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HEAVY METAL DISTRIBUTION IN THE DIFFERENT PARTS OF MOLLUSKS BY USING MULTIVARIATE ANALYSIS

OCENA AKUMULACJI METALI CIĘŻKICH W RÓŻNYCH CZĘŚCIACH MIĘCZAKÓW Z WYKORZYSTANIEM ANALIZY WIELOCZYNNIKOWEJ

Abstract: The mollusk samples were obtained from several locations along the Black Sea coast (gulf of Varna and Gulf of Burgas, Bulgaria). The samples were dissected into five different soft tissues. The soft tissues and the shell were then analysed for heavy metals. It was found that the highest concentrations of Cu (112–178 μg/g dm) and Zn (117–161 μg/g dm) were found in the tentacle; the highest concentrations of Cd (4.41–5.37 μg/g dm), Pb (53.2–63.8 μg/g dm) and Ni (26.1–27.9 μg/g dm) were found in the shell. On the other hand, the highest Fe concentrations (910–2921 μg/g dm) were found in the operculum. The cluster analysis revealed that the accumulation of heavy metals were clustered into a few groups, where the metals found in the shell were significantly different from the other soft tissues. Results from the cluster analysis were further complimented by the correlation analysis and multiple stepwise linear regression which revealed that the accumulation by the different parts were interrelated with one another. It was also found that the soft tissue was the most influential part in accumulation of heavy metal studied. Thus, it indicates the ability of the mollusks to accumulate heavy metal, hence fulfilling the criteria as a good biomonitor.

Keywords: mollusks, heavy metal, multivariate analysis

Currently, the determination of heavy metal concentrations in whole individuals presents little interest, since the main metal accumulating organs such as gills, digestive gland and kidney are but a small part of a total soft tissue [1]. Besides, the spawning season of the mollusks and environmental factors may contribute to the wide variability of heavy metal concentrations in the total soft tissues, thus, the above points strongly

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evidenced the disadvantages of the use of the total tissue in monitoring of metal bioavailability in marine environment.

The present study focuses on the different parts of mollusks as a biomonitor of heavy metal contamination in coastal waters. They are found abundantly in the coastal areas and their ability to accumulate metals fulfilled the criteria as biomonitors of heavy metal contamination. In the literature, their reliability as biomonitors of heavy metal contamination had been studied by many researchers [2–5]. The literature above supported the application of mollusks as biomonitors of heavy metal pollution in the marine environment.

On the other hand, correlation and cluster analysis were applied in this study to observe the differences of metal distribution in the different tissues of the mollusks. Correlation and cluster analysis (CA) are the most usual multivariate statistical methods used in environmental studies [6–12] especially in the studies of heavy metals in sediment. A few studies reported on the use of cluster analysis in the determination of heavy metals in the marine mollusks were found in the literature [13–16].

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 [17–21]. 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 [22–23].

Therefore, present study aims to determine the distribution of heavy metal in the different parts of mollusks and to determine the possible significant relationships between the concentrations of the different parts by using cluster and multiple stepwise linear regression analyses.

Experimental

A sampling was conducted in the gulfs of Burgas and Varna. The identification of the species were followed the descriptions by Lim et al [24]. For the analysis, 30–40 individual mollusks with almost similar sized were randomly taken from the main sample and thawed at room temperature (26–29 ^{o}C) on a clean tissue paper. The soft tissues were then separated from the shell by crunching (using a clean pestle) the shell carefully. Due to the fragile characteristic of the shell, a mild force was sufficient to break the shell (strong force might destroy the internal organs of the snail). 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 ^{o}C in an oven to constant dry weights. The whole analytical procedure was performed at Institute of Oceanology, Bulgarian Academy of Sciences.

About 0.5 gram of sample tissues were digested in 10 cm^3 of concentrated nitric(V) acid (AnalaR Grade; 69 %). They were placed in a hot block digester first at low temperature (40 ^oC) for 1 hour and were then fully digested at high temperature (140 ^oC) for at least 3 hours. The digested samples were then diluted to a volume of 40 cm^3 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) and they were determined for Cd, Cu, Fe, Ni, Pb and Zn by using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Model AAnalyst 800. The samples were analyzed in three replicates. The data were presented in μg/g dry mass (dm) basis. Multilevel calibration standards were analysed to generate calibration curves against which sample concentrations were calculated. Standard solutions were prepared from 1000 mg/dm^3 stock solutions of each metal (Merck Titrisol).

All the glassware and plastic materials used were acid-washed in 10 % acid solution in order to minimize external contamination. Quality control samples made from standard solutions of Cu, Cd, Zn, Pb, Ni and Fe were analyzed once in every ten samples to check for the metal recoveries. The analytical procedures for the snail samples were checked with the Certified Reference Material (CRM) for dogfish liver (DOLT-3, National Research Council Canada) and the recoveries of all metal were satisfactory (Table 1).

Table 1

Analytical results for the Certified Reference Material (CRM) and its certified values for each metal [μg/g dry mass]

Metal	Sample	CRM values	Measured values	Percentage of recovery
Cd	DOLT-3 Dogfish-liver	19.4 ± 0.600	20.5 ± 0.439	106 ± 2.26
Cu	DOLT-3 Dogfish-liver	31.2 ± 1.00	26.5 ± 2.58	85.0 ± 8.28
Fe	DOLT-3 Dogfish-liver	1484 ± 57.0	1070	72.1
Ni	DOLT-3 Dogfish-liver	2.72 ± 0.350	2.77 ± 0.741	102 ± 27.2
Zn	DOLT-3 Dogfish-liver	86.6 ± 2.40	80.9 ± 1.94	93.4 ± 2.24

NA: Pb value is not available.

For the statistical analysis, the distributions of heavy metals in the different parts were determined by using cluster analysis. Multiple stepwise linear regression analysis was used to determine the influence of heavy metal in the different parts toward the allometric parameters. All data were log10 (X + 1) transformed prior to the statistical analysis. STATISTICA 7 was used to conduct the cluster analysis, correlation and multiple stepwise linear regression analyses.

Results and discussions

Heavy metal concentrations in the different parts of the mollusks collected from the three sampling sites (V1 and V2 from Varna Gulf abd B1 fom Burgas Gulf) are shown in Table 2. In general, it was found that the tentacles were highly accumulative

Table 2

Heavy metal concentrations [μg/g dm, mean ± SE] of (Cu, Cd, Zn, Pb, Ni and Fe) in the different soft tissues of mollusks

	Site	Cu	Cd	Zn	Pb	Ni	Fe
Caecum	V1	95.3 ± 15.8	1.79 ± 0.185	113 ± 18.3	10.0 ± 0.193	9.44 ± 0.207	782 ± 86.2
	V2	94.7 ± 2.61	1.43 ± 0.086	113 ± 10.6	7.94 ± 0.433	9.26 ± 0.530	543 ± 47.9
	B1	51.1 ± 1.77	3.45 ± 0.169	318 ± 5.67	16.2 ± 1.00	7.54 ± 0.350	661 ± 71.9
Foot	V1	118 ± 4.21	1.48 ± 0.071	74.4 ± 8.77	5.94 ± 0.234	2.80 ± 0.192	177 ± 15.9
	V2	113 ± 11.3	0.984 ± 0.096	97.7 ± 2.67	5.10 ± 0.606	3.58 ± 0.269	121 ± 4.07
	B1	124 ± 9.36	1.22 ± 0.115	113 ± 0.183	14.0 ± 0.553	5.79 ± 0.615	255 ± 3.47
Muscle	V1	64.8 ± 7.72	1.16 ± 0.111	82.4 ± 6.21	5.88 ± 0.434	0.660 ± 0.086	156 ± 12.1
	V2	83.4 ± 3.79	0.901 ± 0.141	92.4 ± 3.30	4.12 ± 0.178	3.33 ± 0.539	124 ± 10.0
	B1	77.3 ± 0.526	1.39 ± 0.075	79.0 ± 1.57	13.7 ± 0.548	5.08 ± 0.137	254 ± 5.11
Operculum	V1	51.7 ± 0.000	2.78 ± 0.000	43.9 ± 0.000	9.45 ± 0.000	0.222 ± 0.000	2921 ± 0.000
	V2	44.7 ± 0.000	2.13 ± 0.000	45.0 ± 0.000	7.50 ± 0.000	3.47 ± 0.000	910 ± 0.000
	B1	59.1 ± 0.000	2.46 ± 0.000	38.9 ± 0.000	43.0 ± 0.000	7.22 ± 0.000	638 ± 0.000
Remainder	V1	136 ± 13.4	1.54 ±0.202	65.0 ± 5.06	10.7 ± 1.86	6.54 ± 0.848	850 ± 60.8
	V2	143 ± 3.25	1.35 ± 0.158	77.5 ± 1.89	7.62 ± 0.550	6.37 ± 0.253	406 ± 29.6
	B1	133 ± 2.51	1.17 ± 0.144	81.1 ± 5.29	17.2 ± 1.71	6.22 ± 0.052	1137 ± 123
Shell	V1	11.9 ± 0.794	5.12 ± 0.047	5.91 ± 0.255	56.2 ± 1.43	26.1 ± 0.909	240 ± 48.9
	V2	11.1 ± 0.750	5.37 ± 0.205	7.04 ± 0.647	53.2 ± 0.483	27.9 ± 0.295	66.3 ± 4.95
	B1	8.65 ± 0.503	4.41 ± 0.118	6.53 ± 1.39	63.8 ± 0.318	24.4 ± 0.143	52.0 ± 2.35
Tentacle	V1	178 ± 0.000	0.962 ± 0.000	117 ± 0.000	5.70 ± 0.000	20.1 ± 0.000	240 ± 0.000
	V2	173 ± 0.000	1.36 ± 0.000	130 ± 0.000	4.54 ± 0.000	16.2 ± 0.000	177 ± 0.000
	B1	112 ± 0.000	2.32 ± 0.000	161 ± 0.000	34.2 ± 0.000	27.9 ± 0.000	283 ± 0.000

of Cu and Zn from all the sites, where they ranged from 112–178 μg/g dm and 117–161 μg/g dm, respectively. Meanwhile, the operculum were mostly accumulative of Fe, ranging between 638–2921 μg/g dm. On the other hand, the shell was highly accumulative of Cd (4.41–5.37 μg/g dm), Pb (53.2–63.8 μg/g dm) and Ni (24.4–27.9 μg/g dm).

Distribution of heavy metals in the different parts of the mollusks are better explained by cluster analysis as shown in Figure 1. Generally, the accumulation 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 through substitution of the calcium ion in the crystalline phase of the shell or are associated with the organic matrix of the shell

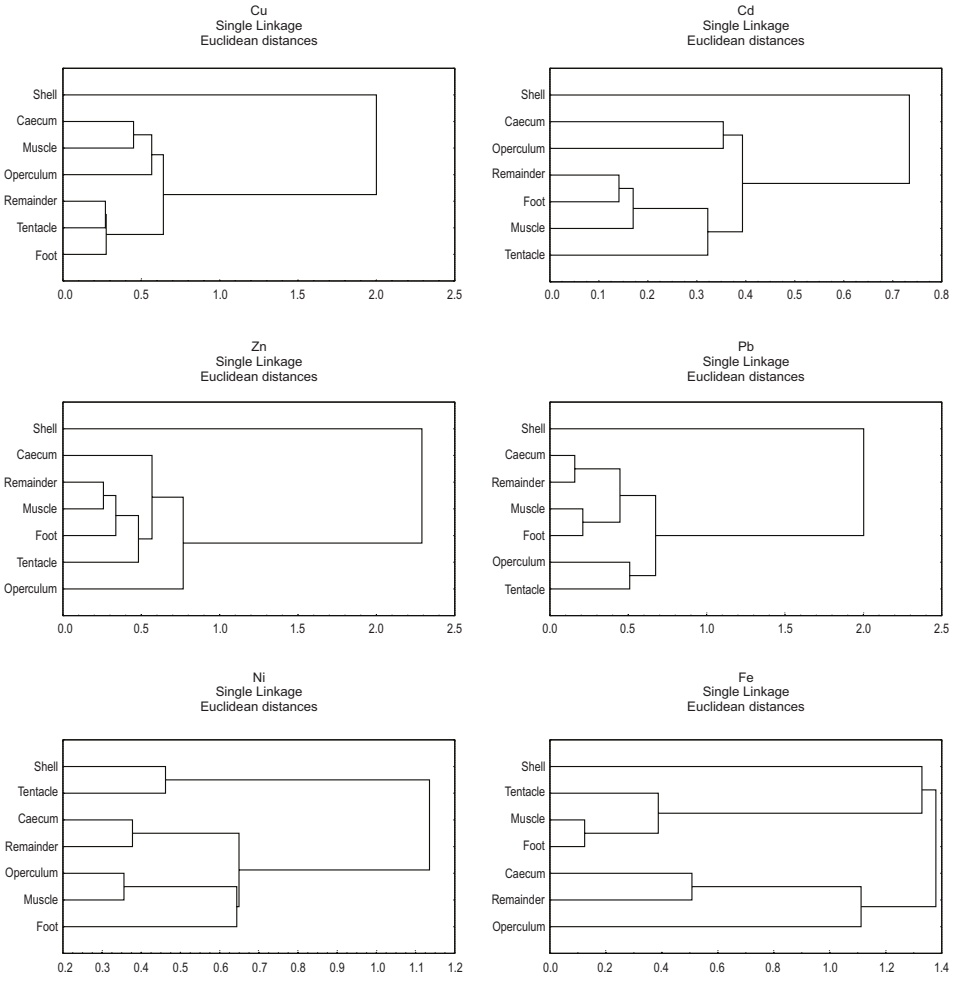

Fig. 1. Cluster analysis on the distribution of heavy metals (Cu, Cd, Zn, Pb, Ni and Fe) in the different parts of mollusks

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 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 accumulate different concentrations of metals.

The relationships between the different parts and the total soft tissues are explained in the multiple stepwise linear regression analysis (Table 3). The caecum was found to be the influential tissues in the accumulation of heavy metals studied besides the remainder and operculum.

Table 3

Multiple linear stepwise regression between the total tissues of mollusks and their different soft tissues

Metal	Multiple stepwise linear regression
Cu	Total tissue = –9.861 – 0.033 (Caecum) + 2.805 (Remainder) + 3.346 (Operculum)
Cd	Total tissue = –0.053 + 0.634 (Caecum)
Zn	Total tissue = –1.479 + 1.492 (Caecum)
Pb	Total tissue = no significant variables were selected
Ni	Total tissue = –0.898 – 0.050 (Caecum) + 1.322 (Remainder) + 0.405 (Operculum)
Fe	Total tissue = –1.502 + 1.189 (Caecum) + 1.264 (Remainder) – 0.917 (Operculum)

Conclusions

From the present study, it was found the ability of mollusks to accumulate and regulate heavy metal concentrations in their body as revealed by the multivariate analysis. From the correlation and cluster analyses, it was found that the accumulation of metal by the shell was significantly different from the remaining soft tissues. Multiple stepwise linear regressions also revealed that the caecum was the most influential organ in the accumulation of heavy metals by mollusks.

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Abstrakt: Próbki mięczaków były pobierane w kilku miejscach wzdłuż wybrzeża Morza Czarnego (Zatoka Warna i Zatoka Burgas, Bułgaria). Z próbek wyodrębniono pięć różnych tkanek miękkich. W tkankach miękkich i skorupach oznaczono metale ciężkie. Stwierdzono, największe stężenia Cu (112–178 μg/g s.m.) i Zn (117–161 μg/g s.m.) w mackach; a największe stężenie Cd (4.41–5.37 μg/g s.m.), Pb (53.2–63.8 μg/g s.m.) i Ni (26.1–27.9 μg/g s.m.) stwierdzono w skorupie. Z drugiej strony, największe stężenia Fe (910–2921 μg/g s.m.) stwierdzono w pokrywie skrzelowej. Analiza klastrów ujawniła, że akumulowane metale ciężkie były pogrupowane w kilka skupień, w których metale oznaczone w skorupkach były inne od tych zidentyfikowanych w tkankach miękkich. Wyniki analizy klastrów były weryfikowane przez analizę korelacji i regresji liniowej wielostopniowej, które wykazały, że akumulacja w różnych częściach była wzajemnie ze sobą powiązana. Stwierdzono również, że w tkankach miękkich kumuluje się najwięcej metali ciężkich. W związku z tym wskazano na zdolność mięczaków do kumulowania metali ciężkich, a tym samym stwierdzono, że spełniają one kryteria charakteryzujące dobre biomonitory.

Słowa kluczowe: mięczaki, metal ciężki, analiza wieloczynnikowa