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Macro- and Microelements and Radionuclides in the Mussel *Mytilus galloprovincialis* from Recreational and Harbor Sites of the Crimean Peninsula (The Black Sea)

Pavel Nekhoroshkov ^{1,*}, Inga Zinicovscaia ^{1,2} , Konstantin Vergel ¹ , Dmitry Grozdov ¹ , Omar Chaligava ^{1,3}  and Alexandra Kravtsova ¹

¹ Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, 141980 Dubna, Russia

² Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, 30 Reactorului, MG-6, Magurele, 077125 Ilfov, Romania

³ Faculty of Exact and Natural Science, Georgian Technical University, 77 Merab Kostava Street, 0171 Tbilisi, Georgia

* Correspondence: nekhoroshkov@jinr.ru

Abstract: The concentrations of 29 elements in the soft tissues and of 24 elements in the shells of mussels (*Mytilus galloprovincialis*) collected in one selected recreational and two relatively polluted zones were determined and are presented. The high levels of elements in the mussels from a relatively polluted zone of the harbor (Kamyshovaya Bay) could be associated with phytoplankton, terrigenous matter and anthropogenic sources, but did not harm the mussel population, according to the calculated average condition factors. The low levels of the studied elements and condition factors in the mussels from another harbor (Sevastopol Bay) can be explained by the high circulation and influence of submarine desalinated waters in this zone. The mussels from a recreational site in the South coast of Crimea (Foros) showed high levels of the examined elements, commonly due to the coastal weathering of typical local rocks. The found levels of the radionuclides ⁴⁰K, ¹³⁷Cs, ²³²Th, ²²⁶Ra and ²³⁵U in mussel soft tissues were below the minimum detectable concentrations. The calculated enrichment factors and soft tissue/shells ratios demonstrated differences between groups of elements among the studied sites.

Keywords: mussels; Black Sea; trace elements; neutron activation analysis; radionuclides; condition factors; enrichment factors



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1. Introduction

Mussels can absorb trace elements from waters and ingest them with phytoplankton and suspended particles [1]. The wide use of mollusks in biomonitoring has led to an increase in the number of studies related to the distribution of potential toxic elements in mussels' tissue [2,3].

In the Black Sea water area, during several decades, micro- and macroelements in mussels were carefully analyzed by different countries [4–6]. The main feature of these studies was the assessment of toxic heavy metals', such as Cd, Hg, Pb accumulation. It was found that in the Black Sea water areas the content of potential toxic elements in the soft tissues of *Mytilus galloprovincialis* decreased in the following order: Zn > Cu > As > Ni > Pb > Cd > Cr > Co > Hg [7]. However, the estimation of the natural levels of other microelements interlinking with their different origins, was not determined in all possible types of water areas and sets of elements.

In the last 5 years, elements accumulation in mussel's tissues in the Black Sea region was investigated in several studies. The majority of them focused on farmed mussels, and the elements were mainly determined using ICP techniques, with the goal of establishing the risk posed by mussel meat consumption to humans [3–7]. The most detailed review of

Fe, Zn, Mn, Cu, Pb, Cd, Hg, Cr, As, Ni content in *Mytilus galloprovincialis* in the Black Sea water area is presented in the study of Arici et al. [4].

An important topic in current research is the determination of the range of elements of natural origin in mussels and the traces of anthropogenic inputs. Among the wide number of elements, Cr, Cu, Zn, Se, Mo and I are considered essential, B, Si, V, Mn and Ni could be considered as probably essential, while Li, F, Al have essential functions but in general are considered potentially toxic [5]. The selected trace elements are considered as essential and could be accumulated at high levels in the soft tissues of mussels for the regulation of their physiological activity. The permanent presence of an excess of such elements in the environment promotes their accumulation in higher, but tolerable, levels in organisms.

The key question of toxicological studies is to define the levels of microelements, including toxic trace elements, in wild mussels from typical pristine areas and recreational and harbor water areas. Issues of concern usually appear when the concentration of elements are higher in wild mussels than in mussels farmed in zones under anthropogenic pressure [8].

The interrelation between the morphometry parameters of the shells (such as length, width and height) and the mass of soft tissue could be reflected by the condition index [9]. The shell length is the main morphometric parameter and is associated with nutritional factors and stress conditions [10]. For example, stress conditions could be caused by tidal activities and a decrease in phytoplankton concentrations. Shell erosion diminishes the inner length of an organism. The maximum length varies in association with nutritional factors and stress conditions [10]. For example, mussels from the upper shore are usually smaller than subtidal individuals. It is worth noting that the weight of the soft tissues could also decline after stress. The condition index is widely used for the characterization of the degree of fullness of the shells volume by the soft tissues in individual mussels. Currently, in the scientific and production fields, the universal condition index is not accepted. Therefore, in different studies, such ratio is calculated individually by using several approaches.

The main aim of this study was to assess the ranges of elements in the soft tissues and shells of mussels from different local zones: a recreational beach and relatively polluted city harbors. To expand additional interlinking data, the bottom sediments and soft tissues of mussels were examined for the activity of radionuclides. For a description of the interrelation of elements, the enrichment factor, soft tissues/shells ratio and condition factor were calculated. The results presented in this study represent the first stage of an environmental biomonitoring project that will be further developed in the future.

2. Materials and Methods

2.1. Study Areas and Sampling

The Crimean Peninsula, situated in Eastern Europe, is washed out by the Black Sea. A specific peculiarity of the water is its low salinity (17 PSU) in comparison with the average values for the World Ocean (35 PSU) [11].

The sampling sites were chosen according to level of anthropogenic influence (Figure 1, Table 1): a recreational zone (st. 1) situated in the South coast of Crimea and two harbors (st. 2 and 3), one in Kamyshovaya Bay on the Sevastopol seaside, and the other in Sevastopol Bay.

The mussels from st. 1 were collected near a resort beach in Foros. The possible sources of metals and other elements in this zone are abrasive erosion, recreational use of the beach, water transport and wastewater flows. The st. 1 is opened to the direct influence of the sea; therefore, waves and upwelling events could affect the mussel communities.

The mussels from st. 2 were collected in the inner side of a mole at the entrance to Kamyshovaya Bay, where a marine oil terminal is situated.

The mussels in st. 2 were collected inside the Kamyshovaya Bay, which is considered a polluted port, with several industrial enterprises.

Stations 2 and 3 differ in that oil tankers and fishing boats regularly enter the Kamyshovaya Bay (st. 2), while marine transport boats, military ships and ships to be repaired enter through st. 3 into the main Sevastopol Bay. In addition, st. 3 could be influenced by freshwater masses of the Black River when east winds blow.

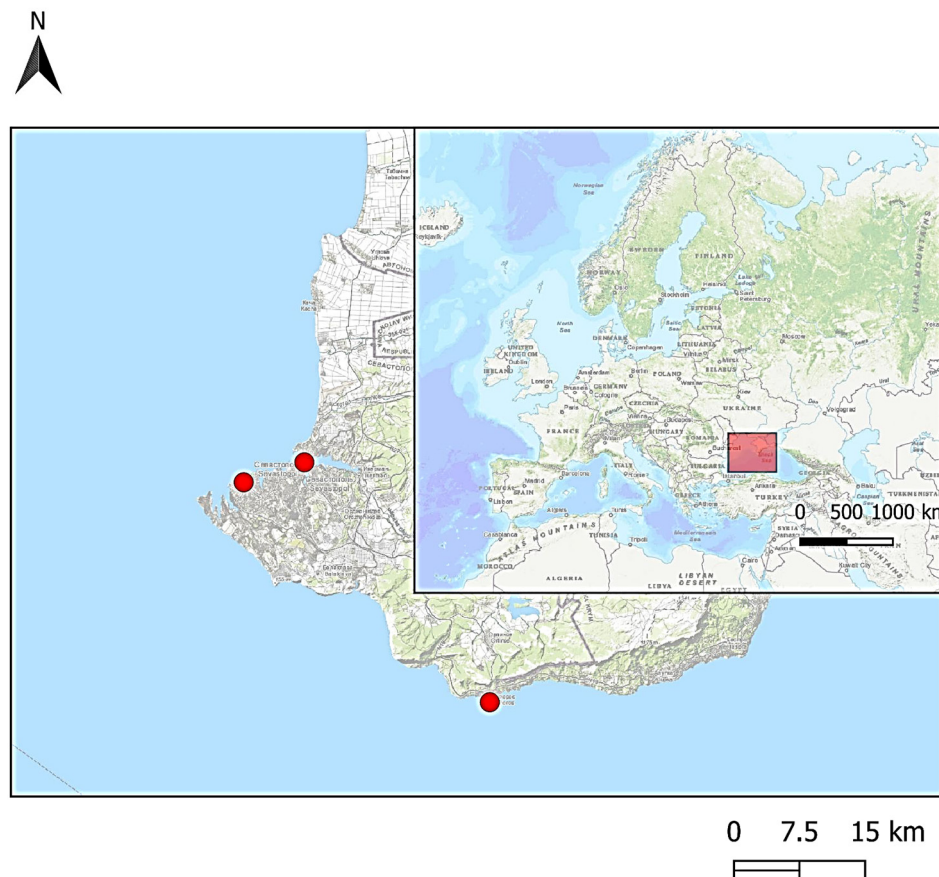


Figure 1. Sampling sites in the Crimean peninsula.

All mussels were wild *Mytilus galloprovincialis* attached to submersible parts of natural rocks (st. 1) and rocks of anthropogenic origin (coastal protection stones at st. 2 and st. 3). A high number of mussels ranging from 40 to 80 mm were collected in August of 2020 before the spawning season (Tables 1 and 2). Mussels ranging from 40 to 55 mm were picked out randomly from those collected.

Table 1. Characteristics of the sampling sites.

Station	Origin of the Substrate	Hydrological Character
St. 1 (Foros)	Natural rocks	Open to direct waves
St. 2 (Kamyshevaya bay)	Coastal protection stones	Closed bay
St. 3 (Sevastopol bay)	Coastal protection stones	Closed bay

The temperature at all stations can vary, depending on the season and on upwelling events, within similar ranges, i.e., from 7 °C in winter to 23 °C in summer (average levels), and salinity usually slightly fluctuates around 18 PSU. However, in summer the upwelling events could increase the salinity by 0.2–1.5 PSU [12]. Spawning for the rock biotope usually occurs in spring at a temperature higher than 8 °C and in autumn at a temperature lower than 15–18 °C [13]. The temperature and salinity during sampling were in the typical ranges.

Table 2. Parameters of the samples.

Station	Number (Individuals)	L (mm)	Ws (g)
St. 1 (Foros)	40	65.6	4.1
St. 2 (Kamyshevaya bay)	118	46.2	1.7
St. 3 (Sevastopol bay)	106	51.6	1.6

Ws—average weight of the soft tissue of one individual; L—average length (mm).

After sampling, the mussels were frozen and placed into plastic bags, then transferred to the laboratory where they were stored at -20°C until dissection. Three pooled samples were prepared from all mussels for each station.

2.2. Analysis of the Elements

The content of 29 elements in the soft tissues and of 24 elements in the mussels' shells were determined by using instrumental neutron activation analysis (INAA) at the REGATA facility of the reactor IBR-2 in the Frank laboratory of Neutron Physics (Dubna, Russia). The main parameters of the irradiation are listed in Table 3.

The mussels were prepared for analysis by several procedures. Individuals were dissected with a plastic knife to separate shells and soft tissues. Purification from sediment particles was performed using deionized water. Then, the soft tissues were weighed in wet state at room temperature (Ws—wet weight of the soft tissues). After lyophilization, the soft tissues were dried to a constant weight at 105°C and then homogenized using a planetary mono mill with agate balls at 400 rpm (Pulverisette, 6, FRITSCH, Idar-Oberstein, Germany). We packed 0.3 g of the obtained material (in triplicate) into polythene bags for the determination of the elements with short-lived isotopes and in aluminum cups for the elements with long-lived isotopes.

The basic properties of neutron activation analysis are described in [14]. For elements determined based on short-lived isotopes (Mg, Al, S, Cl, Ca, Ti, V, Mn, I), the subsamples were irradiated for 3 min at a neutron flux of $1.1 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$. The induced gamma activities were measured for 15 min using Canberra HPGe detectors. The subsamples for the determination of elements with long-lived isotopes (for Sc, Cr, Fe, Co, Ni, Zn, Se, Rb, Sr, Sb, Cs and Th) were irradiated for 4 days at a neutron flux of $3.1 \times 10^{11} \text{ n cm}^{-2} \text{ s}^{-1}$, and induced gamma activities were measured during 30 min and 90 min after 4 and 20 days of decay, respectively.

Spectra recording and analysis and concentrations calculations were performed by using Genie 2000 (v. 3.4.1, Nov 1, 2016, © Mirion Technologies (Canberra), Inc., Zellik, Belgium) and software developed in FLNP JINR [15]. A comparative analysis, in which the analyzed sample and standard were irradiated in the same conditions, was applied for the calculation of the mass fractions of the elements.

Quality assurance (Table 3) was provided by using international certified reference materials, SRMs: JRC-IRMM-BCR-667 (Estuarine sediment), NIST-1566b (Oyster tissue), NIST-1549 (Non-Fat Milk Powder), NIST-1633c (Trace elements in coal fly ash), NIST-2711a (Montana I Soil), NIST 1632c, 1632d (Trace Elements in Coal (Bituminous)), CTA-FFA-1 (Fine fly ash). During the procedure of testing the standards for neutron activation analysis of mussel tissues, several standards were selected to create group standard reference material. The detailed description of the technique is presented in our previous works [16,17]. The final uncertainty of the mass fraction of the determined elements was in the ranges of 2–10% and 20–25% for several elements. These values are presented as recovery rates (Table 3). The elements with uncertainties higher than 30% were excluded from further discussion.

Table 3. Quality assurance of the neutron activation analysis.

	SRMs	Concentrations, ppm		Uncertainties, %		Recovery Rates, %	Detection Limits ppm
		Determined	Certified	Determined	Certified		
Na	2711a	12,100	12,000	8.4	0.01	100.7	50
Mg	1549	1000	1200	7.3	2.5	82.8	50
Al	1632d	8300	9120	6.4	0.5	91.4	6.5
Cl	063R	1050	9940	7.7	3	106	35
K	FFA1	21,400	22,000	10.9	30	97.2	150
Ca	1632d	1400	1440	18	2.1	99.8	500
Sc	2711a	8.47	8.5	5.5	1	99.5	0.006
Ti	2710a	3400	3110	7.1	2.3	110.1	20
V	1633c	275	286	4.8	2.8	96	1.5
Cr	667	170	178	7.8	9	94.7	1.5
Mn	1632d	13.2	13.1	8.9	3.1	101.1	0.16
Fe	2711a	27,800	28,200	5.5	0.04	98.7	20
Co	2711a	9.7	9.89	5.7	2	97.8	0.02
Ni	1632c	9.2	9.32	10.4	5.5	98.7	0.3
Zn	FFA1	600	569	4.1	10.2	107.9	0.4
As	FFA1	53.9	53.6	6	5	100.6	0.3
Se	FFA1	3.8	4.6	13.3	30	81.7	0.05
Br	1632c	22.1	18.7	4.5	2.1	118.2	0.04
Rb	2711a	120	120	16.6	3	103.1	0.15
Sr	2711a	240	242	9.6	4	99.7	5.5
Sb	FFA1	18.0	17.6	8.9	14.2	102.3	0.003
I	1549	3.4	3.4	15.3	0.6	100	0.05
Cs	667	7.9	7.8	6.4	9	101.2	0.003
Ba	2711a	760	730	16.1	2	104.2	0.1
La	667	28.2	27.8	8.6	3.6	101.4	0.1
Tb	FFA1	1.5	1.4	4.4	10.1	105.5	0.001
Ta	667	1.9	2.1	3.7	7.6	90.7	0.002
Th	2711a	14.5	15	5.3	7	96.9	0.01
U	667	2.4	2.3	7.5	6.6	104.7	0.1

The radionuclides activity was measured separately for the shells of the mussels and the bottom sediment powder by using several procedures. The samples were dried, homogenized and packed in 150 mL plastic containers and kept sealed for one month to establish a secular equilibrium between parent ^{226}Ra and daughter ^{222}Rn . The activity concentrations of the radionuclides in the samples were measured by gamma spectrometry using an HPGe detector (Canberra Industries, Inc., Meriden, CT, USA) with 40% relative efficiency and energy resolution of 1.80 keV at the 1332 keV gamma ray energy of ^{60}Co . The detector was calibrated in the same 150 mL plastic container geometry using standard reference material (silica sand and potassium chloride matrix with the homogeneously dispersed radionuclides ^{241}Am , ^{133}Ba , ^{60}Co , ^{137}Cs , ^{152}Eu and ^{40}K ; total activity 16.8 kBq on 3 December 2020; V. G. Khlopin Radium Institute, Saint Petersburg, Russia, Passport №2324/4).

^{40}K and ^{137}Cs were measured directly via their lines with energy of 1460 and 661.66 keV, respectively. It was assumed that ^{238}U and ^{226}Ra were in equilibrium; the activity of ^{226}Ra and ^{232}Th was determined by their decay products ^{214}Bi (609, 1120 and 1764 keV), ^{214}Pb (295 keV) and ^{228}Ac (338 and 911 keV). ^{235}U was determined through the peak with 185.7 keV energy, corrected for ^{226}Ra (186 keV). The spectra were recorded and analyzed using Canberra Genie 2000 software. The counting time was 86,400 s.

2.3. Condition Factor

The condition factor (Cf) was calculated according to previous calculations of the condition index [7,18], by the specific ratio $\text{Cf} = W/L^3$, where W is the wet weight of the soft tissue, and L is the length of shell in mm. The ratio represents differences between

the shell size and the weight of the inner tissues. The condition factor for each station is presented as the average value for all individuals.

2.4. Statistics

By using STATISTICA software (Data Analysis Software System, v.12.0, StatSoft, Inc, Tulsa, OK, USA), the medians, standard deviations, minimum and maximum values of elemental concentrations in soft tissues and shells were calculated and are presented. Outliers were excluded by using the Tukey's fence method. The precision of the determined concentrations is indicated by the coefficients of variation, in %. To reveal the significance of the differences between the average values for the studied stations, the Kruskal–Wallis test (with $p < 0.05$) [19] was applied due to the small number of analyzed observations and pooled samples.

3. Results and Discussion

3.1. Elements in Soft Tissues

The concentrations of 29 elements in the soft tissues of mussels determined in our study are in a good agreement with correspondent literature data [5,20,21] (Tables 4 and S1). However, the higher levels of elements such as Al, Sc, Ti, Cr, Ni, Rb, La, Tb in farmed mussels reported in [5] indicated a contribution of a terrigenous component to the resulting total mass fractions. It means that the mussels in our study were influenced by terrigenous flows to a lesser degree—for example, by river runoff—or were sampled outside the period of intense storms or rainfalls, which could result in the resuspension of the bottom sediments in the studied zones (such as in [16,17,22]). The soft tissues, in general, contained higher mass fractions of elements, except for Ca, Sr and, particularly Cr, which accumulated in shells due to features of their crystallographic texture.

The higher values of Ca, Zn, As, Br, Sr, Ba, U in our samples compared to literature data pointed out specific features of their accumulation in wild mussels in the studied zones. Moreover, the differences between the content of some elements could indicate the peculiarity of the wild mussels to withstand elevated concentrations of elements and convert them in soft tissues. Moreover, wild mussels naturally contain higher levels of all elements in comparison with farmed ones [16,23].

It is interesting to note that even high salinity and different periods of sampling [23–25] did not affect drastically the ability of the mussels to accumulate the elements, as evidenced by the close range of the mass fractions of the majority of elements in the present and previously published studies.

Coastal zones are sensitive to natural and anthropogenic influences associated with to shipyard activities, busy commercial and fishing ports, tourism and human settlements [26]. Thus, the significant higher levels ($p < 0.05$) of Al, Cl, Ca, Mn, Zn, Br, I were found in the soft tissues of mussels from st. 2, whereas the higher levels of Sc, V, Co, As, La, Tb, Ta at st. 1 can be explained by the influence of the natural coastal flows.

These groups of elements indicated specific geochemical patterns, corresponding to sediments and weathering rock products. Al, Ca, Mn could be attributed to a terrigenous component from the coast of the Kamyshevaya Bay. However, such elements as Al, Cl, Mn, Zn, Br and I could be associated with high phytoplankton concentrations [27], marine transport and oil reloading on the facilities of this bay [28]. Sc, V, Co, La, Tb, Ta at st. 1 probably originated from a terrigenous component of coastal weathering [5,6,16]. The coastal rocks at this site consist of siltstones, mudstones, sandstones and reef limestones [29]. In contrast, the coastal rocks at st. 2 include limestones, clays and sands and contain high percentage of Al and Ca. Arsenic could originate from an anthropogenic source, such as pesticides or fertilizers used in agriculture [16].

The mussels from the st. 3 were characterized by the lowest levels of all elements in comparison with those from other sampling sites, due to high circulation of water masses, the lack of resuspended bottom sediments and the influence of waters of a submarine river [30].

Table 4. Mass fractions (in ppm) of 29 elements in the soft tissues of mussels.

	Present Study				Coef. Var. %	Crimea	Crimea	Tyrrhenian Sea
	Median	Minimum	Maximum	Std. Dev.		[5]	[21]	[20]
Na	5800	2440	5900	1648	35.0	11,500 ± 1600	-	-
Mg	1890	1300	2070	310	17.7	2200 ± 300	-	-
Al	193	61	228	74	46.9	3000 ± 2200	-	39–877
Cl	5700	2280	6200	1759	37.9	-	-	-
K	1050	550	1290	252	24.1	7100 ± 700	-	-
Ca	7000	5100	7900	1148	16.9	900 ± 120	-	-
Sc	0.13	0.09	0.21	0.04	29.9	12.0 ± 1.6	-	-
Ti	24	11	67	18	57.2	96 ± 13	-	-
V	0.9	0.6	2.4	0.8	62.3	1.14 ± 0.18	2.3 ± 0.1	2.3–12.5
Cr	1.2	1.0	2.2	0.4	28.3	2.61 ± 0.34	0.48 ± 0.05	0.19–2.17
Mn	8.6	6.6	11.6	1.6	18.5	6.3 ± 1.4	6.01 ± 0.09	3.4–20.6
Fe	329	145	430	98	33.8	197 ± 26	206 ± 5	66–656
Co	1.0	0.5	1.4	0.3	35.8	0.64 ± 0.11	-	0.37–1.37
Ni	2.4	2.0	3.0	0.4	16.7	5.2 ± 0.8	1.36 ± 0.02	0.71–3.39
Zn	372	358	491	60	14.8	308 ± 59	106 ± 2	35–224
As	14.5	13.8	21.2	3.3	20.3	15.1 ± 2.6	1.86 ± 0.03	17–46
Se	1.7	1.6	2.6	0.4	22.7	8.8 ± 1.1	-	1.5–4.3
Br	219	144	252	47	22.8	61 ± 6	-	-
Rb	0.8	0.3	1.2	0.3	36.4	3.2 ± 0.3	-	-
Sr	61	46	79	11	18.3	35 ± 11	-	-
Sb	0.03	0.02	0.06	0.01	41.8	0.086 ± 0.018	-	0.007–0.028
I	12	7	18	5	37.5	13.9 ± 0.9	-	-
Cs	0.04	0.01	0.11	0.03	71.8	0.036 ± 0.009	-	-
Ba	19	16	93	36	87.2	3.8 ± 1.4	4.8 ± 0.1	-
La	0.3	0.1	0.5	0.1	37.2	2.54 ± 0.52	-	-
Tb	0.007	0.003	0.012	0.003	40.6	0.022 ± 0.004	-	-
Ta	0.003	0.001	0.008	0.002	53.0	0.012 ± 0.002	-	-
Th	0.08	0.05	0.14	0.03	31.9	0.13 ± 0.02	-	-
U	0.2	0.1	0.4	0.1	44.8	0.067 ± 0.019	-	-

3.2. The Elements in the Shells of the Mussels

In general, the levels of elements in the shells were in a good agreement with the literature data [17,21,31] (Tables 5 and S1), including data from regions of the Black Sea. The shells of the mussels from the studied sites were enriched in Ca, As and Ba. However, the relatively high levels of several elements (Ca, V, Cr, Mn, Fe, As, Sr, Ba) can be explained by the typical features of the environment hydrochemistry in the studied regions and by differences in the analytical techniques applied in different studies.

It is worth noting that the Mn/Ca ratio was the highest at st. 3. It usually correlates with river discharges [32] associated with runoff events with high concentrations of suspended Mn from soil erosion. The ratio Sr/Ca, linked to differences in the environmental temperature [32], was almost equal among the stations.

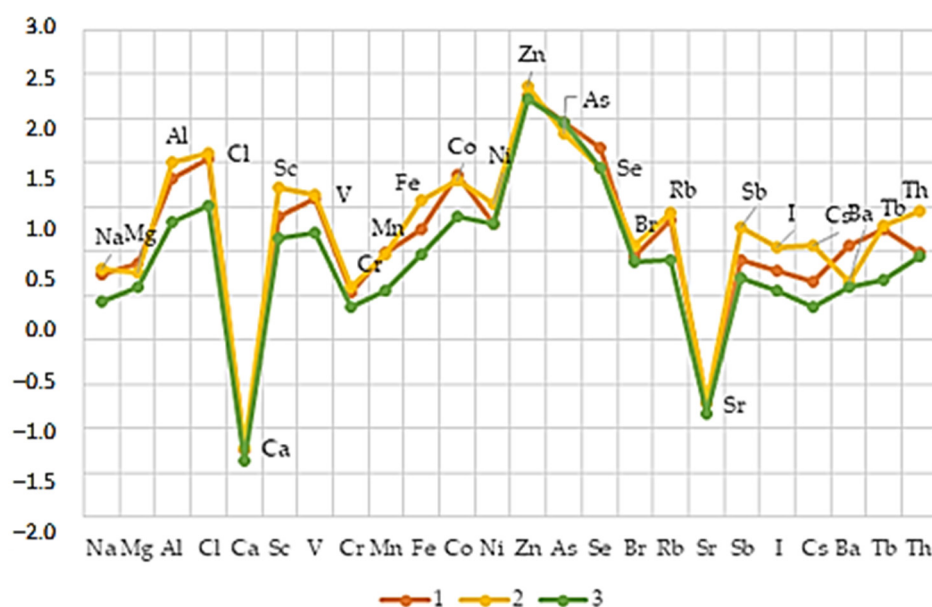
3.3. Soft Tissues/Shells Ratios

The accumulation of such elements as Al, Sc, V, Mn, Fe, Co, Rb, Sb, I, Cs, Ba, Tb and Th in soft tissues vs. shells differed between stations, with the highest levels at st. 2 (except for Co and Ba) and the lowest levels at st. 3 (Figure 2). This indicated the high contribution of the terrigenous component at st. 1 and 2 in comparison with st. 3 [5]. It could be also explained by the relatively high depositional activities of the mussels from st. 3, which transferred the selected elements to the shell matter, thus purifying the soft tissues. It is worth noting that such elements as Cr, Ni, Zn, As, Se and Br were accumulated to the similar levels in soft tissues and shells among studied sites.

Table 5. Mass fractions (in ppm) of 24 elements in the shells of mussels.

	Present Study					[21]	[17]	[31]
	Median	Minimum	Maximum	Std. Dev.	Coef. Var., %	Black Sea	Danger Bay	Adriatic Sea
Na	3037	2640	3400	248	8.2	-	4300 ± 300	2500–4000
Mg	1006	840	1130	103	10.3	-	797 ± 38	800–1400
Al	9	7	11	1	14.2	-	45 ± 6.6	2.5–27
Cl	185	147	260	38	20.7	-	-	-
Ca, %	39.1	36.0	44.0	2.2	5.6	-	36 ± 1	35–37
Sc	0.02	0.01	0.03	0.01	50.4	-	0.05 ± 0.002	-
V	0.1	0.1	0.2	0.06	40.7	0.024 ± 0.001	0.3 ± 0.04	-
Cr	1.3	0.8	1.6	0.30	23.4	<0.1	1.6 ± 0.4	-
Mn	4.1	2.7	6.5	1.46	35.5	6.01 ± 0.09	1.9 ± 0.3	2–155
Fe	53	24	88	21	39.7	4.6 ± 0.4	155 ± 25	15–550
Co	0.06	0.03	0.10	0.02	40.2	<0.1	0.1 ± 0.03	-
Ni	0.3	0.2	0.4	0.08	23.7	<0.1	0.5 ± 0.12	7–33
Zn	2.3	1.6	3.2	0.46	20.1	0.61 ± 0.07	4 ± 0.2	2.5–15
As	0.2	0.1	0.3	0.06	30.1	0.024 ± 0.002	-	-
Se	0.06	0.02	0.08	0.02	32.3	-	-	-
Br	67	54	82	10	15.1	-	67 ± 2	-
Rb	0.14	0.05	0.24	0.05	39.1	-	-	-
Sr	1000	950	1040	25	2.5	-	1523 ± 25	500–800
Sb	0.01	0.00	0.03	0.01	65.6	-	0.03 ± 0.009	-
I	5.9	4.9	6.9	0.7	11.5	-	13 ± 0.4	-
Cs	0.02	0.01	0.05	0.02	70.4	-	0.06 ± 0.02	-
Ba	17	11	27	6	36.8	11.4 ± 0.6	-	-
Tb	0.002	0.001	0.003	0.001	46.8	-	-	-
Th	0.02	0.01	0.04	0.01	55.2	-	-	-

K, Ti, La, Tb, Ta and U were removed from the table due to their insignificant concentrations in the shells (below detectable limits).

**Figure 2.** Ratio of element concentrations in soft tissues and shells, $\text{Log}_{10}(\text{Soft/Shells})$, for mussels from the studied stations.

3.4. Natural Radionuclides in Sediments and Mussel Shells

The activity concentrations of such radionuclides as ^{40}K , ^{137}Cs , ^{232}Th in the sediments at st. 1 were higher than at st. 2 and 3 (Table 6). This was associated with the high percentage of the fine fraction of sediments at st. 1 in comparison with st. 2 and 3.

The activity concentrations of the analyzed radionuclides in mussel shell powder were below the minimum detectable levels. The levels of radioactive K, Cs and Ra in the soft tissues of mussels in the study of Kilic et al. [33] were by an order of magnitude higher than the MDA values obtained in the present study. This indicates extremely low levels of radionuclides in the shells in comparison to the soft tissue. When analyzing Black Sea mussels collected in four different areas of the Crimean coast, Egorov and co-authors [34] showed that ^{40}K concentrations ranged from 65 to 90 Bq/kg, those of ^{137}Cs from 0.66 to 1.5 Bq/kg, and those of ^{90}Sr from 2.4 to 2.5 Bq/kg. According to Thébault et al. [35], the level of ^{137}Cs in mussels collected from the Black Sea was up to two orders of magnitude higher than that found in mussels from the western Mediterranean Basin.

Table 6. Activity concentrations (Bq/kg, dry weight) of radionuclides in mussel shell powder and total sediments fraction.

	^{40}K		^{137}Cs		^{232}Th		^{226}Ra		^{235}U	
	A	U	A	U	A	U	A	U	A	U
	Mussel shells									
St. 1	<2.8	-	<0.16	-	<0.79	-	-	-	-	-
St. 2	<2.7	-	<0.15	-	<0.63	-	-	-	-	-
St. 3	<2.6	-	<0.13	-	<0.67	-	-	-	-	-

A—activity concentrations, U—uncertainty for each station. The values of activity concentrations for the radionuclides in the mussel shells are given as minimum detectable concentrations.

3.5. Enrichment Factors for Mussel Soft Tissues

The accumulation properties in selected zones were described by using the enrichment factor, corresponding to the ratio between the median of the mass fraction of the appropriate element to Sc (as a non-volatile element) in the soft tissues of the mussels and in average shale, which was used as reference terrigenous suspended matter (according to data from [36]). The data of EF in the mussels from the studied stations are presented in Table S2.

The elements with $\text{EF} > 10$ are usually considered as elements with anthropogenic input [37]. In our study, for all sites, elements such as Na, Mg, Cl, Ca, Zn, As, Se, Br, Sr, I were included in this group. Among them, Na, Mg, Cl, Se and I are probably associated with marine sources. Ca and Sr could be related to the sediment features of the studied zones (particles of carbonaceous coastal rocks). Zn, As and Br, with the higher levels of EF in the mussels from st. 1, could have an anthropogenic origin related to recreational and agricultural activities. Elements such as Al, Ti, V, Mn, Fe, Rb, Cs, La, Tb, Ta, Th, with $\text{EF} < 1$, are in the terrigenous group (average shale).

Among the stations, the lowest levels of EF were obtained at st. 1, which can be explained by the closeness of the elemental ratios to that of the average shale (according to the type of rocks present in the local coast) and the low levels of anthropogenic activities.

It is worth noting that all elements could have accumulated from key components of the environment in several ways, including coastal runoff, marine waters, coastal abrasion and resuspension of bottom sediments.

In this way, the enrichment factors indicated the high contribution of elements such as Na, Mg, Cl, Ca, Zn, As, Se, Br, Sr and I at all studied stations, which was associated with high levels of suspended particles (organic and inorganic origin) and food (phytoplankton).

3.6. Condition Factors

In our study, we assumed the ratio between the length and the soft tissue wet weight as a representative parameter of mussels' condition and used it as a condition factor. The average wet weight of wild mussels from open water at st. 1 was higher than of mussels from the bays at st. 2 and 3. It corresponded to a high average length (65.6 mm) in comparison with the lower values for mussels from st. 2 (46.2 mm) and st. 3 (51.6 mm).

The calculated values of the condition factor (Cf) (Table 7) for mussels from st. 2 were found to be higher in comparison with those obtained for mussels from st. 1 and 3. Geographical differences between the stations, expressed in environmental parameters such as temperature, salinity and nutrition, can affect the values of the condition factor. Food concentration, corresponding to higher concentrations of phytoplankton at st. 2 in comparison with st. 1, usually increase the condition index [38]. It is important to note that higher concentrations of the majority of elements at st. 2 could be connected with the growth of phytoplanktonic assemblages in this bay due to the higher levels of biogenic elements dissolved in the water in comparison with st. 1—which is naturally open to direct waves and has a higher underwater slope angle—and st. 3—which is characterized by intense water circulation in the deeper zone of its mouth.

Table 7. Average ratios representing the condition factor for the three stations.

Station	Weight/Length	Cf
1	0.062	1.44×10^{-5}
2	0.038	1.76×10^{-5}
3	0.030	1.16×10^{-5}

On the other hand, the results of the calculation of the condition factor (Cf) in the mussel *Mytilus galloprovincialis* showed that its value was slightly higher in polluted area (at st. 2) than in the relatively pristine recreational area (st. 1). This can be explained not only by the presence of high phytoplankton concentrations (and the probability of frequent phytoplanktonic blooms), but also by higher temperatures and low storm activities in the closed water area in the bay at st. 2. All these environmental conditions increased the Cf of the mussels. However, the Cf of the mussels from the water area of the bay at st. 3 had the minimum values among those of mussels from the different studied zones. This could be due to the introduction of freshwaters of the Chernaya river and the high mixing of waters in the inner zone of the pier at the entrance of the main Sevastopol Bay.

Thus, the low Cf values indicated the presence of environmental stress at st. 1 and 3, probably connected with natural factors, such as decreasing salinity (st. 3), mixing of waters (st. 1 and 3), storm activities (st. 1), and interlinking with the concentrations of the majority of elements in the soft tissues.

4. Conclusions

The highest levels of the majority of elements were found in the soft tissue of the mussels from the st. 2, corresponding to a closed city harbor. The lowest levels were determined in the mussels from st. 3 (main Sevastopol Bay), probably connected with the circulation processes inside the bay, including the influence of the submarine flows of river waters.

The soft/shell ratio indicated higher levels of Al, Sc, V, Mn, Fe, Co, Rb, Sb, I, Cs, Ba, Tb and Th at st. 2 that could be explained by a high accumulation of the mentioned elements in the soft tissues of the mussels at this site. It agreed with the lowest levels of enrichment factors for this sampling site, corresponding to values of the average shale. Among the stations, the lowest soft/shell ratios for almost all elements were obtained for the mussels from st. 3, except for the similar levels of Zn, As, Se. That can be explained by the relatively high depositional activities of mussels, which can transfer selected elements to the shell matter, thus purifying the soft tissues.

The values of the enrichment factors for the mussels from all sampling sites demonstrated excess levels of Na, Mg, Cl, Ca, Zn, As, Se, Br, Sr and I in comparison with the levels in average shale. This was probably due to their excess in the surrounding suspended matter and phytoplankton.

The higher levels of the selected radionuclides in bottom sediments found at st. 1 could be explained by their high percentage in the fine fraction. However, their activity concentrations were lower than the corresponding reference values for the Black Sea region.

The condition factors were interlinked with the levels of the elements in the soft tissues and regulated by environmental factors such as circulation activities, submarine river waters, and presence of nutrient phytoplankton. The Cf values of the mussels at st. 2 indicated favorable environmental conditions.

In general, the mussels from the open recreational zone (st. 1) contained the lowest levels of elements released by anthropogenic sources and the highest levels of several terrigenous elements, whose source could be the coastal weathering of typical local rocks. The mussels from Kamyshovaya Bay (st. 2) accumulated elements associated with phytoplankton, terrigenous matter and anthropogenic sources. However, according to the calculated average condition factor, the levels of these elements did not harm severely the population of mussels. The mussels from st. 3 were characterized by the lowest levels of all elements and, at the same time, a low average condition factor, which indicated the probable influence of specific circulation and submarine desalinated river waters in the zone of the inner part of the pier at the entrance of the Sevastopol Bay.

The investigation of the distribution of elements in the soft tissues and shells of wild mussels in the coastal zone of Crimea will be continued in future studies.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/hydrobiology1030022/s1>, Table S1: Mass fractions (ppm) of the studied elements in the soft tissues and shells of mussels from the different stations; Table S2: Enrichment factors for the soft tissues of mussels from the different studied stations.

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