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Doctoral School Of Chemical Engineering And Biotechnologies
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ABSTRACT

DOCTORAL THESIS

INVESTIGATIONS ON THE SPATIAL VARIABILITY OF HEAVY METALS IN THE SURFACE SEDIMENTS AND BIVALVE MOLLUSCS OF THE NORTHWESTERN BLACK SEA SHELF

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KEY WORDS:

pollution, Black Sea, heavy metals, sediments, pollution indices, bivalve mollusks

CHAPTER I
LITERATURE REVIEW

1.1. Heavy metals/trace elements in the aquatic environment

Heavy metals are inorganic compounds naturally present in the Earth's crust and organisms, many of which play key roles in metabolic processes in the latter. However, the artificial introduction of these elements into the environment can lead to accumulation, sometimes in excess, in the environment and/or biota, leading to exceedances of the limits allowed/recommended by environmental or health norms and standards.

1.1.1. Arsenic

Arsenic (As) is a chemical element with atomic number 33 and an atomic mass of 74.92 U.

The main sources of As in the marine environment are river inputs, groundwater and marine sediments (Neff, 1997; Belivermiset al., 2016). Seaweed accumulates arseniate (AsO^{4-}_{3}) in water, reduces As^{5+} to As^{3+} , converts arsenite (AsO^{3-}_{3}) into various organoarsenic compounds, and then releases the AsO^{3-}_{3} and organoarsenic compounds into seawater (Neff, 1997).

1.1.2. Chrome

Chromium (Cr) belongs to the transition metal group and has atomic number 24, atomic mass 51.996 U and four stable isotopes, the most common being ^{52}Cr (84%) (Adriano, 1986).

In the environment, Cr is present in the form of chromite and can also replace Fe3+ and Al^{3+} in most minerals (Faust and Aly, 1981). As for Cr pollution in the aquatic environment, it mainly comes from paint factories, paint shops and industrial metal finishing processes (Krishnamurthy and Wilkens, 1994).

1.1.3. Nickel

Nickel (Ni) belongs to the group of transition metals, has atomic number 28, atomic mass 58.71 U and five stable isotopes, of which the most abundant are ^{58}Ni (67.9%) and ^{60}Ni (26.2%). The concentration of Ni in the lithosphere is 55 ppm (Li, 2000), in river water 0.8 μg/L, and in oceans 0.47 μg/L.

1.1.4. Copper

Copper (Cu) has atomic number 29, atomic mass 63.546 U, two stable isotopes, *i.e.*, ^{63}Cu (69.2%) and ^{65}Cu (30.8%), and belongs to the group of transition metals. It has a moderate abundance in marine sediments (Li, 2000), in which it is associated with clay mineral fractions, particularly those containing organic carbon and manganese oxides (Garrels and Christ, 1965).

1.1.5. Zinc

Zinc (Zn) belongs to the transition metal group, has atomic number 30, atomic mass 65.39 u, five stable isotopes, the most abundant being ^{64}Zn (49%), and a radioisotope (^{65}Zn). The concentration of Zn in the lithosphere is about 80 ppm, the main sources being ZnS, $ZnCO_3$, Zn_2SiO_4 and ZnO (Reimann and de Caritat, 1998).

1.1.6. Mercury

Mercury (Hg) has atomic number 80, atomic mass 200.59 you and belongs to the group of metallic elements. Hg has seven stable isotopes (^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg and ^{204}Hg) and is one of the most toxic elements due to the biogeochemical cycle and bioamplification capacity in the food chain, having major consequences on the aquatic ecosystem and the human body. In the lithosphere, the Hg concentration is about 0.05 ppm.

1.1.7. Cadmium

Cadmium (Cd) has atomic number 48, atomic mass 112,40 U and has seven stable isotopes, of which ^{112}Cd and ^{114}Cd are the most abundant (Nriangu, 1980a).

In both ocean and river waters, the concentration of Cd is 0.08–0.80 μg/L (Gaillardet et al., 2003; Chester, 2000).

1.2. Aspects related to heavy metals/trace element contamination of marine sediments

Marine sediments play a crucial role in the transport and storage of heavy metals/trace elements. On the one hand, elements are stored in sediments as a result of sedimentation and chelation/adsorption, and on the other hand, they can be released by desorption (Wang et al, 2020; Xie et al., 2019).

Heavy metals/trace elements determined in marine ecosystems have become a serious concern due to their multiple sources of origin, non-biodegradability, toxicity, as well as bioaccumulation and bioamplification along the aquatic food chain (Zhao et al., 2017; Zhou et al., 2022). They come either from natural sources (*e.g.*, river discharge, rainfall, volcanic eruptions, wildfires, sand/dust storms, weathering and rock/soil erosion) or anthropogenic (*e.g.*,

port activities, tourism, undertreated water, agricultural and industrial effluents, fossil fuel burning, waste incineration, construction activities, mining and extraction, crude oil refining and other industrial processes, offshore oil/gas drilling and related transport) (Sánchez-García et al., 2010; Romano et al., 2022). These elements can also be produced *in situ* by marine organisms (Rubio et al., 2000).

1.3. Aspects relating to heavy metal/trace element contamination of molluscs *Mytilus galloprovincialis*

Mytilus galloprovincialis has aroused great interest among scientists because it has become an emblematic mollusc species in the Mussel Watch Program, one of the oldest initiatives on pollution monitoring (Goldberg et al., 1983; Cantillo, 1998). These molluscs are bioindicators ("sentinel organisms") of environmental quality due to their ability to accumulate pollutants in different tissues through intensive filtration activity, their sedentary nature, abundance, geographical distribution and tolerance to environmental change and pollution (Çevik et al., 2008).

1.4. Potential sources of heavy metals/trace elements contamination in the Romanian Black Sea area

The Black Sea is a semi-enclosed sea, being part of the Mediterranean Sea, connected to the main basin by various straits and basins, including the Bosphorus Strait, the Sea of Marmara, the Dardanelles Strait and the Aegean Sea (Tătui F., 2017). With an area of 423488 km2 and an average depth of 1271 m, the Black Sea communicates to the south with the Mediterranean Sea, through the Bosphorus Strait, and to the north it is connected to the Sea of Azov, through the Kerch Strait.

The Romanian Black Sea area is subject to multiple natural and anthropogenic pressures caused by Danube discharges, coastal erosion, various port, tourist, industrial activities, untreated or insufficiently treated wastewater discharges (Catianis et al., 2016; Oros, 2019). After the 2000s, however, when Romania started to implement the Water Framework Directive (2000/60/EC) and the Marine Strategy Framework Directive (2008/56/EC), there was a downward trend in the concentration of heavy metals/trace elements in the marine ecosystem.

CHAPTER II
STUDY AREA, MATERIALS AND METHODS

2.1. Study area

In order to assess the quality of the marine environment in terms of sediment and biota contamination with heavy metals and to identify the main sources of pollution in each area of interest, a series of data collected over 2 years (2018-2019) were processed, during field campaigns organized by NIRD GeoEcoMar in Constanta within the Core **project PN 19 20 01 02: "Multidisciplinary research to improve knowledge of the interaction between climate change and anthropogenic pressures and its effects on the Black Sea ecosystem".**

The study area was chosen to provide a complete spatial coverage of the Romanian shelf and to capture as well as possible the human impact and diversity of hydrological, geo- and hydrochemical conditions that are the result of both the influence of human activities and climatic variations or geographical context, such as the vicinity of the Danube and its fresh water intake. The study area and map of sampling stations are shown in Fig. 2.1 and Fig. 2.2.

The sediment samples were collected in August 2018, from 22 stations located at water depths between 13.5–67 m and in August–September 2019, from 32 stations located at water depths between 12.7 and 149 m.

Also, between February and June 2019, Mytilus galloprovincialis bivalve molluscs (mussels) were collected from two areas of the Romanian seaside (northern area - Sfântu Gheorghe and southern area - Agigea) and the concentration of inorganic contaminants in their soft tissue was determined.

Mussels in the Sfântu Gheorghe area were collected in May 2019 from 5 sampling stations (S1–S5), located at water depths between 43.2 and 54.2 m. Mussels in the southern area of Agigea were collected between February and June 2019 from 2 stations (A and B) located on the two artificial breakwaters within the outer berths of Agigea Port.

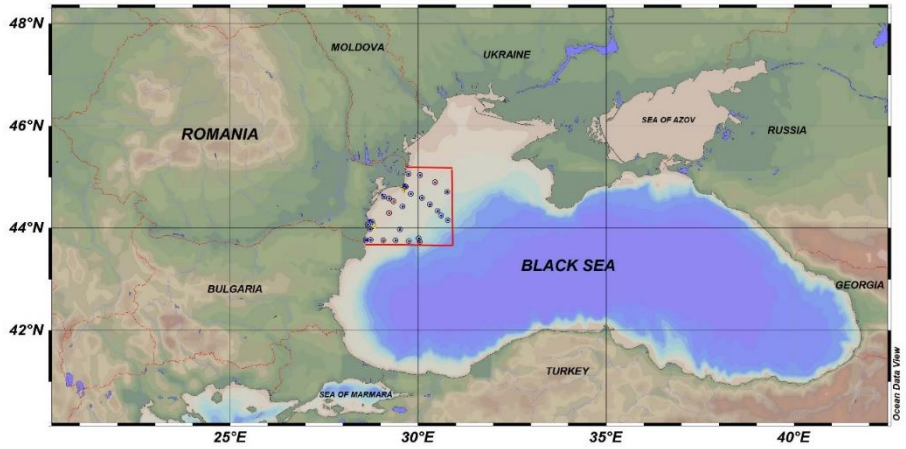


Figure 2.1. Study area and stations in the research program.

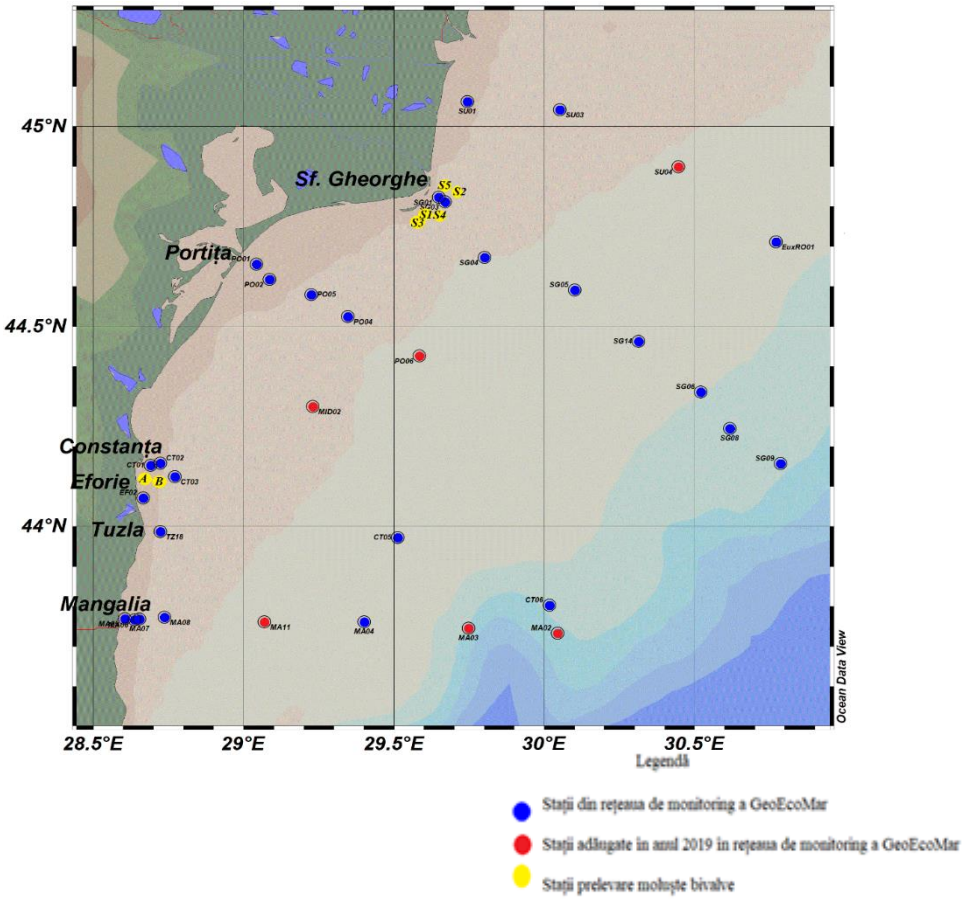


Figure 2.2. Map of stations investigated during 2018–2019.

2.2. Sampling

2.2.1. Sediment sampling

The marine sediment samples were collected during field campaigns carried out in August 2018 and September 2019, onboard the oceanographic vessel for multidisciplinary scientific research *R/V Mare Nigrum*, equipped with laboratories specific to each field for

marine research and specialized equipment, necessary to collect samples, both from the water column and from sediment or biota.

The investigations in the Black Sea during the two field campaigns involved bathymetric data acquisition, physicochemical measurements *in situ* and sediment and biota sample collection, which were subsequently analyzed in the Geochemistry, Sedimentology and Biology laboratories of the National Institute for Marine Geology and Geoecology – GeoEcoMar, Constanta branch.

Sediment sampling was carried out using Van Veen and Multicorer MARK II sampling systems.

2.2.2. Sampling of bivalve molluscs

Samples of *M. galloprovincialis* bivalve molluscs taken from the northern area of the Romanian shelf were collected using a dredge (for biological samples) with a length of 6 m and mesh size of 4 cm. The sampling was done during a research expedition with the *R/V Mare Nigrum* in May 2019.

The *M. galloprovincialis* molluscs collected from the Port of Agigea area were taken manually, with the help of a team of professional divers, from an artificial rocky substrate, the distance between sampling points being about 0.4 km and the water depth of about 8 m. The sampling was done monthly, between February and June 2019.

2.3. Preparation of samples for laboratory analysis

The sediment samples were dried in the oven for 24–48 hours at 105 °C (time varying according to sediment type), then ground using an electric mortar Fritsch/RM 200, sieved with a mesh diameter of 250 μm and placed in paper envelopes pending further analysis.

The mollusc samples were frozen until analysis. The length (*L*) and mass (*mw*) of wet mussels (after thawing and removal of excess water by swabbing) were measured using a caliper and analytical balance (Kern, Germany). The contents of the shell were weighed (after removal of excess fluid by swabbing), freeze-dried at -55°C for 48 h using an ilShin BioBase freeze-drying machine, Netherlands, then shredded with a mortar and pestle prior to heavy metal analysis.

2.4. Determination of metal concentrations in samples of sediments and bivalve molluscs *M. galloprovincialis*

To determine the concentration of elements (Cr, Cu, Ni, Zn, As, Pb, Rb, Sr Zr and V) and metal oxides (Al_2O_3, Fe_2O_3, TiO_2 and MnO) an X-ray fluorescence spectrometer with

dispersed energy ED-XRF SPECTRO XEPOS (SPECTRO Analytical Instruments GmbH, Kleve, Germany) was used to determine the concentration of Hg, a mercury analyzer Milestone DMA-80 (Milestone Srl, Sorisole, BG, Italy), and an inductively coupled plasma mass spectrometer (ICP-MS) was used to determine Co and Cd in molluscs.

2.5. Determination of total organic carbon content and calcium carbonate content

The total organic carbon content (TOC) was determined using a titrimetric method (Gaudette et al., 1974), which consisted of oxidation of carbon with excess $K_2Cr_2O_7$, in the presence of concentrated sulfuric acid, and titration of excess with Mohr salt, using diphenylamine as indicator.

The calcium carbonate ($CaCO_3$) content was determined using a titrimetric method. The sediment sample was treated with a determined volume of 0.9 N HCl and the excess acid was determined by titration with 0.9 N NaOH in the presence of phenolphthalein (Black, 1965).

2.6. Quality control of data obtained

The accuracy (accuracy) of sediment element analyses was assessed using a certified reference material (CRM) from NCS Testing Technology Co., Ltd. (Beijing, China), *i.e.*, NCS DC 73022. The measured and certified values of element concentration and Al_2O_3 in NCS DC 73022, indicate a high accuracy of analytical methods (recovery coefficient values (*CR)* in the range 96.0–104.8%).

The accuracy (accuracy) of element analyses in biological material was assessed using certified reference materials from the National Institute of Standards and Technology (NIST) and the International Atomic Energy Agency (IAEA), *i.e.*, SRM 2976 (mussel tissue as a matrix) and IAEA 407 (fish tissue as a matrix).

The recovery coefficient (*CR*) values for both certified reference materials range from 82 % to 116 %, indicating an acceptable accuracy of the analytical procedures applied.

2.7. Determination of marine sediment particle size

For sediment particle size analysis, the sifting-diffraction method was used using vibrating sieve AS 200 Basic (Retsch, Haan, NRW, Germany) and laser analyzer Mastersizer 2000E (ver.5.20) (Malvern Instruments, Malvern, Worcs, UK).

2.8. Pollution indices

The contamination status of sediments was assessed on the basis of contamination factor (*CF)* (Håkanson, 1980), geo-accumulation index (*Igeo*) (Müller, 1969) and enrichment factor (*EF)* (Taylor, 1964). The pollution indices were determined using equations (2.4)–(2.6), where *E* is the concentration of the element (Al, As, Cr, Cu, Hg, Ni, Pb and Zn) in the sample and *Eb* the reference concentration of the element (in uncontaminated sediment). The mean element concentrations reported by Turekian and Wedepohl (1961) were used as reference values (*Al* = 8% = 80000 mg/kg, *As* = 13 mg/kg, *Cr* = 90 mg/kg, *Cu* = 45 mg/kg, *Hg* = 0.40 mg/kg, *Ni* = 68 mg/kg, *Pb* = 20 mg/kg, and *Zn* = 95 mg/kg).

$$CF = CF_E = \frac{E}{E_b},\ E = Al, As, Cr, Cu, Hg, Ni, Pb, Zn \tag{2.4}$$

$$I_{geo} = I_{geo,E} = \log_2\left(\frac{CF_E}{1.5}\right) \tag{2.5}$$

$$EF = EF_E = \frac{CF_E}{CF_{Al}} \tag{2.6}$$

2.9. Data processing

2.9.1. Graphical data processing

The graphical processing of data in order to highlight the spatial and temporal distribution of heavy metals in sediments was performed using the program OCEAN DATA VIEW (version 4.7.10) (Schlitzer, 2002).

2.9.2. Statistical data processing

Statistical data processing was performed using STATISTICA ver. 10 (StatSoft, Tulsa, OK, USA) and XLSTAT ver. 2019.1 (Addinsoft, New York, NY, USA). The Pearson correlation coefficient (*r*) was used to evaluate the strength and direction of linear correlations between different parameters.

CHAPTER III

HEAVY METALS/TRACE ELEMENTS CONTAMINATION OF SURFACE SEDIMENTS IN THE ROMANIAN BLACK SEA AREA

3.1. Spatial distribution of heavy metals/trace elements and potential sources of contamination in sediments collected in August–September 2019

3.1.1. Objectives

The objectives of this study were: (i) to assess the spatial distribution of Al and some heavy metals/trace elements, *i.e.*, As, Cr, Cu, Hg, Ni, Pb and Zn, in surface sediments collected from 32 stations located at water depths of 12.7–149 m in the Romanian Black Sea area; (ii) assessment of sediment contamination status based on contamination factor (*CF);* (iii) establishing the effects of the main environmental factors (water depth, sediment texture, organic matter and calcium carbonate content) on the accumulation of heavy metals/trace elements and identifying possible sources of contamination by applying statistical analysis. The results obtained in this study were published in the journal *Frontiers in Marine Science* (Bucse et al., 2024).

3.1.2. Study area

The distribution of the 32 sampling stations in the investigated area is illustrated in Fig. 2.2. The stations in the sampling areas were positioned as follows: 28 stations were positioned along 5 transects, *i.e.*, Sulina–E (SU01, SU03 and SU04), Sf. Gheorghe–SE (SG01, SG03, SG04, SG06, SG08, SG09 and SG14), Portița–SE (PO01, PO02 and PO04–06), Constanta–SE (CT01–03, CT05 and CT06) and Mangalia –E (MA02–08, and MA11), one between Sulina–E and Sf. Gheorghe–SE transects (EuxRO01) and 3 others were in Midia, Eforie and Tuzla areas (MID02, EF02 and TZ18).

3.1.3. Results and discussion

3.1.3.1. Particle size analysis, total organic carbon content and CaCO3 content in surface sediments of the investigated area

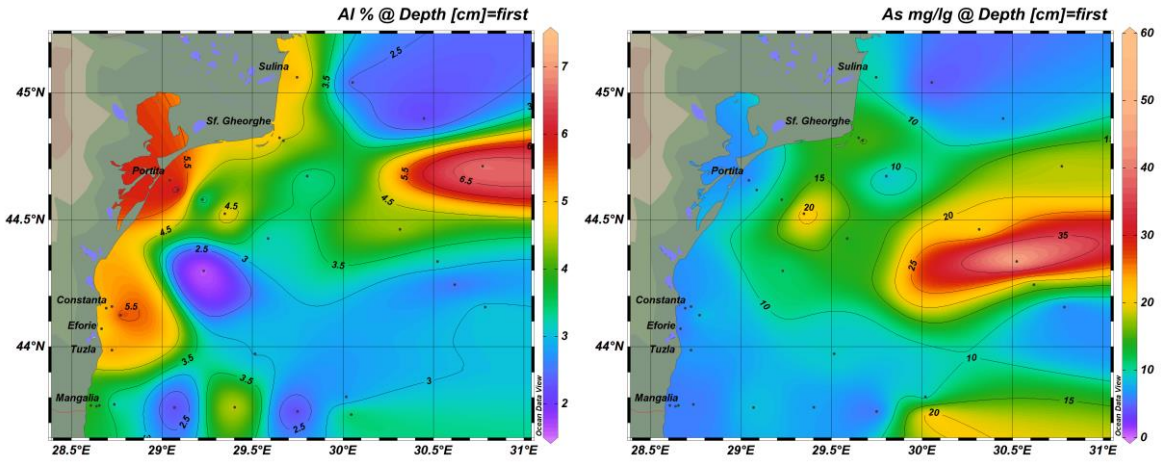
Depending on the percentages of sand, silt, and clay, the surface sediments collected were mostly clayey silt and sandy silt.

Higher levels of sand content (48.3–82.7%), as well as lower levels of clay content (3.1–7.8%) and TOC (0.02–0.43%) were found in sediments from low-depth stations (water depths of 16–27 m) located south of the Danube mouth areas, *i.e.*, PO01, CT01, EF02, MA05 and MA06 (near Portița, Constanta, Eforie and Mangalia). Higher *TOC* values (>2%) were determined in surface sediments collected from EuxRO01 (2.60%), PO04 (2.54%), PO06 (2.11%), SG04, SG06 and SG08 (2.04–2.17%) stations, located at greater water depths (42–119 m).

The concentration of CaCO3 (*CaCO3*) ranged from 10.1% to 57.9%, showing an increasing trend towards the sea. Slightly calcareous sediments (*CaCO3* in the range 10–30%) and calcareous sediments (*CaCO3* in the range 30–50%) were detected at 16 stations and 6 stations, respectively. The lowest levels of *CaCO3* (10.1% and 10.5%) were identified in sediments from stations near the mouth of Sulina (SU01) and Sf. Gheorghe (SG01) (water depths of 16.6 m and 20.6 m respectively).

3.1.3.2. Heavy metals/micronutrient concentrations in surface sediment and potential sources of contamination

The spatial distributions of concentrations of aluminium (Al), arsenic (*As*), chromium (*Cr*), copper (*Cu*), mercury (*Hg*), nickel (*Ni*), lead (*Pb*) and zinc (*Zn*) in surface sediments in the study area are shown in fig. 3.3.



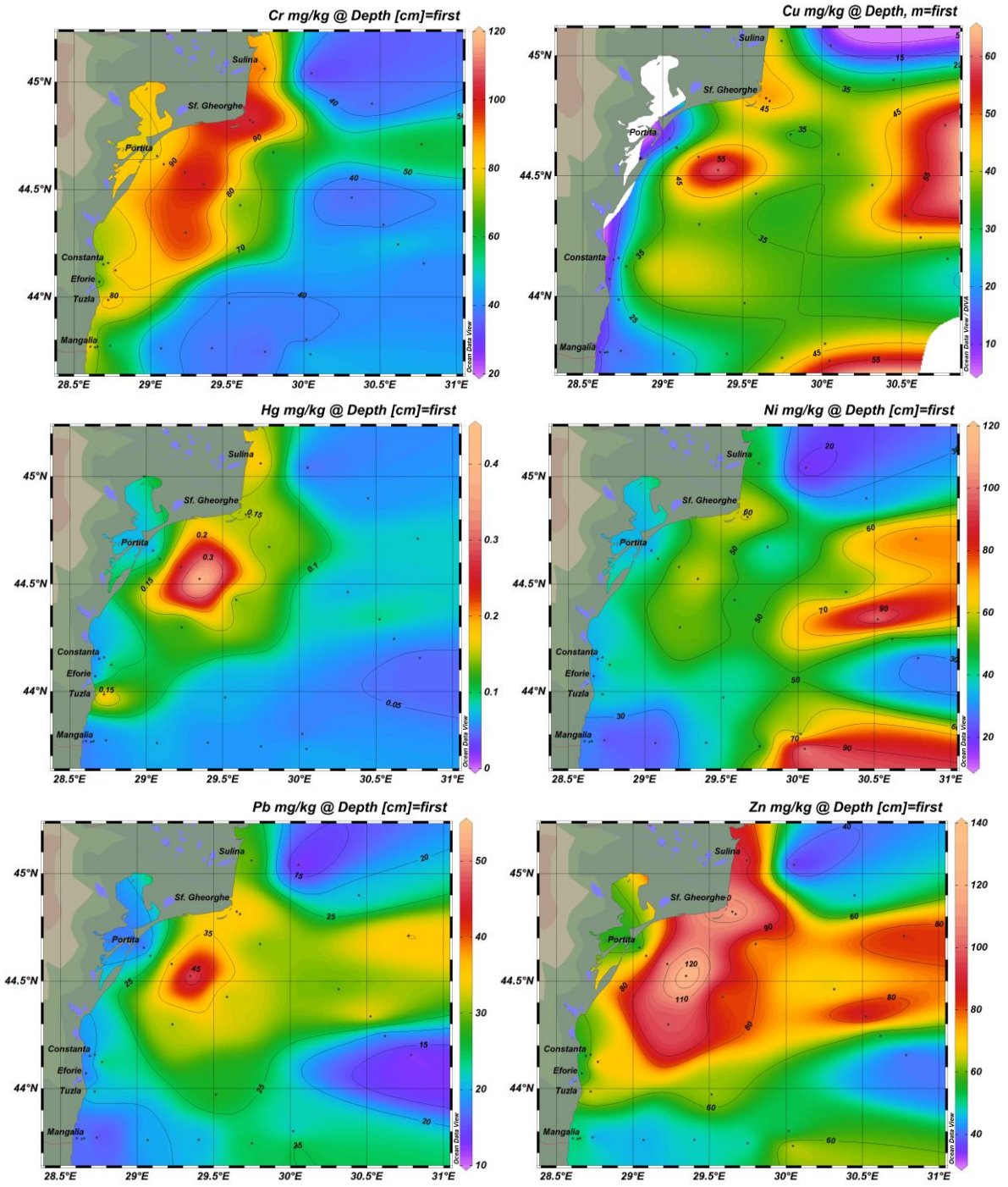


Figure 3.3. Spatial distributions of Al, As, Cr, Hg, Cu, Ni, Pb and Zn concentrations in surface sediments.

Higher concentration levels of As (10.0–57.1 mg/kg), Cu (33.7–62.9 mg/kg), Ni (48.0–118.0 mg/kg), Pb (26.1–50.8 mg/kg), and Zn (68.8–135.0 mg/kg) were determined in sediments from PO04–PO06, SG01, SG03, SG06, EUXRO01, MID02, SU01, CT03 and MA02 stations. Higher Hg concentration levels (0.08–0.45 mg/kg) were determined in sediments from PO02, PO04–PO06, TZ18, SG01, SG03, SG04, SG06, EUXRO01, MID02,

SU01, CT02, CT03 and MA07 stations. Also, peak concentrations of Cu (62.9 mg/kg), Hg (0.45 mg/kg), Pb (50.8 mg/kg) and Zn (135.0 mg/kg) were found in sediments at PO4 station. Higher concentration levels of Al (5.2–7.0%) and Cr (59.8–103.0 mg/kg) were determined in sediments collected from PO01, PO02, EUXRO01, SG01, CT02, CT03, TZ18, SU01 and PO04 stations. Surface sediments collected from stations MA05–07, SG03, PO05, PO06 and MID02 also had increased Cr concentration levels (60.8–114.0 mg/kg).

The eigenvalues corresponding to the first 3 main components (PC), *i.e.*, PC1 (6.72), PC2 (3.74) and FP3 (1.21) were > 1. The top 3 PCs explained 83.3% (48.0% + 26.7% + 8.6%) of the total variance. Only FP1 and FP2, which explained 74.7% of total variance, were still used in multivariate analysis. The results shown in Table 3.4 and Fig. 3.4 indicate that the most important variables are As, Cu, Hg, Ni, Pb, Zn, TOC, sand, silt, clay and, to some extent, water depth for PC1 and Al and Cr content, water depth, CaCO3 content, and, to some extent, Hg, Pb and Zn concentrations for PC2.

Table 3.4. Coordinates of variable projections on PC1 and PC2 axes.

No.	Variable		FP1	FP2
	Name	Symbol		
1	Al concentration	*Al*	-0.02	**-0.62**
2	As concentration	*As*	**0.70**	-0.01
3	Cr concentration	*Cr*	0.16	**-0.88**
4	Cu concentration	*Cu*	**0.95**	-0.02
5	Hg concentration	*Hg*	**0.59**	-0.57
6	Ni concentration	*Ni*	**0.79**	-0.06
7	Pb concentration	*Pb*	**0.87**	-0.39
8	Zn concentration	*Zn*	**0.78**	-0.59
9	Water depth	*h*	0.42	**0.76**
10	TOC content	*TOC*	**0.85**	0.27
11	Sand content	*Sand*	**-0.85**	-0.30
12	Silt content	*Silt*	**0.82**	0.30
13	Clay content	*Clay*	**0.81**	0.25
14	$CaCO_3$ content	$CaCO_3$	0.09	**0.93**

Significant coordinate levels on the PC1 and PC2 axes are highlighted in bold.

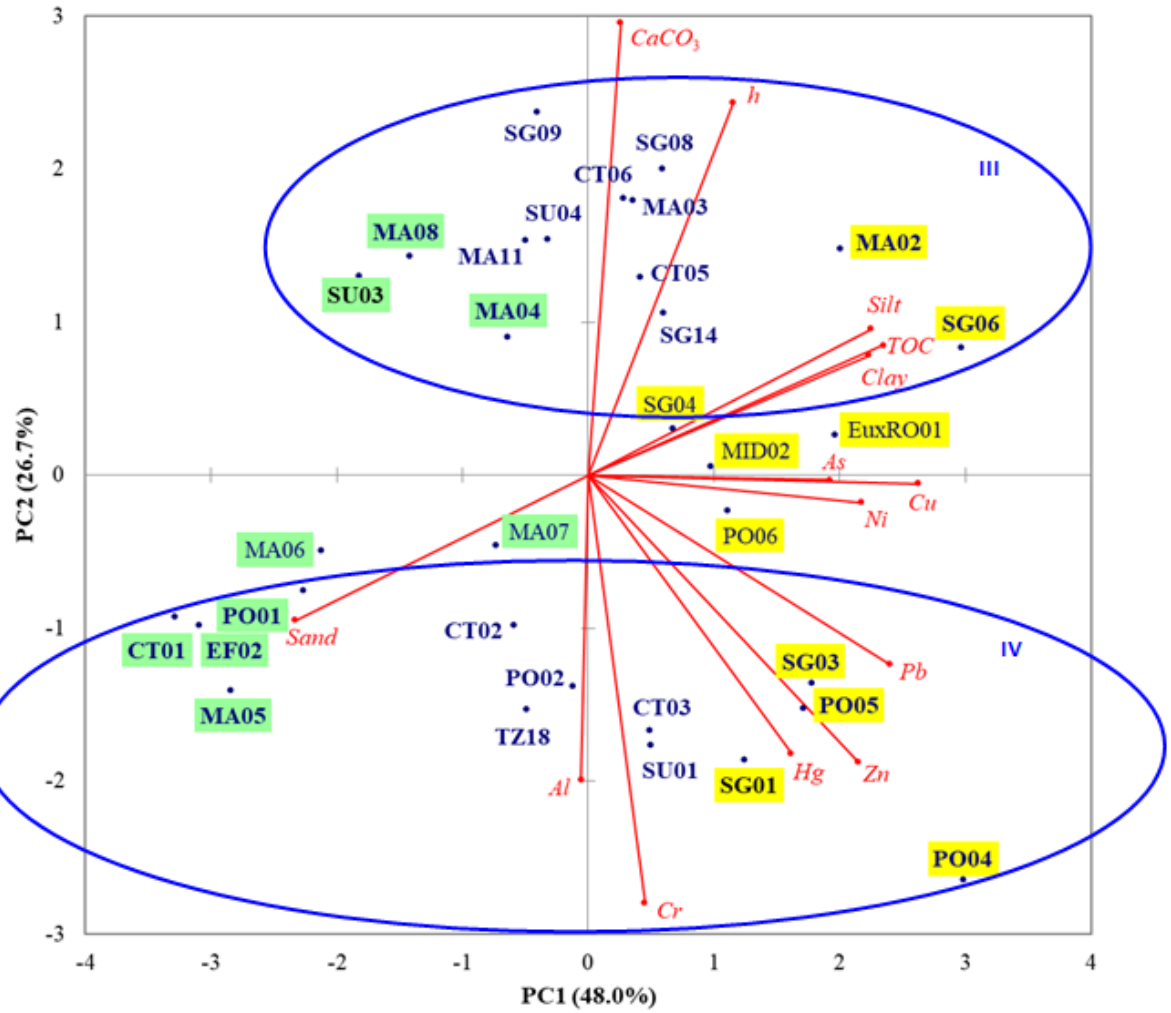


Figure 3.4. Projections of variables (*Al*, *As*, *Cr*, *Cu*, *Hg*, *Ni*, *Pb*, *Zn*, *h*, *TOC*, *Sand*, *Silt*, *Clay*, and $CaCO_3$) and samples (collected from 32 stations) on axes PC1 and PC2; group I (9 stations highlighted in green): SU03, PO01, CT01, EF02 and MA04–08; group II (10 stations highlighted in yellow): EuxRO01, SG01, SG03, SG04, SG06, PO04–06, MID02 and MA02; group III (13 stations highlighted in bold): SU03, SU04, SG06, SG08, SG09, SG14, CT05, CT06, MA02–04, MA08 and MA11; group IV (13 stations highlighted in bold): SU01, SG01, SG03, PO01, PO02, PO04, PO05, CT01–03, EF02, TZ18 and MA05.

According to the coordinates of the projections of the PC1 axis variables specified in Table 3.4, As, Cu, Hg, Ni, Pb and Zn (coordinates in the range 0.59–0.95) were associated with clay, silt and particles of organic matter (coordinates in the range 0.81–0.85). The correlation analysis also indicated that *As*, *Cu*, *Hg*, *Ni*, *Pb*, *Zn*, *Clay*, *Silt*, and *TOC* were directly correlated with each other ($0.28 \leq r \leq 0.93$), and each of these variables was inversely correlated with *Sand* ($-0.99 \leq r \leq -0.34$).

Consequently, sediment particle size and total organic carbon content had a significant influence on the distribution of As, Cu, Hg, Ni, Pb and Zn content in surface sediments. Numerous studies published in the literature have shown that clay, silt and organic matter

particles are important carriers of heavy metals/trace elements in sediments (Rubio et al., 2000). On the one hand, finer sediment fractions (clay and silt) have a higher specific surface area than the coarser fraction (sand), thus a higher adsorption capacity of organic elements and matter (Xu; et al., 2016; Zhou et al., 2022). On the other hand, the organic matter covering these fine grains can retain a wide variety of heavy metals/trace elements.

3.1.3.3. Contamination factor

The results obtained for the contamination factor indicate that:

- considerable contamination with As (*CFAs* = 4,39) and moderate contamination with Cu (*CFCu* = 1,24), Ni (*CFNi* = 1,71), Pb (*CFPb* = 1,89) and Zn (*CFZn* = 1,05) for sediments collected from station SG06;
- moderate contamination ($0 < CFE < 3$ for all heavy metals investigated and $1 \leq CFE < 3$ for at least one element) in sediments collected from 22 stations (SU01, SU04, EuxRO01, SG01, SG03, SG04, SG14, PO02, PO04-06, MID02, CT02, CT03, CT05, CT06, TZ18, MA02-05 and MA07);
- sediments collected from MID02 station, located in the Danube influence zone, showed moderate contamination with Cr, Pb and Zn, mainly due to Danube discharges, anthropogenic inputs of pollutants from Midia Port, refinery and wastewater discharges;
- sediments collected from stations SG01 and SG03 (located near the mouth of the St. George arm) and PO04 and PO05 (located near oil and gas drilling platforms) were moderately contaminated with Cr, Cu, Pb and Zn; sediments taken from stations SG03 and PO04 also showed moderate contamination with As, while sediments collected from station PO04 showed moderate Hg contamination; heavy metals found in these sediments originated mainly from Danube discharges, wastewater discharges, tourism, oil and gas extraction, rock/soil erosion;

Based on mean CFE, *i.e.*, *CFPb* = 1.23, *CFAs* = 0,86, *CFCu* = 0.73, *CFCr* = 0.72, *CFZn* = 0.72, *CFNi* = 0.67, *CFHg* = 0.26, sediments in the study area showed moderate Pb contamination and low contamination with the other investigated elements.

3.1.4. Conclusions

This study provides new information on the concentration levels of some elements (Al, As, Cr, Cu, Hg, Ni, Pb and Zn) in surface sediments collected in August–September 2019 from

32 sampling stations located in the Romanian Black Sea area, at water depths in the range of 12.7–149 m. The mean concentrations of the investigated elements were as follows: *Al* = 3.9%, *As* = 11.2 mg/kg, *Cr* = 64.8 mg/kg, *Cu* = 32.7 mg/kg, *Hg* = 0.10 mg/kg, *Ni* = 45.3 mg/kg, *Pb* = 24.6 mg/kg and *Zn* = 68.0 mg/kg.

3.2. Spatial distribution of heavy metals/trace elements and potential sources of contamination in sediments collected in August–September 2018

3.2.1. Objectives

The objectives of this study were: (i) to assess the spatial distribution of Al and some heavy metals/trace elements, *i.e.*, As, Cr, Cu, Hg, Ni, Pb and Zn, in surface sediments collected from 22 stations located at water depths of 13.5–67 m in the Romanian Black Sea area; (ii) assessment of sediment contamination status based on the following indices: enrichment factor (*EF*), contamination factor (*CF*) and the geoaccumulation index (*Igeo*); (iii) establishing the effects of the main environmental factors (water depth, sediment texture, organic matter content) on heavy metals/trace elements accumulation and identifying possible sources of contamination by applying statistical analysis. The results obtained in this study were published in *Journal of Chemistry* (Bucse et al., 2020).

3.2.2. Study area

The sediment samples from the study area were taken from 22 stations (water depths in the range of 13.5–67 m), covering the waters of Romania's continental shelf (NW of the Black Sea), during the multidisciplinary research expedition onboard the *R/V Mare Nigrum* carried out in August 2018 (fig. 2.2). The stations considered are in Sulina (SU01 and SU02), Sfântu Gheorghe (SG01, SG03–05), Portița (PO01, PO02, PO04 and PO05), Constanta (CT01–05), Eforie (EF02), Tuzla (TZ18) and Mangalia (MA04–08) areas.

3.2.3. Results and discussion

3.2.3.1. Particle size analysis and total organic carbon content in surface sediments of the investigated area

Depending on the percentages of sand, silt, and clay, the surface sediments collected were mostly clayey silt.

TOC values ranged from 0.09% to 1.78%, with a minimum level in the Constanta Port area (CT01) and a maximum in the Portița area (PO04). Low concentrations (<0.5%) were recorded at stations PO01 and MA05, and concentrations higher than 1.5% were determined at

stations SG04, SG05, PO04 and CT05, under the influence of the Danube, but also in Mangalia area, at stations MA06 and MA07.

3.2.3.2. Heavy metals concentrations in surface sediment and potential sources of contamination

Element concentration values (*Al*, *As*, *Cr*, *Cu*, *Hg*, *Ni*, *Pb* and *Zn*) in surface sediments taken from the investigated stations are shown in Fig. 3.10.

The highest values of Al concentration (60640–64930 mg/kg) were detected in the Danube mouth area (SU01, SG01 and SG03), and minimum values (19910 and 22510 mg/kg) were identified in the deepest stations in the studied area, *i.e.*, MA04 (67 m) and CT05 (64.8 m).

The highest values of Cr concentration (> 90 mg/kg) were determined in the Danube mouth area (SU01, SG01 and SG03), in Portița area (PO02, PO04 and PO05) and Constanta area (CT03); Similar to Al, the lowest Cr concentration values (26.3 and 27.7 mg/kg) were detected at the deepest stations in the study area (MA04 and CT05).

Ni, Cu, and Zn had similar concentrations, with elevated values (58.4–59.3 mg/kg, 46.7–52.5 mg/kg, and 110.5–118.5 mg/kg) in the Danube mouth area (SU01, SG01 and SG03) and Portița area (PO04). Trough concentrations of Ni and Zn (19.3 mg/kg and 22.7 mg/kg) were determined at station MA04, and lower Cu concentration levels (6.9–7.5 mg/kg) were detected at stations EF02, MA05 and MA06.

The highest As concentration levels (15.7 mg/kg and 17.2 mg/kg) were determined at SG01 and SG03 stations, while maximum Pb and Hg concentration values (42.1 mg/kg and 0.23 mg/kg) were recorded at PO04; the minimum concentration values of As and Pb (3.42 mg/kg and 11.6 mg/kg) were at the MA04 station, and the lowest Hg concentration level (0.02 mg/kg) was recorded at the EF02 station.

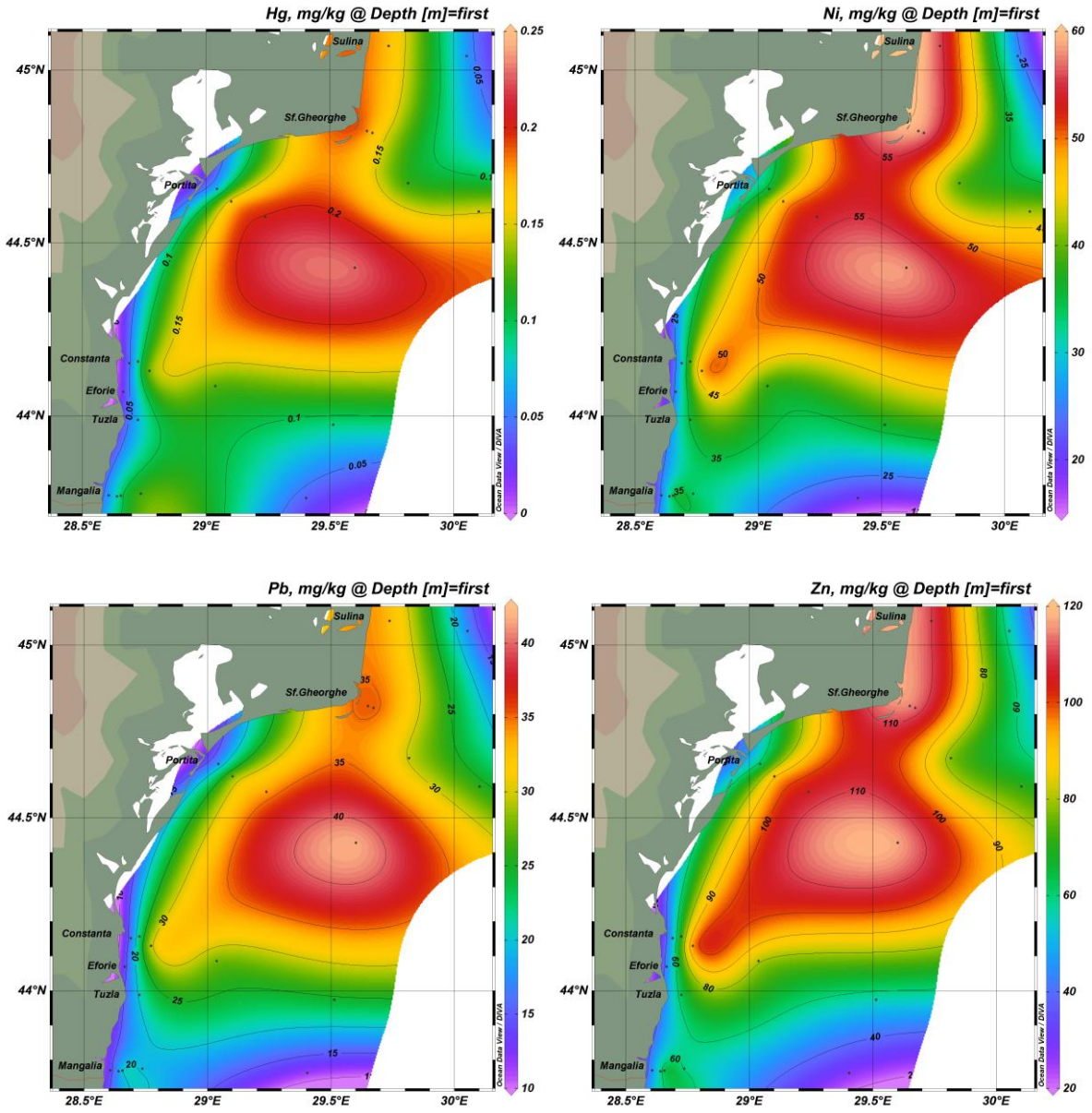


Figure 3.10. Spatial distribution of Al, As, Cr, Cu, Hg, Ni, Pb and Zn in the surface sediments of the Romanian seaside.

The eigenvalues corresponding to the first 2 main components (PC), *i.e.*, PC1 and PC2, were > 1. The first 2 PCs, which explained 84.5% (61.96% + 22.56%) of total variance, were further used in multivariate analysis. The coordinates of the projections of the variables on the PC1 and PC2 axes are specified in Table 3.12.

The correlation matrix indicates the following relevant aspects:

- strong/very strong positive correlations between *As*, *Cu*, *Hg*, *Ni*, *Pb*, *Zn*, and *Clay*, very strong negative correlations between *Clay* and *Sand* (r = -0.84) and *Silt* and *Sand* (r =

-0.85), and negative correlations strong between *Sand* and each concentration of As, Cu, Hg, Ni, Pb and Zn;

- very strong positive correlation between *Al* and *Cr* (r = 0.95), as well as strong negative correlations between water depth and *Al* (r = -0.67) and water depth and *Cr* (r = -0.70).

Table 3.12. Coordinates of variable projections on PC1 and PC2 axes.

No.	Variable		FP1	FP2
	Name	Symbol		
1	Water depth	*Depth*	-0.01	**0.95**
2	OCD concentration	*TOC*	-0.46	**0.55**
3	Al concentration	*Al*	-0.62	**-0.74**
4	As concentration	*As*	**-0.90**	-0.12
5	Cr concentration	*Cr*	-0.57	**-0.75**
6	Ni concentration	*Ni*	**-0.98**	-0.14
7	Cu concentration	*Cu*	**-0.96**	0.11
8	Zn concentration	*Zn*	**-0.98**	-0.15
9	Pb concentration	*Pb*	**-0.98**	0.04
10	Hg concentration	*Hg*	**-0.93**	0.03
11	Sand content	*Sand*	**0.83**	-0.43
12	Silt content	*Silt*	**-0.59**	0.50
13	Clay content	*Clay*	**-0.81**	0.22

Significant coordinate levels on the PC1 and PC2 axes are highlighted in bold.

Figure 3.11. Projection of variables on PC1 and PC2 axes.

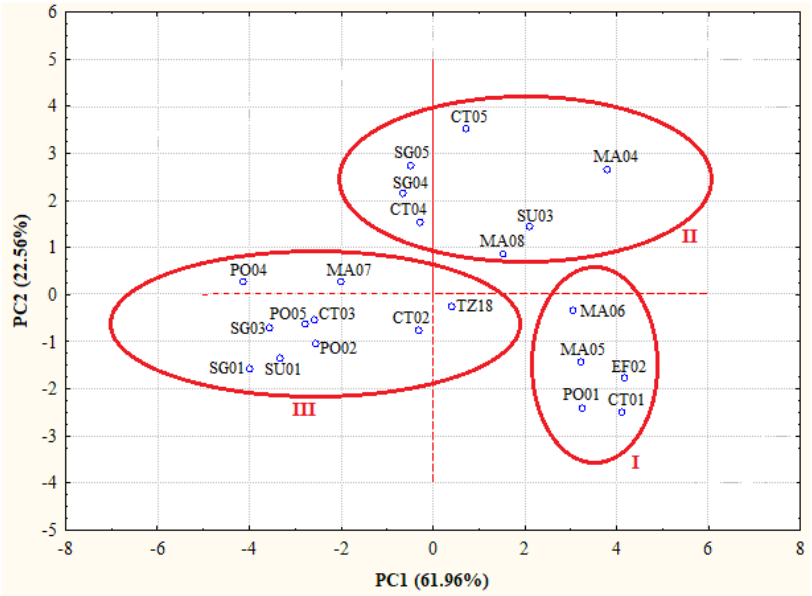


Figure 3.12. Projection of samples on PC1 and PC2 axes.

3.2.3.3. Pollution indices

Values greater than 3 of *EF* were determined only for Pb (a maximum value of 4,36) and Hg (a maximum value of 4,39). Increased values of this index for elements Pb and Hg, which were recorded at stations SG04 (3.66 and 3.61), SG05 (3.03 and 4.39), CT04 (2.97 and 3.34) and CT05 (4.36 and 4.00), are most likely an effect of Danube discharges.

Lower values of *EFPb* (1.13–1.30) and *EFHg* (0.43–0.79) were determined for sediments taken from shallow PO01, CT01, EF02 and MA06 stations, characterised by lower clay content (4.50–11.43%) and higher sand content (36.75–66.02%). Also, the lowest EF factor values for Pb (1.13) and Hg (0.43) were recorded at the EF02 station, from which samples with the lowest clay content (4.50%) and the highest sand content (66.02%) were collected.

Like the *EFPb* and *EFHg* factors, the lowest values of *EFAs* (0.56), *EFCu* (0.27), *EFNi* (0.62) and *EFZn* (0.73) factors were identified at the EF02 station. Also, lower levels of *EF* factor, *i.e.*, *EFAs* = 0.56–0.64, *EFCu* = 0.27–0.48, *EFNi* = 0.61–0.74 and *EFZn* = 0.73–0.86, were also determined for sediments taken from PO01, CT01, EF02, MA05 and MA06 stations, characterised by low clay and high sand content.

All *EFCr* values (1.10–1.86) were in the range 1–3 (minor enrichment), higher values being determined at shallow stations in the Mangalia area, *i.e.*, MA05 (1.61), MA06 (1.86) and MA08 (1.72), as well as at some stations in the Danube influence zone *i.e.*, SU03 (1.63) and SG04 (1.64).

68% of the values of *EFNi* factors (0.61–1.92) and *EFCu* (0.27–2.64) are between 1 and 3, with higher values at stations SG04 (1.41 and 1.79), SG05 (1.46 and 1.83) and CT05 (1.92 and 2.64). 73% of the values of *EFAs* (0.56–2.20) and *EFZn* (0.73–2.09) are between 1 and 3, the maximum levels being recorded in the Danube zone of influence, at stations CT05 for Zn and SG04 for As.

The spatial distribution of the contamination factor (*CF* = 0.15–2.88) reveals low (*CF* < 1) and moderate ($1 \leq CF < 3$) contamination of sediments taken from all 22 stations.

The *CF* values for Pb and Hg (*CFPb* = 0,58–2,10) and *CFHg* = 0,25–2,88) indicate moderate contamination with both elements at 13 stations (SU01, SG01, SG03–05, PO02, PO04, PO05, CT02–05 and MA07) and also moderate Pb contamination at station TZ18 and moderate Hg contamination at station MA08. The relatively high factor values at stations CT02, CT03, TZ18, MA07 and MA08 can be attributed to anthropogenic coastal pressures resulting from port activities, tourism or wastewater discharges.

CFNi values (0.28–0.87) indicate low contamination. *CFCr* = 0.29–1.12 and *CFZn* = 0.29–1.25 values were greater than 1 in the Danube mouth area (SU01, SG01 and SG03), Portița area (PO2, PO4 and PO5) and Constanta area (CT03). *CFCu* values = 0.15–1.17 and *CFAs* = 0.26–1.32 were greater than 1 in the Danube mouth area (SU01, SG01 and SG03), as well as at the eastern boundary of Portița area (PO04) for Cu and MA07 station for As.

The lowest values of contamination factors for As, Cr, Ni, Pb and Zn were identified at station MA04 (*CFAs* = 0.26, *CFCr* = 0.29, *CFNi* = 0.28, *CFPb* = 0.58 and *CFZn* = 0.29), at which the influence of the Danube is not so pronounced. CF values for Cu and Hg were minimal at stations characterized by high sand content (*CFCu* = 0.15 at EF02 and MA06 stations, and *CFHg* = 0.25 at EF02 station).

Geo-accumulation index values (*Igeo*) for As, Cr, Ni, Cu and Zn obtained in this study are below 0, indicating uncontaminated sediment.

3.2.4. Conclusions

This study provides information on concentration levels of some elements (Al, As, Cr, Cu, Hg, Ni, Pb and Zn) in surface sediments collected in August–September 2018 from 22 sampling stations located in the Romanian Black Sea area, at water depths in the range of 13.5–67 m. The mean values of the concentrations of the investigated elements were as follows: *Al* = 4.6%, *As* = 8.9 mg/kg, *Cr* = 74.8 mg/kg, = 28.3 mg/kg, *Hg* = 0.11 mg/kg, *Ni* = 39.7 mg/kg, *Pb* = 24.4 mg/kg and *Zn* = 74.1 mg/kg. These average values were not significantly different ($p > 0.05$) from those reported for surface sediments (0–2 cm deep) collected in 2019 from 32 stations located in the same area but at greater water depths (12.7–149 m).

CHAPTER IV

HEAVY METAL/TRACE ELEMENT CONTAMINATION OF BIVALVE MOLLUSCS *MYTILUS GALLOPROVINCIALIS* FROM THE ROMANIAN BLACK SEA AREA

4.1. Heavy metal concentration levels in the soft tissue of M . *galloprovincialis molluscs* in the Black Sea, Sfântu Gheorghe area

4.1.1. Objectives

The objectives of the study were to determine the concentrations of Cd, Co and Hg in the soft tissue of M. galloprovincialis bivalve molluscs (mussels) collected from 5 sampling stations in the NW of the Black Sea, in the Sf. Gheorghe area, an area heavily polluted due to the influence of the Danube, and to establish the effect of water depth and TOC content in surface sediments collected from sampling stations on heavy metal concentrations in molluscs. The results obtained in this study were published in *U.P.B. Sci. Bull.* (Bucse et al., 2021).

4.1.2. Study area

Nineteen samples of M. *galloprovincialis* bivalve molluscs were collected from 5 sampling stations, located at water depths between 43.2–54.2 m, at *approx.* 30 km away from the mouth of the Sfântu Gheorghe arm, during a research expedition onboard the *R/V Mare Nigrum* (fig. 2.2).

4.1.3. Results and discussion

The heavy metal concentrations in mollusc soft tissue, TOC content in surface sediments and water depth in each station (S1–S5) are shown graphically in figure 4.2.

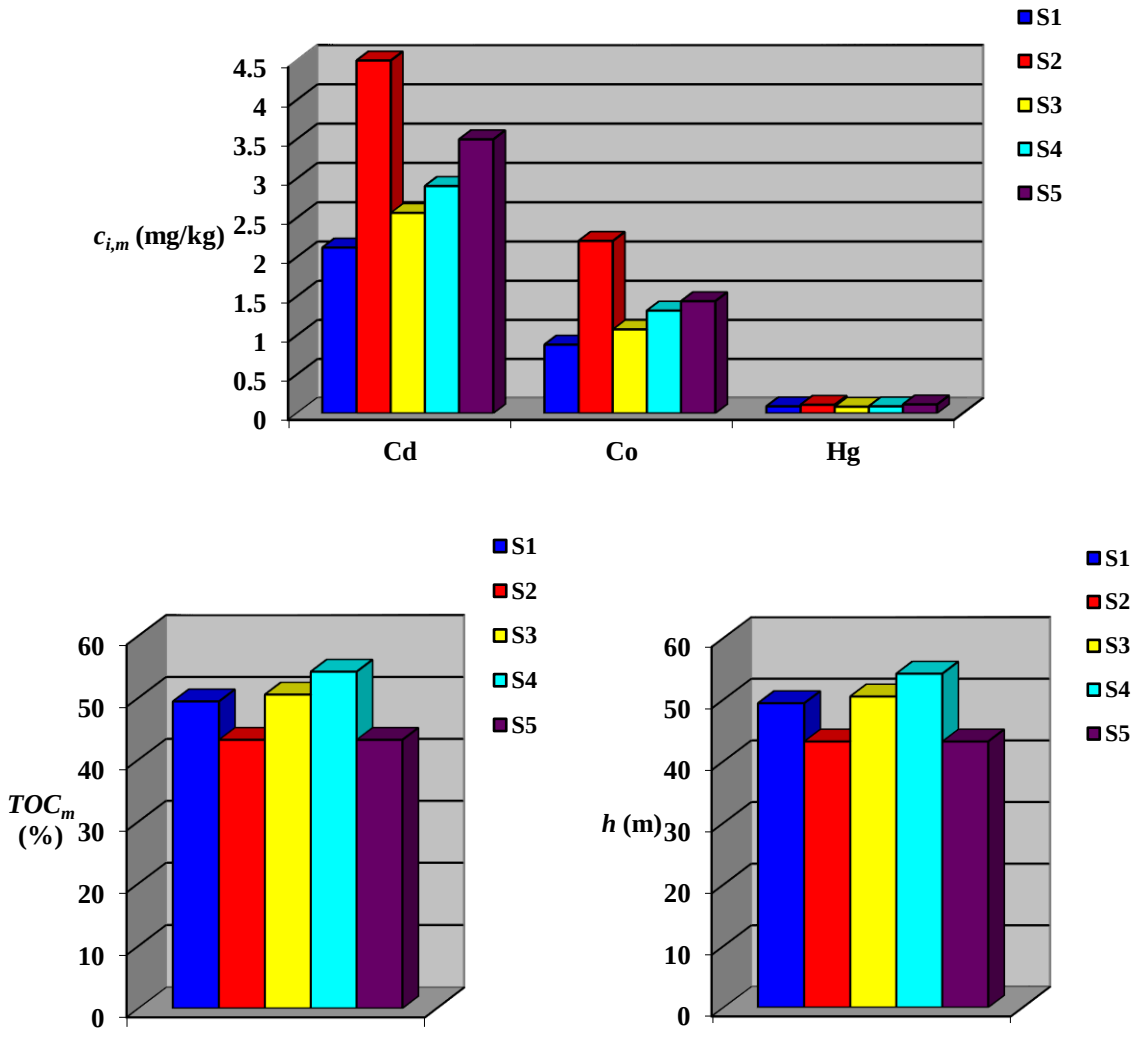


Figure 4.2. Mean values of heavy metals concentration in mollusc soft tissue of molluscs *M. galloprovincialis* (*ci*, $_i$ = Cd, Co, Hg) and TOC content (*TOC*) in sediments and water depth values (*h*) in the stations studied.

The highest values of concentrations of Cd (4.70 mg/kg and 5.37 mg/kg) and Co (1.91 mg/kg and 3.00 mg/kg) were detected in mussels collected from shallow stations, *i.e.*, S5 and S2 (43.2 m), under the influence of the Danube, and the lowest values (1.69 mg/kg and 1.91 mg/kg for Cd concentration, respectively, 0.72 mg/kg and 0.75 mg/kg for Co) concentration were identified in mussels collected from stations S3 (49.4 m) and S1 (50.5 m). From the data shown in figure 4.2 it can be seen that the mean values of Cd concentration (2.12–4.55 mg/kg) are 2.1–2.4 times higher than those of Co concentration (0.88–2.21 mg/kg). All Cd concentration values (1.69–5.37 mg/kg) exceeded the maximum level set by European Commission Regulation (EC) No. 1881/2006, *i.e.*, 1 mg/kg.

The mean Hg concentration values in mussels collected from the 5 sampling stations (0.09–0.11 mg/kg) are not significantly different from each other ($p > 0.5$) and are 10–20 times lower than the Co concentration and 24–41 times lower than the Cd concentration.

Higher values of metal concentrations in mussel soft tissue can be caused by intense eutrophication and phytoplankton blooms, which usually occur as a result of high flows of the Danube (Bondar and Panin, 2001). These phenomena can increase the metabolic speeds of mussels and, in particular, their filtering capacity. The eigenvalues and principal component variance (PC) values, which are specified in Table 4.2, highlight two PCs with eigenvalues greater than 1. Only these two PCs, which explain 80.60% (57.99% + 22.61%) of total variance, will still be used in multivariate analysis.

Table 4.2. Eigenvalues and principal component variance (PC) values

Main component	Own values		Variance (%)	
	PC	Cumulative	PC	Cumulative
FP1	2.90	2.90	58.99	57.99
FP2	1.13	4.03	22.61	80.60
FP3	0.63	4.66	12.53	93.13
FP4	0.19	4.85	3.84	96.96
FP5	0.15	5.00	3.04	100.00

The coordinates of the projections of variables on the axes PC1 and PC2, which are specified in Table 4.3, indicate that PC1 is dominated by heavy metals concentrations in mollusc samples and water depth, and PC2 is dominated by TOC content in sediment samples.

Table 4.3. Coordinates of variable projections on PC1 and PC2 axes

No.	Independent variable	PC1	PC2
1	*Cd*	**0.81**	–0.48
2	*Co*	**0.77**	–0.56
3	*Hg*	**0.73**	0.18
4	*TOC*	–0.59	**–0.66**
5	*h*	**–0.87**	–0.34

Significant coordinate levels on the PC1 and PC2 axes are highlighted in bold.

The coordinates of the projections of variables and samples on the axes PC1 and PC2 (fig. 4.3) and the correlation coefficients (r) highlight the following:

- discrimination on the PC1 axis between mollusc samples collected from shallow stations S2 and S5 (43.2 m), characterised by higher concentrations of Cd (4.55 ± 0.70 mg/kg and 3.50 ± 1.06 mg/kg), Co (2.21 ± 0.64 mg/kg and 1.44 ± 0.30 mg/kg) and Hg (0.11 ± 0.01 mg/kg) and shown in yellow in Fig. 4.3, and mollusc samples collected

from deeper stations S1 (49.4 m) and S4 (54.2 m), characterised by lower concentrations of Cd (2.12 ± 0.19 mg/kg and 2.90 ± 0.45 mg/kg), Co (0.88 ± 0.13 mg/kg and 1.32 ± 0.20 mg/kg) and Hg (0.09 ± 0.01 mg/kg and 0.09 ± 0.02 mg/kg) and shown in green in Fig. 4.3;

- discrimination on the PC2 axis between samples collected from station S3, with the lowest *TOC* in surface sediment samples (0.74%), and those collected from station S4, with the highest *TOC* (2.14%); samples collected from stations S3 and S4 are highlighted in bold in Fig. 4.3;
- a very strong positive linear correlation between *Cd* and *Co* (r = 0,84);
- a strong positive linear correlation between *TOC* and *h* (r = 0.67);
- a strong negative linear correlation between *Hg* and *h* (r = -0.65) and moderate negative linear correlations between *Cd* and *h* (r = -0.53) and *Co* and *h* (r = -0.47).

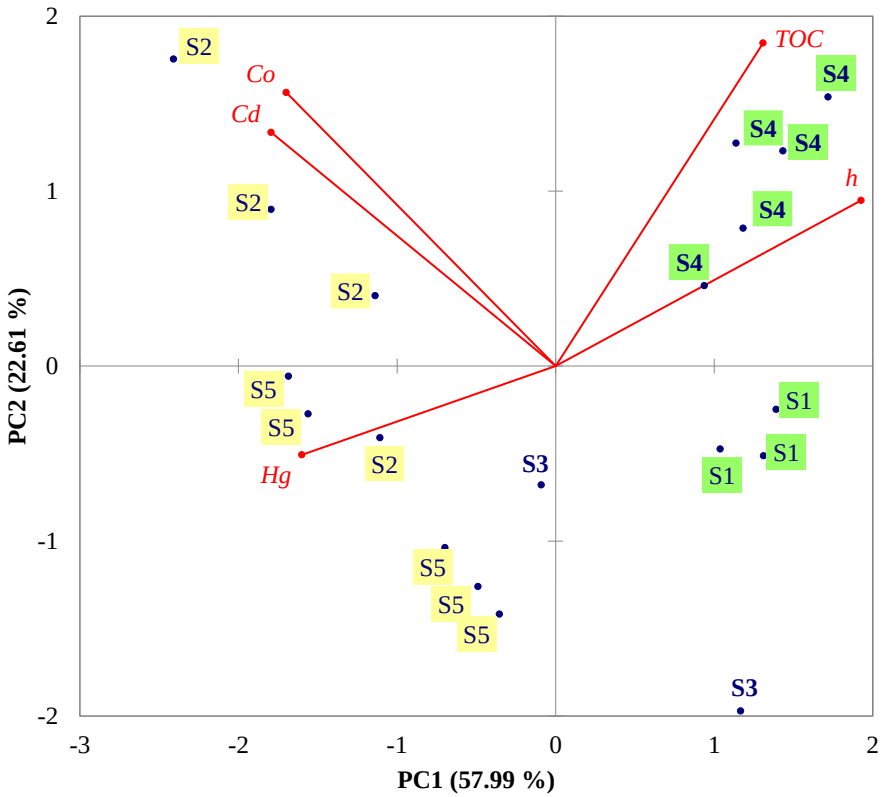


Figure 4.3. Coordinates of the projections of variables (*Cd*, *Co*, *Hg*, *TOC* and *h*) and of the 19 samples (collected from stations S1–S5) on the PC1 and PC2 axes.

4.1.4. Conclusions

This study provides information on the concentration levels of heavy metals, *i.e.*, Cd, Co and Hg, in the soft tissue of *M. galloprovincialis* bivalve molluscs in the NW of the Black Sea, in the Sf. Gheorghe area, an area heavily polluted as a result of river discharges. The

investigated heavy metals were detected in all samples collected from 5 sampling stations, located at water depths in the range of 43.2–54.2 m.

4.2. Concentration levels of heavy metals/trace elements in the soft tissue of *M. galloprovincialis molluscs* in the Black Sea, Port of Agigea area

4.2.1. Objectives

The main objective of the study was to determine the concentrations of As, Br, Cu, Hg, Se and Zn in the soft tissue of *M. galloprovincialis* bivalve molluscs collected from 2 sampling stations located near the Port of Agigea, an important industrial hub in the Romanian Black Sea area. The results obtained in this study were published in the journal *Frontiers in Marine Science* (Bucse et al., 2022).

4.2.2. Study area

The mussel samples were taken from two points (*i.e.*, stations A and B), located in front of the Danube-Black Sea Channel, along the two artificial breakwaters in the outer berths of Agigea Port (fig. 2.2). Stations A and B are 2.7 km and 2.3 km away, respectively, from the exit of the Danube–Black Sea Channel. The average water depth in the study area was about 8 m.

4.2.3. Mussel classification, analysis and parameters

Depending on length (L), mussels have been classified into the following groups (classes): A (L = 3–5.9 cm), B (L = 6–8.9 cm) and C (L = 9–12 cm). The meat yield (MY) is with equation (4.1), where M_{wf} represents the mass of soft tissue, and M_w It is the mass of whole mussels (soft tissue and shell).

$$MY = 100\frac{m_{wf}}{m_w} \tag{4.1}$$

4.2.4. Results

4.2.4.1. Physico-chemical characteristics of sea water

The water temperature (t = 4−24 °C) showed normal seasonal variation, with low values in February (4−6 °C) and high values in June (20−24 °C).

Salinity (S = 10.4−19 PSU) showed significant differences, with fluctuations mainly caused by higher flows of the Danube.

The pH values (8.20−8.40) were lower in June (8.20−8.26) and higher in February (≈8.38) and April (8.30−8.38).

Chlorophyll a *concentrations* (c_c = 0.1−6 mg/m3) were lower in June (0.1−0.6 mg/m^3) and higher in February, early March and mid-April (2.5−6 mg/m^3), as a result of phytoplankton blooming in the Black Sea (Vasiliu et al., 2012).

Dissolved O_2 concentrations (*cDO* = 289−357 mmol/m^3) varied significantly. Higher values (330−357 mmol/m^3) were recorded during periods of lower seawater temperature and higher solubility of O_2, *i.e.*, February and early March, as well as mid-April caused by phytoplankton blooms.

4.2.4.2. Mass of soft tissue and mass index (MY) of molluscs analysed

The changes in wet soft tissue mass (m_{wf} = 5.1−23.4 g) and mass index (*MY* = 34.1−69.5%) during the study period (February−June 2019) highlight the following aspects:

- for Class A individuals, m_{wf} (5.1−9.1 g) decreased slightly from February to May and *the* m_{wf} value in June was 1.8 times higher than in May;
- *MY* (34.6−69.5%) increased slightly from February to April, *MY* values in April and May were almost equal (≈41%), and *MY* value in June was 1.7 times higher than that determined in May;
- for class B individuals, m_{wf} (8.6−14.8 g) showed an increase in March (1.4 times), a larger decrease in April (1.7 times), an increase in May (1.4 times), and a smaller decrease in June (1.2 times); *MY* (34.1−40.2%) increased slightly by April, *MY* values in April and May were almost equal (≈37%), and *MY* value in June was 1.1 times lower than in May;
- for class C individuals, m_{wf} (15.8−23.4 g) increased from February to April; *MY* (35.1−41.7%) increased through April, with a significant increase in March.

4.2.4.3. Content of heavy metals/trace elements in soft tissue of mussels

According to the obtained results, the order of accumulation of elements in the soft tissue of mussels was: Zn>Br>As>Cu>Se>Hg.

4.2.4.4. Results of statistical analysis

The PCA revealed 3 eigenvalues > 1, *i.e.*, those corresponding to the main components (PC) PC1 (5.06), PC2 (2.86) and FP3 (1.77). The top three PCs explain 80.7% (42.2% + 23.8% + 14.7%) of the total variance. Only PC1 and PC2, which explain 66% (42.2% + 23.8%) of total variance, were still used in PCA.

Table 4.7. Coordinates of variable projections on PC1 and PC2 axes.

j	Variable		Coordinates	
	Name	**Symbol**	$l_{j,PC1}$	$l_{j,PC2}$
1	Zn concentration	c_{Zn}	0.149	**0.708**
2	Br concentration	c_{Br}	**0.476**	**0.523**
3	Ace Centration	c_{As}	**0.481**	0.309
4	Cu concentration	c_{Cu}	-0.139	**0.940**
5	Se concentration	c_{Se}	-0.354	**0.810**
6	Hg concentration	c_{Hg}	0.212	-0.267
7	Temperature	t	**-0.934**	0.109
8	Salinity	S	**0.582**	**-0.533**
9	Ph	pH	**0.981**	-0.004
10	Chlorophyll concentration *of*	c_c	**0.943**	0.253
11	Dissolved oxygen concentration	c_{DO}	**0.935**	0.110
12	Meat yield	MY	**-0.675**	-0.068

4.2.5. Discussion

4.2.5.1. Physico-chemical parameters of sea water

The correlation coefficients (r) between the monthly average values of water temperature (t), salinity (S), pH, chlorophyll concentration a (cc) and dissolved O_2 concentration (cDO) indicate the following:

- strong negative correlation between t and S (r = -0.73);
- very strong negative correlation between t and pH (r = -0.96); increase in water pH with temperature decrease has been reported in the literature (Dickson, 1993);
- very strong positive correlation between cc and pH (*r=0.93); in the process of photosynthesis,* CO_2 in water is taken up by algae, the process of decomposition of HCO^{-3} into CO_2 and HO^{-} is favored, resulting in an increase in *water pH* (Zang et al., 2011); also, cc and t are strongly negatively correlated (r = -0.84);
- very strong positive correlations between cc and cDO (*r=0.95), cDO,* and pH (*r=0.92),* as reported in the literature (Zang et al., 2011), as well as strong negative correlations between cDO and t (*r=-0.78).*

4.2.5.2. Phases of mussel gametogenesis

The following phases of mussel gametogenesis *M. galloprovincialis* have been reported (Suarez et al., 2005):

- 0 – sexual rest and accumulation of reserve substances (glycogen, lipids, proteins) in adipogranular cells (ADG) and vesicular connective tissue cells (VCTs);
- I – the beginning of gametogenesis (multiplication of gonia, development of follicles);
- II – development of gametes (immature eggs and sperm, more apparent follicles);

- IIIa – maturation of gametes (gonadal follicles filled with mature gametes);
- IIIb – spawning (release of gametes);
- IIIc – gamete restoration;
- IIId – follicle cleansing process (gamete degradation).

4.2.5.3. Content of heavy metals/trace elements in mussel soft weaving

Zn and Cu concentrations in February (171−226.5 mg/kg and 11.89−14.94 mg/kg, respectively) and June (199−203 mg/kg and 12.85−15.46 mg/kg, respectively) were among the highest of all months, and class A and B specimens had the highest concentrations (Fig. 4.7). The data specified in Table 4.6 indicate a positive correlation (r = 0,56) between *cZn* and *cCu*, suggesting the possibility of similar sources of contamination (*e.g.*, port activities, waste water discharges) (Roméo et al., 2005).

The highest Se concentration levels were recorded in February (≈3.7 mg/kg) and June (3.2 mg/kg/kg and 5.9 mg/kg, respectively) for class A and B specimens (Fig. 4.7). Fowler and Benayoun (1976) found that absorption of Se can be reduced in the presence of Hg. A negative correlation (r = -0.45) has been observed between concentrations of Se and Hg, which is insignificant at a significance level α = 0.05.

As, Br and Hg are considered non-essential elements for mussel metabolism, and Br and Hg are harmful even at very low concentrations.

Br present in the environment usually has industrial origins, with the main anthropogenic sources being brominated flame retardant compounds (BFRs). According to the data shown in Fig. 4.7, higher Br concentration levels were determined in February (220−309 mg/kg) and June (152−156 mg/kg), and lower levels were measured in April (124−129 mg/kg). The positive correlation between *cBr* and *cc* (r = 0.65) (Table 4.6) indicates that phytoplankton was an important food source for mussels.

The Hg concentration peaked in April (0.07 mg/kg) for mussels in classes A and B. The graphs in figure 4.7 indicate that mussels in classes A and B show higher concentrations of As in February and March than those in class C. Also, larger mussels in class C recorded the highest concentration of As (19.26 mg/kg) in April, corresponding to the highest levels of *mwf* and *MY* parameters. Positive correlations between *cAs* and *cc* (r = 0.49), even though statistically insignificant at α = 0.05, and between *cAs* and *cOD* (r = 0.59) (Table 4.6) suggest that phytoplankton blooms contributed to the absorption of As by mussels.

4.2.5.3.1. PCA

Depending on the significant values of their coordinates (highlighted in bold in Table 4.7), the most important variables are:

- *pH* (l_8,*PC1* = 0.981), *cc* (l_9,*PC1* = 0.943), *cDO* (l_{10},*PC1* = 0.935), *t* (l_7,*PC1=-0.934)*, *MY* (l_{12},*PC1=-0.675)*, *S* (l_{11},*PC1* = 0.582), *cAs* (l_3,*PC1* = 0.481) and *cBr* (l_2,*PC1* = 0.476) for PC1;
- *cCu* (l_4,*PC2*=0.940), *cSe* (l_5,*PC2*=0.810), *cZn* (l_1,*PC2*=0.708), *S* (l_{11},*PC2*=-0.533*)*, and *cBr* (l_2,*PC2*=0.523) for PC2.

The coordinates of the sample projections on the PC1 and PC2 axes (Table 4.8 and Fig. 4.8) indicate discrimination between groups of mussels (MS) collected in February and June in the direction of FP1.

PC1 is positively associated with *cAs, cBr, cc*, *cDO*, *pH* and *S* and negative associated with *t* and *MY* indicating that phytoplankton was an important source of As and Br contamination for smaller mussels collected in February (group MS1) compared to those collected in June (group MS5).

PC2 is positively associated with Cu, Se, Zn and Br concentrations and negatively associated with *S*, suggesting that Cu, Se, Zn and Br originate from similar anthropogenic sources and their higher concentrations reported in February and June were driven by higher freshwater flows of the Danube during these two months.

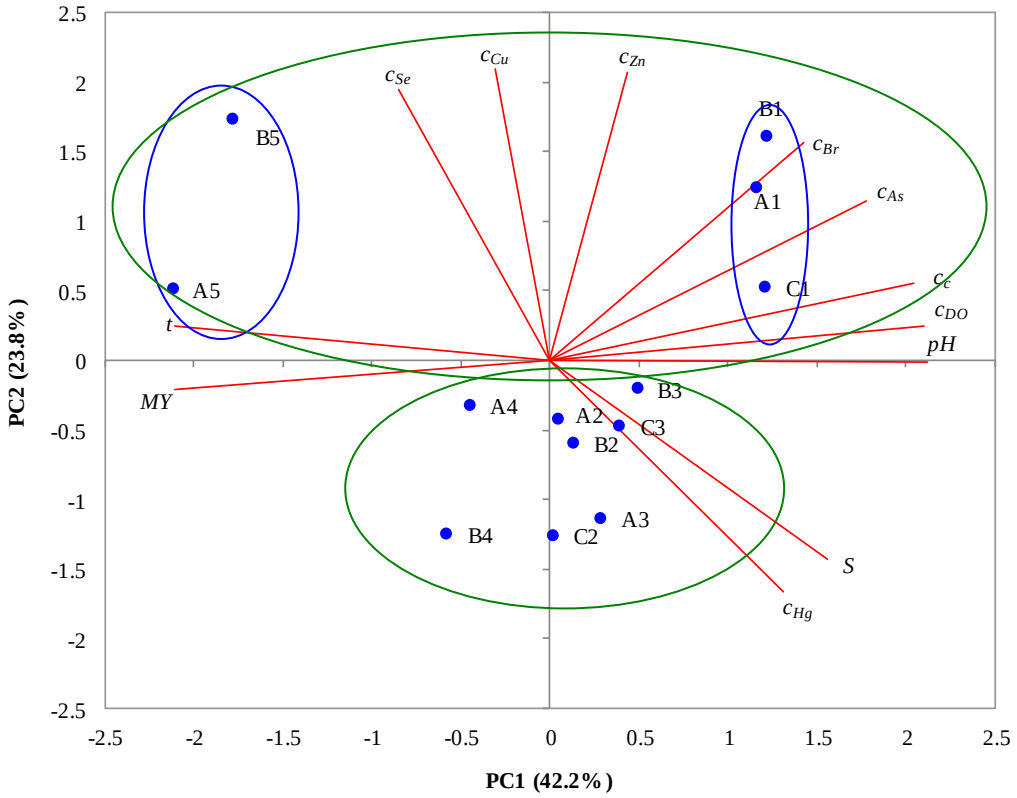


Figure 4.8. Coordinates of the projections of variables and samples on the PC1 and PC2 axes.

4.2.5.3.2. Comparison with other studies and maximum permitted concentration levels of analysed

The *cCu* values determined in this study were up to 1.5 times higher and those of *cHg* 1.5 times lower than the values reported by Roméo et al. (2005) in the same area, from 2001 to 2002. Also, the levels of *cAs*, *cCu* and *cZn* reported in this study were similar to those determined for mussels taken from Turkey (Black Sea, Sea of Marmara and Aegean Sea) (Belivermis et al., 2016), Montenegro (Adriatic) (Stanković et al., 2011), Croatia (Gulf of Mali Ston, Adriatic Sea) (Kljaković-Gašpić et al., 2007), Slovenia (Gulf of Trieste, Adriatic Sea) (Kristan et al., 2014), Spain (Atlantic Ocean and Mediterranean Sea) (Deudero et al., 2009) and Morocco (Atlantic Ocean) (Maanan, 2007).

4.2.6. Conclusions

The current study provides new information about the concentration of elements As, Br, Cu, Hg, Se and Zn in the soft tissue of *M. galloprovincialis* mussels collected from an artificial rocky substrate in the Port of Agigea area. The order of accumulation was as follows: Zn > Br > As > with > > Hg. Increased concentrations of elements were generally determined for mussels of classes A and B compared to larger mussels of class C.

CHAPTER V
GENERAL CONCLUSIONS AND RESEARCH PERSPECTIVES

The assessment of heavy metals/trace element contamination of marine and coastal ecosystems has become a serious concern due to their risks and effects on the entire aquatic food chain. Surface sediments and the bivalve mollusc species *M. galloprovincialis* are relevant indicators of marine quality.

Average values of concentrations of some elements (Al, As, Cr, Cu, Hg, Ni, Pb and Zn) in surface sediments (0–2 cm deep) collected in August–September 2019 from 32 sampling stations located in the Romanian Black Sea area, at water depths in the range of 12.7–149 m, *i.e.*, *Al* = 3.9%, *As* = 11.2 mg/kg, *Cr* = 64.8 mg/kg, = 32.7 mg/kg, *Hg* = 0.10 mg/kg, *Ni* = 45.3 mg/kg, *Pb* = 24.6 mg/kg and *Zn* = 68.0 mg/kg, were similar ($p > 0.05$) to those reported for surface sediments collected in August 2018 from 22 stations located in the same area, but at shallower water depths (13.5–67 m), *i.e.*, *Al* = 4.6%, *As* = 8.9 mg/kg, *Cr* = 74.8 mg/kg, = 28.3 mg/kg, *Hg* = 0.11 mg/kg, *Ni* = 39.7 mg/kg, *Pb* = 24.4 mg/kg and *Zn* = 74.1 mg/kg.

PCA results and correlation analysis revealed that As, Cu, Ni, Hg, Pb and Zn were associated with finer carriers, *I.E.*, clay, silt and organic matter, suggesting that these elements come mainly from Danube discharges contaminated with various effluents (municipal, agricultural, industrial), but also from local anthropogenic sources. The results of statistical analysis also indicated that Al and Cr originate from rock/soil erosion. On the one hand, concentrations of As, Cu, Hg, Ni, Pb, Zn, TOC, silt, and clay were higher in sediments collected from stations generally located at greater water depths, suggesting that elements associated with finer carriers were carried offshore by currents and waves.

The values of the *EF*, *CF* and *Igo* indices revealed a higher pollution of sediments by Pb and Hg in the Danube influence zone, in the areas of offshore oil and gas extraction platforms (eastern edge of Portița area) and partly in Constanta and Mangalia areas, indicating the influence of port, industrial and construction activities, wastewater discharges and tourism.

The values of concentrations of heavy metals (Cd, Co and Hg) in the soft tissue of *M. galloprovincialis* bivalve molluscs (relative to the dry mass of shellless mussels) collected from 5 sampling stations (S1–S5) in the area of Sf. Gheorghe, an area heavily polluted as a result of river discharges, varied within the following limits: *cCd* = 1.69–5.37 mg/kg, *cCo* = 0.72–3.00

mg/kg and *cHg* = 0.06–0.12 mg/kg. The concentration values of Cd exceeded the limit set by the European Commission Regulation (no. 1881/2006), *i.e.*, 1 mg/kg.

The mean values of concentrations of heavy metals/trace elements (As, Br, Cu, Hg, Se and Zn) in the soft tissue of *M. galloprovincialis* mussels (relative to soft tissue dry mass), collected from an artificial rocky substrate in the Port of Agigea area, were as follows: *cAs,m* = 12.5 mg/kg, *cBr* = 173.6 mg/kg, *cCu* = 11.2 mg/kg, *cHg* = 0.02 mg/kg, *cSe* = 2.86 mg/kg and *cZn* = 179.5 mg/kg. Higher concentrations of elements were generally determined for mussels of classes A (3−5.9 cm) and B (6−8.9 cm) compared to larger mussels of class C (9−12 cm).

The PCA results indicated the following relevant aspects:

- PC1 was positively associated with *cAs*, *cBr*, *cc*, *cDO*, *pH*, and *S*, as well as negatively associated with *t* and *MY*, suggesting that phytoplankton was an important source of As and Br for smaller mussels collected in February (when temperature levels were lower and those of pH and water salinity higher) compared to more mussels large samples taken in June;
- PC2 was positively associated with *cCu*, *cSe*, *cZn* and *cBr*, as well as negatively associated with *S*, indicating that Cu, Se, Zn and Br originated from similar sources.

The information provided in the doctoral thesis may be useful in complementing existing data on heavy metals/trace element pollution of sediments and mussels in the Black Sea and for developing/improving pollution control strategies. Also, the research undertaken so far can be continued/deepened in future studies. The main directions/themes/objectives that I aim at below refer to:

- assessment of contamination/pollution of the aquatic ecosystem (sediment, water and different species of the food chain) by heavy metals/trace elements/radionuclides and organic compounds;
- Improving programs
- use of new quality indices/indicators to assess contamination/pollution of the aquatic ecosystem;
- establishing natural levels ('background values') of element concentrations in Black Sea sediments.

PERSONAL CONTRIBUTIONS

Scientific articles in ISI or BDI rated / indexed journals:

1. **Andra Bucşe**, Oana Cristina Pârvulescu, Dan Vasiliu, Florina Rădulescu, Naliana Lupascu, Bogdan Adrian Ispas (2023) Spatial distribution of trace elements and potential contamination sources for surface sediments of the North-Western Black Sea, Romania. *Frontiers in Marine Science*, 10, 1310164, 1−17, WOS:001150642500001, **FI (2022): 3.7** (Q1). https://doi.org/10.3389/fmars.2023.1310164

2. **Andra Bucşe**, Oana Cristina Pârvulescu, Dan Vasiliu, Mihaela Mureșan (2022) The contents of some trace elements (As, Br, Cu, Hg, Se, and Zn) in *Mytilus galloprovincialis* mussels from Agigea Port, Romania. *Frontiers in Marine Science*, 9, 899555, 1−13, WOS:000811647100001, **FI (2021): 5,247** (Q1). https://doi.org/10.3389/fmars.2022.899555

3. **Andra Bucşe**, Oana Cristina Pârvulescu, Dan Vasiliu, Naliana Lupascu, Cezara Voica (2021) Levels of heavy metal concentration in *M. galloprovincialis* mollusc species from NW Black Sea (Romania). *U.P.B. Sci. Bull.* Series B, 83(3), 51–60, WOS:000692192600005.

4. **Andra Bucşe,** Dan Vasiliu, Sorin Bălan, Oana Cristina Pârvulescu, Tănase Dobre (2020) Heavy metal spatial distribution and pollution assessment in the surface sediments of the North–Western Black Sea shelf. *Journal of Chemistry*, 71(4), 155–170, **FI (2019): 1,775**. https://doi.org/10.37358/RC.20.4.8054

5. Dan Vasiliu, Laura Tiron Dutu, **Andra Bucşe**, Naliana Lupascu, Florin Duțu (2021) Geochemical characteristics of riverbed sediments in the Danube Delta, Romania. *Scientific Papers Series E–Land Reclamation Earth Observation & Surveying Environmental Engineering*, 10, 258–264, WOS:000704605600035.

6. Adrian Teacă, Mihaela Mureşan, Selma Menabit, **Andra Bucşe**, Tatiana Begun (2020) Assessment of Romanian circalittoral soft bottom benthic habitats under Danube River influence. *Regional Studies in Marine Science*, 40, 1–15, WOS:000603374600009, **FI (2019): 1,462**. https://doi.org/10.1016/j.rsma.2020.101523

7. Dan Vasiliu, **Andra Bucşe**, Naliana Lupascu, Bogdan Ispas, Cătălin Gheablău, Ion Stănescu (2020) Assessment of the metal pollution in surface sediments of coastal Tasaul Lake (Romania). *Environmental Monitoring and Assessment*, 192(12), 749, 1–15, WOS:000590048400002, **FI (2019): 1,903**. https://doi.org/10.1007/s10661-020-08698-0

FIC: 14,1

Oral presentations at international conferences/symposia:

1. **Andra Bucşe,** Dan Vasiliu, Oana Cristina Pârvulescu, Daniela Florea, Naliana Lupascu, Ana Bianca Pavel, *Heavy metal pollution in surface sediments of the Tăbăcărie Lake (Romania),* International Symposium of Chemistry and Chemical Engineering (SICHEM), 17–18 November 2022, Bucharest, Romania.
2. **Andra Bucşe**, Dan Vasiliu, Naliana Lupascu, Sorin Balan, Daniela Florea, Teodor Muşat, Florina Rădulescu, *Heavy metals contamination in the surface sediments of the NW Black Sea Shelf, Romania,* Modern approaches of the environment-climate change feedback, 20–23 September 2023, Galați, Romania.
3. **Andra Bucşe,** Dan Vasiliu, Naliana Lupascu, Sorin Balan, Daniela Florea, Teodor Muşat, Florina Rădulescu, Bogdan Ispas, *Trace metals assessment in the surface sediments of the NW Black Sea shelf*; International Symposium "Ancient and Present River-Delta-Sea Systems Modifications Under Impacts of Human Interventions and Climate Change", October 17–18, 2023, Bucharest, Romania.

Oral presentations in summer schools:

1. **Andra Bucşe,** Dan Vasiliu, Sorin Balan, Oana Cristina Pârvulescu, Tănase Dobre, *The contents of some trace elements (As, Br, Cu, Hg, Se, and Zn) in Mytilus galloprovincialis mussels from Agigea Port, Romania,* Ambiacva project (contract 23pfe/2021 funded by the Ministry of Research, Innovation and Digitalization) - Highlighting environmental and biotic changes in ancient and current aquatic systems, 2023, May 14-17, Romania
2. **Andra Bucşe,** Dan Vasiliu, Sorin Balan, Oana Cristina Pârvulescu, Tănase Dobre, *Heavy metals spatial distribution and pollution assessment in the surface sediments of the North-Western Black Sea shelf,* Proiect Biocontam, Réseaux trophiques marins et mécanismes BIOlogiques de transfert des CONTAMinants, Marseille (Station Marine d'Endoume & Océanomed Luminy), 2023, June 5-9, France.
3. **Andra Bucşe,** Dan Vasiliu, Sorin Balan, Oana Cristina Pârvulescu, Tănase Dobre, *Heavy metals spatial distribution and pollution assessment in the surface sediments of the North-Western Black Sea shelf,* Fluvimar Project – Research of Excellence (contract 8pfe/2018 funded by the Ministry of Education and Research), 2020, 12-15 July, Romania.

Poster presentations at international conferences:

1. **Andra Bucşe**, Dan Vasiliu, Oana Cristina Pârvulescu, Naliana Lupascu, Cezara Voica, *Heavy metal concentration in M. galloprovincialis from NW Black Sea shelf (Romania),* International Symposium of Chemistry and Chemical Engineering (SICHEM), 17–18 September 2020, Bucharest, Romania.
2. **Andra Bucşe,** Dan Vasiliu, Sorin Balan, Oana Cristina Pârvulescu, Tănase Dobre, *Heavy metals spatial distribution and pollution assessment in the surface sediments of the North-Western Black Sea shelf,* 21st Romanian International Conference on Chemistry and Chemical Engineering (RICCCE), 4–7 September 2019, Constanta, Romania.

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