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ASSESSMENT OF THE CONTENT OF MAGNESIUM, POTASSIUM, PHOSPHORUS AND CALCIUM IN WATER AND ALGAE FROM THE BLACK SEA IN SELECTED BAYS NEAR SEVASTOPOL

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Abstract

The content of macroelements such as magnesium, calcium or phosphorus in tissues of aquatic organisms is often used as an indicator of the quality of an aquatic environment. The aim of this paper was to assess the content of magnesium, potassium, calcium and phosphorus in water and in algae in selected bays of the Black Sea near Sevastopol. The samples of water and algae were collected in August 2012 from eight bays of Sevastopol (Gałubaja, Kozacha, Kamyshova, Strieletska, Kruhla, Pishchana, Pivdenna, Sevastopol Bay). One sample was obtained from the open sea near Fiolent. *Cystoseira barbata* and *Ulva rigida* algae were collected from the same sites. The collected water was conserved *in situ* and, after being brought to a laboratory, it underwent determinations of the content of magnesium, potassium, phosphorus and calcium. Laboratory samples of the algae were subjected to mineralization in a closed system with the use of microwave energy. The content of the elements in water and in digested algae samples was determined using the ICP-OES method. The magnesium content in water ranged between 461.6 and 638.9 mg Mg dm^{-3}, potassium between 332.8 and 457.6 mg K dm^{-3}, phosphorus 0.072-0.143 mg P dm^{-3}, and the calcium content was within the range from 209.5 to 288.6 mg Ca dm^{-3}. A higher content of calcium and phosphorus was found in *Cystoseira barbata*, whereas a higher level of magnesium was detected in *Ulva rigida* algae. No statistically significant correlation between the content of the elements in water and in algae was observed. The content of the elements in water decreased in the order Mg>K>Ca>P, whereas in algae the order was K>Ca>Mg>P. Despite the differences in amounts of the accumulated elements in algae, there was a significant relation between the content of individual elements in *Cystoseira barbata* and *Ulva rigida*, which suggests that both species of algae are suitable for biomonitoring.

Keywords: Black Sea, water, *Cystoseira barbata*, *Ulva rigida*, macroelements, bioaccumulation.

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INTRODUCTION

Heavy industry, some segments of municipal management (e.g. handling of municipal sewage) and agriculture are the main sources of pollution in the Black Sea near Sevastopol. The release of considerable amounts of pollutants into an aquatic environment has an invariably adverse impact on its individual compartments. Xenobiotics can produce either direct, toxicological effects on living organisms or indirect ones, by interfering with the uptake and metabolism of macroelements. The geostrategic situation in the Black Sea basin is shaped by various factors. Water acidification is among the most powerful anthropogenic effects on the marine environment. Acidification of seawater, which can be observed worldwide, is considered to be one of the most important causes of their degradation. Changes of the water acidification level often result in a disordered ionic regulation system in living organisms (Pérez-gil 2013). Due to their location and often highly dynamic development, coastal areas frequently struggle with the problem of the management and treatment of both industrial and municipal sewage. A coastal zone is a specific site, where negative effects of urban development are often aggrevated. Much has changed in the land use in the Black Sea region over the last 50 years. The coastal zone is increasingly polluted due to the progressing agricultural intensification, development of industries, power generation, navigation and tourism (Langmead et al. 2009). The Black Sea catchment area is very rich in natural resources and has a unique strategic location between Central Asia, Europe and the Middle East. At the same time, the region is constantly struggling with numerous problems, for instance the so-called “frozen” conflicts, or difficultes in protecting the natural environment. Taking regional actions in the areas of environmental protection, energy, transportation or safety may have a beneficial influence on the lives of citizens of the Black Sea countries and may improve their prosperity, political stability and safety (the Black Sea synergy…). Monitoring of the marine environment is of considerable importance in the development of an environment protection policy and evaluation of the efficiency of ongoing actions. Sea algae are thought to be good indicator organisms because of their high potential for the bioaccumulation of trace elements and a noticeable influence of stressors on shaping of their chemical composition (Brito et al. 2012). Assessment of the chemical composition of algae is also important because numerous species of seaweeds are used as food (Qiu 2015).

The aim of this paper was to assess the content of magnesium, potassium and calcium in water and in algae in selected bays of the Black Sea near Sevastopol.

MATERIAL AND METHODS

To reach the set goal, samples of water from 8 bays of Sevastopol as well as one sample from the open sea near Fiolent were collected in August 2012. The samples were drawn from the top layer of water (from the depth of 0-120 cm). Sample collection sites were selected so as to obtain representative samples for the whole bay. To this aim, data on water movement and flow of sea currents were used. Samples were taken at a mid-depth profile of the bay within the entire width of the bay from the sites of occurrence of algae. The samples were collected from the following bays (geographical coordinates of the depth profile of each bay): Gałubaja (44°33′43″ N 33°23′56″ E), Kozacha (44°34′46″ N 33°24′33″ E), Kamyshova (44°34′57″ N 33°25′26″ E), Striletska (44°35′59″ N 33°26′44.05″ E), Kruhla (44°36′04″ N 33°28′08″ E), Pishchana (44°06′06″ N 33°28′58″ E), Pivdenna (44°36′25″ N 33°31′52″ E), and Sevastopol Bay (44°38′0″ N 33°34′29″ E) as well as from the open sea in the region of Fiolent (44°30′55″ N 33°27′11″ E) – Figure 1.

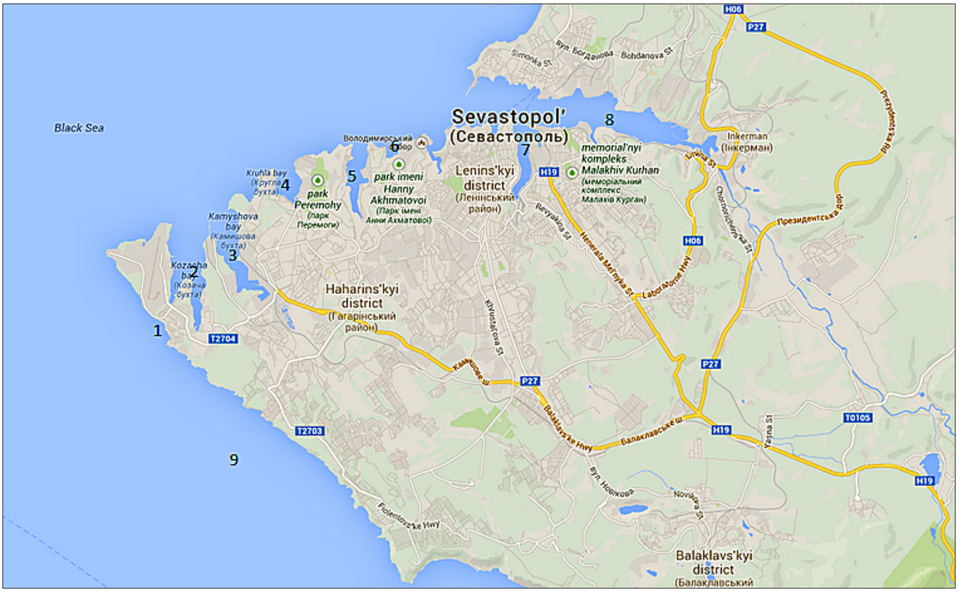

Fig. 1. Sampling sites

In order to ensure reliable research result, elements of biomonitoring were included into the research. Hence, samples of *Cystoseira barbata* and *Ulva rigida* algae were collected in the same sites. These species of algae are common in the studied area. In addition, they can be found in the same habitats. Once collected, water was conserved by adding nitric acid in the amount of 2 cm^3 per each 100 cm^3 water, then the samples were transported to a laboratory. The algae were washed in distilled water, dried and homogenized. Five laboratory samples of water and algae were collected from each bay. The laboratory samples of algae were subjected to wet mineralization in a closed system with the use of microwave energy. Anton Paar Multiwave 3000 microwave system was used for mineralization. Each analytical sample contained approximately 0.5 g in terms of dry matter. The material was digested in a mixture of HNO_3 and H_2O_2, in the 5:1, v/v ratio.The collected water was conserved *in situ* and, after being brought to the laboratory, submitted to determinations of the content of magnesium, potassium, phosphorus and calcium. The collected algae were rinsed in distilled water, dried, homogenized and mineralized. The content of elements in water and in digested algae samples was determined using the ICP-OES method, on an Optima 7600 DV apparatus manufactured by Perkin Elmer.

The wavelengths used to determine the concentrations of the elements and limits of determination for the applied methods are presented in Table 1.

Table 1

Paremeters of the analytical method

Parameter	Wavelengths (nm)	Detection limit (g dm^{-3})	Content in certified material (g kg^{-1})	Measured (g kg^{-1})	Recovery (%)
Mg	285.208	0.0016	2.72	2.904	106.8
P	213.617	0.076	-	-	-
Ca	317.933	0.01	27	28.33	104.9
Na	589.592	0.069	13.1	12.88	98.32
K	766.490	-	13.1	12.91	98.55

Certified reference material CRM 16-050 was used to check the correctness of all analyses of the elements. Table 1 presents results of our analyses of the reference material and an estimated value of recovery, based on the analyses replicated four times. Concentrations of the elements in water and algae were compared with the Tukey's test. The difference in the content of each element in the samples was evaluated at a significance level of $p = 0.01$. Bioaccumulation coefficients were calculated for individual elements by dividing the dry matter concentration of an element in algae by the content of that element in water. Moreover, correlation coefficients between the content of the elements in water and algae were calculated using the Pearson's correlation.

RESULT AND DISCUSSION

Assessment of the quality of the environment supported by bioindication methods has great cognitive value as it provides information on the content of elements in individual, biotic parts of an ecosystem, which allows one to estimate the actual effect of environmental pollution on living organisms. Among bioindication methods for the marine environment assessment, solutions which make use of algae are the most popular ones (Brito et al. 2012). However, the use of algae as indicators of environmental pollution requires selection of appropriate species, whose growth will not be inhibited by such factors as changes in salinity, water temperature or velocity of tides (Luo, Liu 2011). For instance, Padina algae have a great capacity to accumulate macroelements but are sensitive to changes in salinity, which considerably limits their usability (Chakraborty et al. 2014). This is of particular importance in zones of strong mixing of river and marine waters, particularly in areas of considerable seasonal fluctuations of the flow of river waters. There is another, practical aspect of using macroalgae as research material, namely about 6 million tons of fresh biomass of sweaweed is grown for food and industry ever year (Naser 2013).

Algae of the *Ulva* and Cystoseira genera are widespread organisms that live in shallow marine waters and in estuaries. They are resistant to changes in salinity and temperature, and also to an elevated content of xenobiotics in water. Thus, they satisfy all the criteria set for organisms used in biomonitoring (Mamboya et al. 2009, Kravtsova et al. 2015). Disruption of an ecosystem's homeostasis caused by pollution, a change in environmental parameters or an appearance of pathogens typically cause disorders in mineral metabolism. Hence, the content of macroelements and microelements in tissues of marine organisms is an important indicator of an ecosystem's quality (Sánchez-Rodríguez et al. 2001, Besada et al. 2009). Macroalgae are an important part of marine ecosystems. Through photosynthesis, they provide an ecosystem with energy and oxygen, indispensable to all living organisms. Through the roots grown in the bottom of a water body algae can absorb elements bound in benthic sediments, which is important in the biogeochemical cycle of elements, particularly the ones which occur in trace amounts in the marine environment (Horta-Puga et al. 2013). Moreover, algae that spread in shallow coastal waters and in estuaries form an environment for life and reproduction of many aquatic organisms; they are their food and they buffer the intensity of waves.

The magnesium content in the water sampled from the Black Sea bays ranged between 461.56 and 638.9 mg dm^{-3}, the potassium concentration varied between 332.8 and 457.6 mg dm^{-3}, the phosphorus concentration was between 0.07 and 0.14 mg dm^{-3}, and calcium occurred at the concentration between 209.5 and 288.6 mg dm^{-3} in the studied water samples (Table 2). Differences in the content of the elements in water from the individual bays were found to be insignificant. The least potassium, magnesium and calcium was determined in the water collected from Sevastopol Bay, whereas the least phosphorus was in the water collected in the open sea. Data on the statistical significance of differences in the concentrations of the elements in water from the bays are provided in Table 2. The chemical composition of

Table 2

Content of elements in water from bays (mg dm^{-3})

Sites of sampling	Mg	K	Ca	P
Gałubaja	598.7*c*	385.5*b*	273.9*b*	0.086*a*
Kazacha	564.7*bc*	412.5*b*	256.9*ab*	0.093*a*
Kamyshova	584.6*bc*	445.6*bc*	234.6*a*	0.102*a*
Kruhla	523.6*b*	399.5*b*	246.0*ab*	0.094*a*
Striletska	541.4*b*	432.5*bc*	221.7*a*	0.091*a*
Pishchana	529.8*b*	436.8*bc*	209.9*a*	0.121*ab*
Pivdenna	511.9*ab*	376.8*b*	211.2*a*	0.113*ab*
Sevastopolska	461.6*a*	332.8*a*	209.5*a*	0.143*b*
Open Sea near Fiolent	638.9*c*	457.6*c*	288.6*b*	0.072*a*

Different letters beside the mean values indicate statistically significant differences among the samples from different bays at a significance level of $p = 0.01$.

seawater from coastal areas depends on the effect of water and sewage that flow off the land and by the intensity of the mixing of coastal water with open sea water (CHAKRABORTY et al. 2014), which explains the differences in the chemical composition determined in the current study. The highest phosphorus content was found in the water from Sevastopol Bay, exposed to a high level of human impact. The macroelement contents determined in this research were at a level detected in seawater from different regions of the world (CULKIN, COX 1966). The ecosystem's properties linked to the geographical and climatic conditions are similar in all the examined bays (KUZMINOVA et al. 2014). Therefore, the detected differences in the content of the macroelements in algae are most probably a result of the diverse strength of human impact.

The mean magnesium content in the studied *Cystozeira barbata* algae was 5.485 g kg^{-1} DM and varied from 4.698 to 6.259 g Mg kg^{-1} DM (Table 3). The average amount of magnesium in *Ulva rigida* algae was approximately 70% higher and reached 9.348 g Mg kg^{-1} DM. The magnesium content in these algae varied from 8.567 to 11.20 g Mg kg^{-1}. The highest amount of magnesium was observed in algae of both species collected from Kamyshova Bay. Calcium is an element whose content in marine algae may change under the influence of stressors, and it is often used as an indicator of environmental quality (SÁNCHEZ-RODRÍGUEZ et al. 2001). This study showed a significant difference in the content of this element depending on the genus of seaweeds. The mean calcium content in *Ulva rigida* algae was 8.012 g Ca kg^{-1} DM, whereas the average amount of calcium in *Cystoseira barbata* algae was 15.34 g Ca kg^{-1}. Most of this element was observed in samples from Striletska Bay (Table 3). Algae from Sevastopol Bay contained the least calcium. The calcium concentration in *Ulva rigida* was within the range from

Table 3

Content of elements in algae (g kg^{-1})

Sites of sampling	*Cystoseira barbata*				*Ulva rigida*			
	Mg	K	Ca	P	Mg	K	Ca	P
Gałubaja	4.798*a*	43.46	10.73*a*	0.807*b*	9.595*b*	15.77*b*	4.343*a*	0.657*c*
Kazacha	5.871*c*	25.61*b*	13.41*bc*	0.806*b*	9.727*b*	11.36*a*	7.360*b*	0.633*bc*
Kamyshova	6.259*d*	53.22*d*	19.50*d*	0.874*b*	11.12*c*	12.51*a*	7.833*b*	0.679*c*
Kruhla	5.792*bc*	50.68*d*	12.09*b*	0.522*a*	9.060*ab*	19.81*c*	7.708*b*	0.509*b*
Striletska	5.779*bc*	43.76*cd*	24.83*e*	0.474*a*	8.567*a*	19.04*c*	11.88*d*	0.385*a*
Pishchana	4.698*a*	35.83*c*	17.94*d*	0.503*a*	8.968*a*	13.51*a*	11.02*d*	0.381*a*
Pivdenna	5.394*b*	19.91*a*	15.71*cd*	0.625*ab*	9.510*b*	12.51*a*	7.204*b*	0.526*b*
Sevastopolska	5.563*b*	38.75*c*	9.026*a*	1.244*c*	8.777*a*	23.66*e*	6.468*b*	0.635*bc*
Open Sea near Fiolent	5.212*b*	38.68*c*	15.10*cd*	0.503*a*	8.804*a*	20.16*d*	8.296*c*	0.411*a*

Different letters beside the mean values indicate statistically significant differences among the samples from different bays at a significance level of $p = 0.01$.

4.343 to 11.88 g Ca kg^{-1} DM (Table 3). The least of this element was found in algae from Gałubaja Bay, and the highest leevl was determined in in algae from Striletska and Pishchana bays. SÁNCHEZ-RODRÍGUEZ et al. (2001) reported that the calcium content in different algae species from Loreto Bay in Mexico was between 10 and 54 g Ca kg^{-1} DM. These authors found considerable differences in the content of this element in algae of individual species depending on the habitat. Analogously to our results, the cited authors observed a higher calcium content in brown algae than in green ones. Like calcium, significantly more potassium was found in *Cystoseira barbata* algae, in which the mean content of this element was 38.88 g K kg^{-1} DM. The mean potassium content in *Ulva rigida* algae was 16.48 g K kg^{-1} DM. The potassium content in *Cystoseira barbata* algae varied from 19.91 to 53.22 g K kg^{-1} DM (Table 3). *Cystoseira barbata* collected in Kamyshowa and Kruhla bays accumulated the greatest amounts of this element, whereas in the case of *Ulva rigida* the highest content of potassium was in the samples collected in Sevastopol Bay (Table 3). Potassium plays a special role in marine macroalgae, and its content in these organisms is used as an indicator of one of the most important parameters of the aquatic environment quality, i.e. the salinity level (TOUCHETTE 2007). Next to eutrophication, pollution by heavy metals and by organic compounds, a change in water salinity is a major factor that causes degradation of macroalgae communities on many marine areas (RUDNICK et al. 2005). Disorder of processes associated with osmoregulation is the consequence of changes in the amount of salts (particularly sodium) dissolved in water. A change in the potassium content in plant cells is an immediate effect of this disorder. Under osmotic stress, a decrease in the potassium content in plant tissues is usually observed, which is widely believed to be a sensitive indicator of the marine environment quality, particularly in coastal areas and within estuaries (Carpaneto et al. 2004). The natural potassium content in marine algae varies from 30 to 115 g P kg^{-1} DM. Bushy algae (Touchette 2007) contain significantly more potassium. The phosphorus content in the studied algae showed the highest diversity among the sample collection sites. The mean phosphorus content in *Ulva rigida* algae was 0.534 g P kg^{-1} DM and varied within a range from 0.381 to 0.679 g P kg^{-1} DM (Table 3). *Cystoseira barbata* algae, in which the mean content of this element was 0.706 g P kg^{-1} DM, contained on average approximately 25% more of this element. The highest content of this element in both species of algae was found in Sevastopol, Gałubaja, Kozacha and Kamyshova bays, whereas the least phosphorus was found in the samples collected in Striletska and Pishchana bays (Table 3).

Owing to their chemical, physical and biological properties as well as a great capacity for reproduction, algae are widely used in various spheres of human activities. They are valuable raw material for the manufacture of medicines, cosmetics or food. In many regions of the world algae are used as animal fodder, fertilizers and raw material in industry (Latique et al. 2013, Paul, Sheeba 2014). In addition to their antiseptic properties, the unique value of marine algae is associated with a high content of protein characterized by a beneficial amino acid, vitamin and micro- and macroelement composition (Aguilera-Morales et al. 2005). Marine macroalgae have a high capacity to accumulate potassium, therefore using them as fodder or fertilizer may constitute an unconventional source of this element. Yamashita et al. (2009) discovered that the value of the bioaccumulation coefficient for potassium in *Ulvae* algae from the Yokohama region was approximately 20, which is slightly lower than calculated in our research. The content of calcium and magnesium in *Ulva rigida* algae from the region of Hare Island in India was 50.08 mg Ca kg^{-1} and 31.72 mg Mg kg^{-1} , respectively (Paul and Sheeba 2014). In terms of dry matter, these results yield values close to ours. Żbikowski et al. (2007) report the macroelement content in Cladophora from Gdańsk Bay in the amounts of 3.9 g Ca kg^{-1}, 15 g Mg kg^{-1}, and 38 g K kg^{-1}. The calcium, magnesium and potassium content in thalli of *Cystoseira barbata* (brown algae) collected in three places in the region of Sevastopol was between 6.1 and 6.9 g Mg kg^{-1}, between 12.4 and 22.6 g Ca kg^{-1}, between 30.8 and 62.0 g K kg^{-1} (Kravtsova et al. 2014). These authors obtained results comparable to ours. Moreover, they found a significant impact of the place and season on the chemistry of thalli of these algae.

For many years, the region of Sevastopol has been exposed to a high level of human impact associated with dynamic urbanization. The heavy industry sector, certain elements of municipal management, e.g. the handling of municipal sewage, and agriculture are the main sources of pollution of the Black Sea near Sevastopol. The release of considerable amounts of pollutants into an aquatic environment has a negative effect on its individual compartments. Xenobiotics can produce either direct, toxicological effects on living organisms or indirect ones, by interfering with the uptake and metabolism of macroelements. The content of macroelements such as magnesium, calcium or phosphorus in tissues of aquatic organisms is often used as an indicator of the quality of an aquatic environment. Literature indicates a considerable impact of the chemical composition of water on shaping the content of elements in algae (OSTAPCZUK et al. 1997, MALEA, HARITONIDIS 2000, RODRÍGUEZ-FIGUEROA et al. 2009). No statistically significant correlation between the content of the elements in the water and algae thalli was observed in the current research. Other authors confirm that changes in the macroelement content in algae can be observed in a marine environment polluted by heavy metals, radioactive elements or organic compounds (RUPÉRE et al. 2002, CARPANETO et al. 2004). ÇETINGÜL et al. (1997) discovered that the content of calcium and magnesium in the algae from a polluted area near Izmir in Turkey was several times lower than in the same organisms from unpolluted areas. Compared to the content of the studied macroelements in the algae collected from areas near Fiolent, which had the lowest human impact factor and limited influence of the mainland, samples from most of the bays showed a lower potassium and calcium content in both species of algae. The magnesium content in the algae collected in the open sea were the same or higher than in the samples collected in the bays. The majority of algae samples from the research sites located in the bays had a higher phosphorus content than the algae from the open sea. In order to determine the possibility of binding the elements in algae organisms, bioaccumulation coefficients for magnesium, potassium, calcium and phosphorus were calculated. The value of the bioaccumulation coefficient for magnesium ranged between 8.015 and 19.02 (Table 4). The highest value of this parameter was found in the algae from Sevastopol Bay, whereas the lowest bioaccumulation coefficient for magnesium was observed in the samples collected from the open sea. The highest value of the bioaccumulation coefficient for potassium in *Cystoseirabarbata* algae was

Table 4

Bioaccumulation coefficient in algae

Sites of sampling	*Cystoseira barbata*				*Ulva rigida*			
	Mg	K	Ca	P	Mg	K	Ca	P
Gałubaja	8.015	112.73	39.18	9385	16.03	40.90	15.85	7636
Kazacha	10.40	62.08	52.21	8671	17.22	27.54	28.65	6810
Kamyshova	10.71	119.43	83.13	8572	19.02	28.07	33.40	6655
Kruhla	11.06	126.87	49.13	5555	17.30	49.58	31.34	5415
Striletska	10.67	101.18	112.0	5205	15.82	44.03	53.57	4230
Pishchana	8.868	82.02	85.49	4156	16.93	30.94	52.52	3145
Pivdenna	10.54	52.84	74.36	5529	18.58	33.20	34.11	4653
Sevastopolska	12.05	116.44	43.08	8700	19.02	71.09	30.87	4441
Open Sea near Fiolent	8.157	84.54	52.33	6983	13.78	44.05	28.75	5705

observed in the samples collected from Kruhla Bay, whereas the highest value of this parameter for calcium was found in the algae collected in Kamyshova Bay (Table 4). In the case of *Ulva rigida* algae, the highest values of the bioaccumulation coefficient for potassium and calcium was found in the algae from Sevastopol and Striletska bays, respectively. The highest value of the bioaccumulation coefficient for phosphorus in both of the algae studied was found in the samples from Gałubaja Bay. The biggest differences in the values of the bioaccumulation coefficient between samples from individual collection sites were found in the case of calcium and potassium. The results of our research suggest considerable differences in the content of the elements as well as differences in the value of bioaccumulation coefficients in algae samples from individual collection sites. However, data analysis indicates a significant relationship between the content of individual elements in *Cystoseira barbata* and *Ulva rigida* algae, which suggests that both species of algae are suitable for biomonitoring.

CONCLUSIONS

1. The content of the macroelements determined in water samples showed considerable diversity depending on a sample collection site. A high content of magnesium, calcium and potassium, as well as a low phosphorus concentration were found in the water collected from the open sea.
2. Large differences in the content of the elements in individual bays of the Black Sea near Sevastopol were found, which indicates the occurrences of stressors in the research regions.
3. A higher content of magnesium, calcium and phosphorus was found in *Cystoseira barbata* algae, whereas a higher content of magnesium was detected in *Ulva rigida* algae.
4. No statistically significant correlation between the content of the elements in the water and in the algae was observed.
5. The content of the elements in the water decreased in the order Mg >K> Ca >P, whereas in the algae the order was K>Ca>Mg>P.
6. Despite the differences in amounts of accumulated elements in the algae, a significant relationship between the content of individual elements in *Cystoseira barbata* and *Ulva rigida* algae was found, which suggests that both species of algae are suitable for biomonitoring.

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