LEVELS OF HEAVY METAL CONCENTRATION IN *M. GALLOPROVINCIALIS* MOLLUSC SPECIES FROM NW BLACK SEA (ROMANIA)

Andra BUCȘE[1], Oana Cristina PÂRVULESCU[2*], Dan VASILIU[3*], Naliana LUPAȘCU[4], Cezara VOICA[5]

Bivalve molluscs were collected from five sampling stations (water depths within 43.2–54.2 m), 30 km offshore Sfantu Gheorghe mouth (NW Black Sea), during a research cruise aboard R/V Mare Nigrum conducted in May 2019. Concentrations of Cd (1.69–5.37 mg/kg), Co (0.88–2.2 mg/kg), and Hg (0.085–0.114 mg/kg) were determined by spectrometric techniques. Only Cd exceeded the maximum limit set by Commission Regulation (EC) No. 1881/2006. In order to discriminate between samples collected from five sampling stations, PCA was applied, where the heavy metal concentration in the mollusc tissue, TOC content in the sediment, and water depth were selected as variables.

Keywords: Heavy metals, *Mytilus galloprovincialis*, TOC, PCA, ICP-MS analysis, DMA 80 analysis

1. Introduction

The coastal environment of NW Black Sea, especially the northern part of the Romanian shelf, has been faced with strong anthropogenic influence, especially due to the discharges of the Danube [1]. One of the most severe categories of pollutants in the marine environment is that of heavy metals. These types of pollutants are discharged by the Danube River into the Black Sea, either dissolved or particulate (retained by the fine fraction of sediments). Moreover, these pollutants are transported and redistributed along the Black Sea shelf by the

[1] Ph.D. Student, Dept. of Chemical and Biochemical Engineering, University POLITEHNICA of Bucharest, Romania, National Institute for Research and Development on Marine Geology and Geoecology – GeoEcoMar, 23-25 Dimitrie Onciul Str., 024053, Bucharest, Romania.
[2] Assoc. Prof., Dept. of Chemical and Biochemical Engineering, University POLITEHNICA of Bucharest, Romania. oana.parvulescu@yahoo.com.
[3] Ph.D., National Institute for Research and Development on Marine Geology and Geoecology – GeoEcoMar, 23-25 Dimitrie Onciul Str., 024053, Bucharest, Romania. dan.vasiliu@geoecomar.ro
[4] Ph.D., National Institute for Research and Development on Marine Geology and Geoecology – GeoEcoMar, 23-25 Dimitrie Onciul Str., 024053, Bucharest, Romania.
[5] Ph.D., National Institute for Research and Development of Izotopic and Molecular Technologies, 67-103 Donat Str., 40029, Cluj-Napoca, Romania

currents and storm waves, thus contributing to local metal pollution in front of the Danube Delta, especially in the area between Sulina and Sf. Gheorghe [2].

Mytilius galloprovincialis, one of the most common mollusc species from the NW Black Sea part, is considered a sensitive organism to heavy metals, some elements being harmful even at low concentrations [3]. Due to their well-known capacity to accumulate the pollutants in different tissues through intense filtering feeding activity, the abundant mussel population is a good indicator and sentinel of the environmental quality [4].

Due to their suspected toxicity and potential harm for human exposure, Cd, Co, and Hg are included on Agency for Toxic Substances and Disease Registry (ATSDR) Substance Priority List [5]. Cd is one of the most toxic contaminants which can generate DNA damage, protein denaturation, and oxidative stress to the aquatic organisms [6]. It may also accumulate in the human body through the consumption of mussels and can generate carcinogenic forms kidney or reproductive dysfunctions [7]. Although Co is considered an essential element for the organism, being constituent of several key enzymes and playing important roles in various cellular reactions [8], an excess amount of this metal may produce cellular and tissues damage [9]. Hg may affect the nervous system, metabolism of cells and peroxidation of lipids [10].

This paper aims to describe the distribution of Cd, Co, and Hg in *M. galloprovincialis* collected from five sampling stations in the NW Black Sea. To discriminate between samples collected from different stations, Principal Component Analysis (PCA) was applied, where the heavy metal concentration in the mollusc tissue, TOC content in the sediment, and water depth were selected as variables.

2. Materials and methods

2.1. Study area

Twenty samples of *M. galloprovincialis* were collected from five sampling stations (water depths between 43.2 and 54.2 m), 30 km offshore Sfantu Gheorghe mouth (NW Black Sea), during a research cruise aboard *R/V* Mare Nigrum conducted in May 2019. NW Black Sea is subject to high riverine inputs, which bring significant amounts of contaminants, including heavy metals that are transported and deposited along with the terrigenous particulate organic matter at the sea bottom up to 30–40 km offshore [11]. *Mytilus* community thrives at depths of 20–55 m on mixed circalittoral sediments consisting of terrigenous mud mixed with recent or subfossil shells, where it forms one of the largest benthic habitats, *i.e.*, the biogenic reefs with *Mytilus*, by the accumulation of mussel shells and their aggregation by byssal threads [12,13].

The map of the sampling stations considered in this study is shown in Fig. 1.

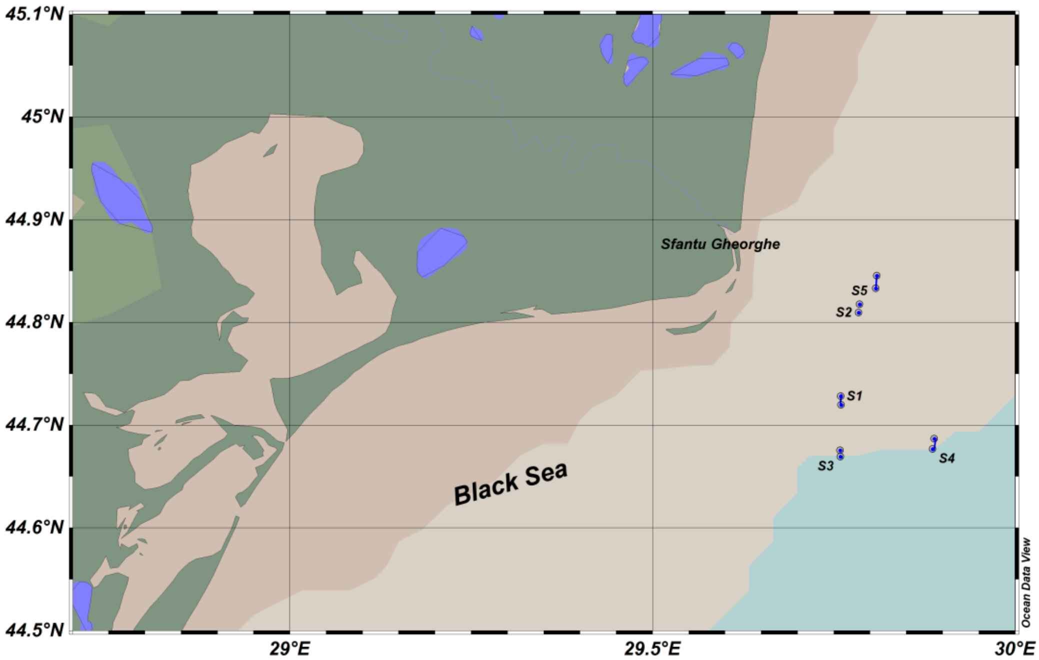

Fig. 1. Study area and sampling stations

2.2. Sampling and chemical analysis

Mollusc samples (Fig. 2) were collected using a dredge with a length of 6 m, opening mouth of 250 cm and mesh size of 4 cm. The samples were dissected with a clean scalpel blade to separate the soft tissues from the shells and then the soft tissues were rinsed with distilled water and freeze-dried at –55 °C for 48 h in an IlShin freeze-drier before the analysis of heavy metals.

Fig. 2. *M. galloprovincialis* samples

The analysis of Hg was carried out with a Direct Mercury Analyser (DMA 80, Milestone, Italy). The samples were weighed in sample cups according to USEPA (2007) [14], then dried and thermally decomposed under air flow and controlled heating. Hg and residual combustion products flowed through the catalyst, where the interferences were removed and Hg species were reduced and trapped on a gold amalgamator, which was heated at 700 °C, where Hg was quantified by atomic absorption spectrophotometry at 253.7 nm. Precision and accuracy of the analytical methods were determined using certified material from the National Institute of Standards & Technology (SRM 2976).

Concentrations of Co and Cd were determined by inductively coupled plasma quadrupole mass spectrometry (ICP-Q-MS). A Perkin Elmer ELAN DRC (e) instrument was used with a Meinhart nebulizer and silica cyclonic spray chamber and continuous nebulization. The operating conditions were as follows: nebulizer gas flow rate: 0.90 L/min; auxiliary gas flow rate: 1.2 L/min; plasma gas flow rate: 15 L/min; lens voltage: 7.25 V; ICP RF power: 1100 W; CeO/Ce=0.030; Ba++/Ba+=0.025. A solution with Mg, Cu, Rh, Cd, In, Ba, Ce, Pb, and U (10 µg/L) from Perkin-Elmer was used for inductively coupled plasma–mass spectrometry (ICP-MS) optimization procedures. Calibration standard solutions and internal standards were prepared by successive dilution of one standard solution, a high purity ICP-multielement calibration standard (10 mg/L of 29 elements including Co and Cd, matrix: 5% HNO_3, Atomic Spectroscopy Standard, PerkinElmer Pure Plus). Standard deviations of measurements were less than 10%. TOC content in the sediments was determined using a titration method in accordance with Gaudette et al. (1974) [15].

2.3. Statistical analysis

Principal Component Analysis (PCA) was performed using Statistica 10 software (StatSoft, Inc). A data matrix with 19 rows (number of samples) and 5 columns (number of variables) was used in PCA.

3. Results and discussion

3.1. Experimental data

Heavy metal concentrations in mollusc soft tissue, TOC content in sediments, and water depths for each station (S1-5) are specified in Table 1 and Fig. 3.

The highest values of Cd concentration (4.70 and 5.37 mg/kg) were detected in the mussels collected from the shallowest stations (S5 and S2), whereas the lowest ones (1.69 and 1.91 mg/kg) in the mussels collected from stations S3 (49.4 m) and S1 (50.5 m). All Cd concentrations (1.69–5.37 mg/kg) exceeded the maximum level set by Commission Regulation (EC) No. 1881/2006, *i.e.*, 1 mg/kg.

The highest values of Co concentration (1.91 and 3.00 mg/kg) were noticed also at stations S5 and S2 and the lowest ones (0.72 and 0.75 mg/kg) were measured at stations S3 and S1. Data presented in Table 1 and Fig. 3 reveal that the mean values of Co concentration (0.88-2.2 mg/kg) are 2.1–2.4 times lower than those of Cd concentration.

The highest values of Hg concentration (0.12 mg/kg) were detected in the mussels collected at stations S5, S2, and S4, whereas minimum values (0.06 and 0.08 mg/kg) were found at stations S4, S3, and S1, respectively. The mean values of Hg concentration (0.085–0.114 mg/kg) were 10–20 times lower than those of Co concentration and 24–41 times lower than those of Cd concentration.

Higher metal concentrations in mussel tissues may be related to intense eutrophication and phytoplankton blooms [16], usually occurring as result of high Danube's flows [17]. These phenomena can enhance mussel metabolic rates and filtering capacity in particular. Fowler and Benayoun [18] reported that the sorption rate of Cd was directly proportional to its concentration in seawater, without taking into account the amount already accumulated in the mussel tissue. In turn, Bryan [19] found that the concentrations of some metals (including Co) in *M. galloprovincialis* decreased with an increase in primary productivity and water temperature.

The highest TOC (2.14%) was found at station S4 with the deepest level of water depth (54.2), as indicated in Table 1 and Fig. 3.

Table 1

Mean±standard deviation and range (min-max) of heavy metal concentrations in molluscs and mean values of TOC content in sediments at various water depth

Station	Water depth *h* (m)	Concentration (mg/kg)			TOC content (%)
		Cd	**Co**	**Hg**	
S1	49.4	2.12±0.19 (1.91-2.28)	0.88±0.13 (0.75-1.02)	0.090±0.01 (0.08-0.10)	1.82
S2	43.2	4.55±0.70 (3.89-5.37)	2.21±0.64 (1.46-3.00)	0.110±0.01 (0.10-0.12)	1.38
S3	50.5	2.56±1.23 (1.69-3.43)	1.08±0.50 (0.72-1.43)	0.085±0.01 (0.08-0.09)	0.74
S4	54.2	2.90±0.45 (2.22-3.44)	1.32±0.20 (1.03-1.57)	0.090±0.02 (0.06-0.12)	2.14
S5	43.2	3.50±1.06 (2.24-4.70)	1.44±0.30 (1.14-1.91)	0.114±0.01 (0.11-0.12)	1.17

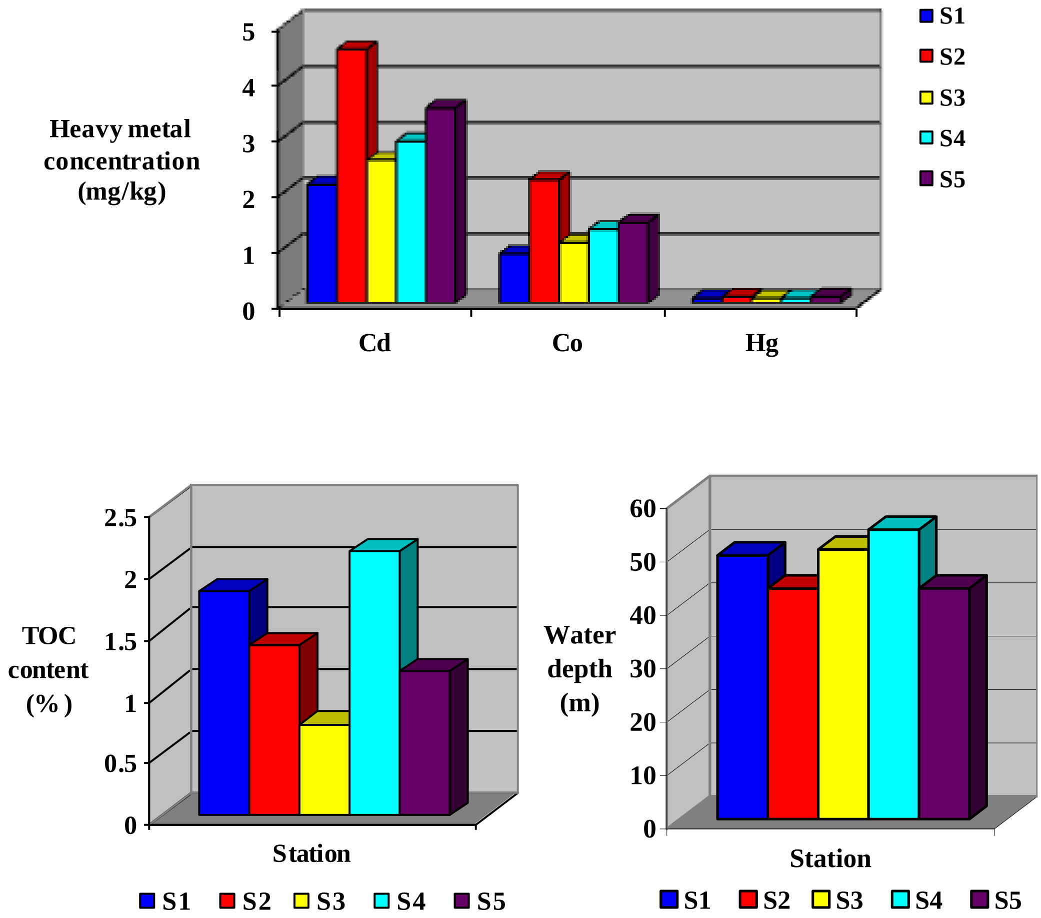

Fig 3. Heavy metal concentrations in molluscs, TOC content in sediment, and water depth for different sampling stations (S1−S5)

3.2. Statistical processing of experimental data

PCA was performed using 5 independent variables, *i.e.*, Cd, Co, and Hg concentrations, TOC content, and water depth. Correlation coefficients (r) specified in Table 2 indicate the following linear relationships: (i) strong positive between Cd and Co concentrations (r=0.84); (ii) moderate positive between TOC content and water depth (r=0.67); (iii) weak positive between Hg and Cd concentration (r=0.40) and Hg and Co concentration (r=0.39); (iv) moderate negative between the concentration of each heavy metal and water depth ($-0.67 \leq r \leq -0.47$); (iv) very weak negative between the concentration of each heavy metal and TOC ($-0.32 \leq r \leq -0.15$).

Table 2

Correlation coefficients

Variable	Cd	Co	Hg	TOC	h
Cd	1.00	0.84	0.40	-0.24	-0.53
Co	0.84	1.00	0.39	-0.15	-0.47
Hg	0.40	0.39	1.00	-0.32	-0.65
TOC	-0.24	-0.15	-0.32	1.00	0.67
h	-0.53	-0.47	-0.65	0.67	1.00

PCA results in terms of eigenvalues and explained variances of principal components (PCs), which are summarized in Table 3, highlight two PCs with eigenvalues greater than 1. These first two PCs explain 80.60% (57.99%+22.61%) of the total variance.

Table 3

Eigenvalues and explained variance of principal components (PCs)

PC	Eigenvalue		% Total variance	
	PC	Cumulative	PC	Cumulative
PC1	2.900	2.900	57.99	57.99
PC2	1.130	4.030	22.61	80.60
PC3	0.626	4.656	12.53	93.13
PC4	0.192	4.848	3.84	96.96
PC5	0.152	5.000	3.04	100.00

Factor coordinates of variables are specified in Table 4. Tabulated data indicate that PC1 is dominated by heavy metals concentrations and water depth, whereas PC2 is dominated by TOC content.

Table 4

Factor coordinates of variables

No.	Variable name	PC1	PC2	PC3	PC4	PC5
1	Cd	**0.814**	–0.479	0.171	–0.018	0.281
2	Co	**0.770**	–0.561	0.122	0.103	–0.258
3	Hg	**0.726**	0.182	–0.645	0.150	0.034
4	TOC	–0.594	**–0.662**	–0.408	–0.207	–0.012
5	h	**–0.874**	–0.340	0.008	0.340	0.071

Projections of variables and cases on factor plane PC1–PC2 (Figs. 4 and 5) emphasize the following issues:

(i) PC1 discriminates between samples collected at stations S2 and S5, characterized by highest values of concentrations of Cd (4.55±0.70 and 3.50±1.06 mg/kg), Co (2.21±0.64 and 1.44±0.30 mg/kg), and Hg (0.110±0.01 and 0.114±0.01 mg/kg) as well as by lowest levels of water depth (43.2 m), and those collected at stations S1 (49.4 m) and S4 (54.2 m);

(ii) PC2 discriminates between samples collected at station S3 (50.5 m), characterized by lowest level of TOC content (0.74%), and those collected at station S4 (54.2 m) characterized by highest level (2.14%).

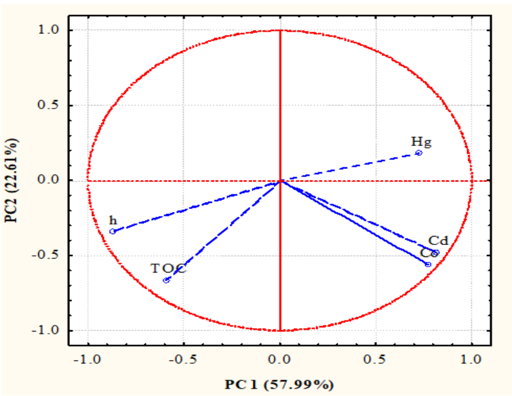

Fig. 4. Projections of variables on factor plan PC1–PC2

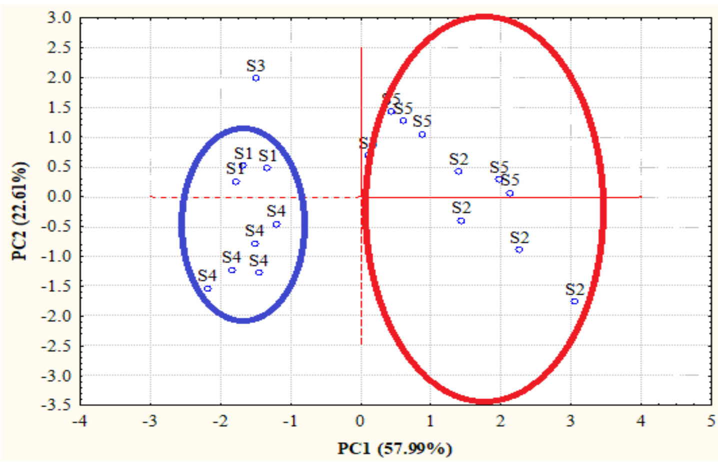

Fig. 5. Projections of cases on factor plan PC1–PC2

4. Conclusions

The present study provides new data about concentrations of some heavy metals, *i.e.*, Cd, Co, and Hg, in *M. galloprovincialis* from the NW Black Sea shelf, specifically the Sf. Gheorghe area, which is known to be polluted as a result of riverine discharges. The investigated heavy metals were detected in all samples collected from five sampling stations (water depths in the range of 43.2−54.2 m). The order of metal accumulation was: Cd (1.69–5.37 mg/kg) > Co (0.88–2.2 mg/kg) > Hg (0.085–0.114 mg/kg). The values of Cd concentration in the mussel flesh exceeded the limit set by the European Communities (1 mg/kg). The mean values of Hg concentration were 10–20 times lower than those of Co concentration and 24–41 times lower than those of Cd concentration. The highest values of metal concentrations were detected in the mussels collected from stations S5 and S2, found under the influence of the Danube and characterized by lowest levels of water depth (43.2 m), whereas minimum values were found in the mussels collected from stations S3 (49.4 m) and S1 (50.5 m). The highest value of TOC content in sediment (2.14%) was found at the station S4 with the highest level of water depth (54.2).

In order to discriminate between samples collected from different sampling stations, PCA was applied, where the heavy metal concentration in molluscs flesh, TOC content in sediment, and water depth were selected as variables. According to PCA, the 5-dimensional feature space was reduced to a 2-dimensional space characterized by two PCs with eigenvalues greater than 1, which explain 80.60% of the total variance. PC1, accounting for 57.99% of total variance, was dominated by metal concentration and water depth, whereas PC2, explaining 22.61% of total variance, was dominated by TOC content. Two well separated groups of samples, *i.e.*, a group consisting of mollusc samples collected from S2 and S5 stations (found under the influence of the Danube and characterized by lowest levels of water depth) and another containing mollusc samples collected from S1 and S4 stations, on the PC1 direction were obtained by projecting the mollusc samples on the factor-plane PC1–PC2. PC2 discriminated between sediment samples collected at station S3 (50.5 m), having the lowest level of TOC content (0.74%), and those collected at station S4 (54.2 m), having the highest TOC level (2.14%).

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