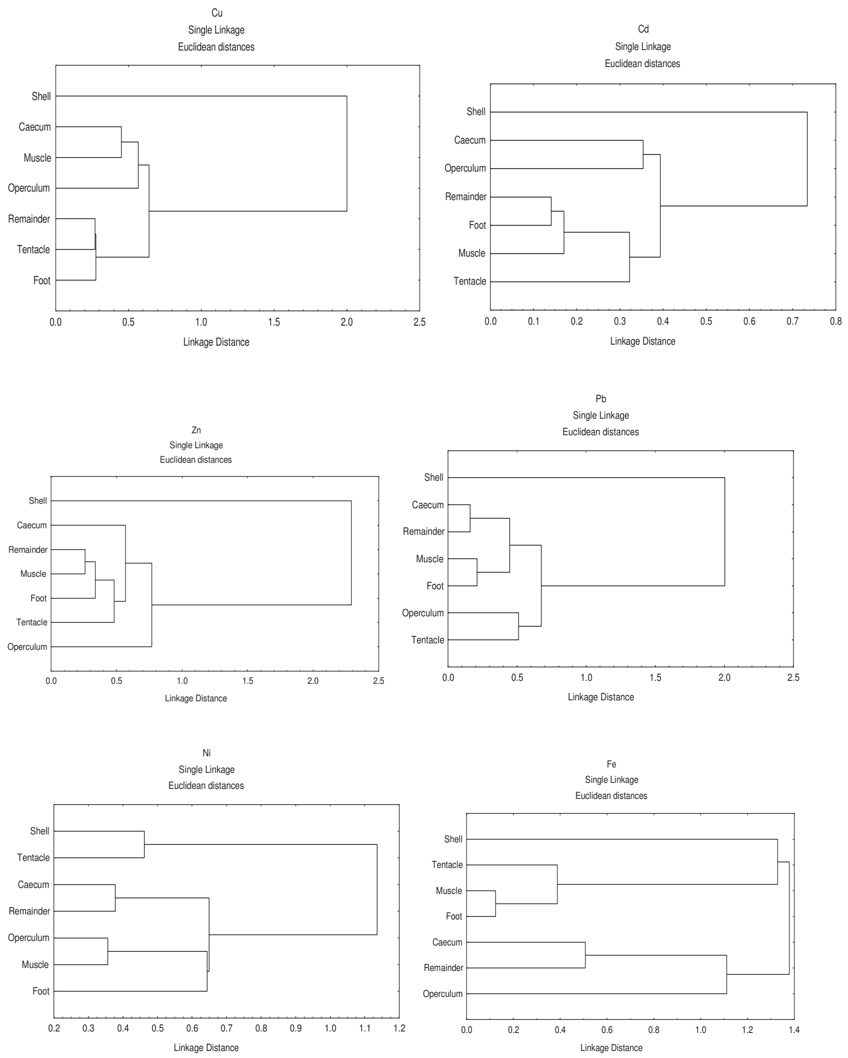

Figure 1: Hierarchical dendrograms for clustering of heavy metals with respect to their distribution in snail tissues.

while the operculum and tentacle were clustered into another group. Meanwhile for Ni, the first group of the soft tissues consisted of the caecum and remainder and the second group consisted of the operculum, muscle and foot. Two distinct cluster groups were also observed in the accumulation of Fe by the soft tissues, where the first group consisted of the tentacle, muscle and foot while the second group consisted of the caecum, remainder and operculum. Generally, the cluster analyses indicated the differences of heavy metal accumulation by the different parts, in other words, each tissue accumulates different concentrations of metals.

4 Conclusion

From the present study, it was found the ability especially of the snails to accumulate and regulate heavy metal concentrations in their body as revealed by the cluster analysis. It was found that the accumulation of metal by the shell was significantly different from the remaining soft tissues.

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