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ELEMENTAL ACCUMULATION IN THE BLACK SEA BROWN ALGAE *Cystoseira* STUDIED BY NEUTRON ACTIVATION ANALYSIS

OCENA AKUMULACJI PIERWIASTKÓW W CZARNOMORSKICH GLONACH BRUNATNYCH *Cystoseira* Z WYKORZYSTANIEM NEUTRONOWEJ ANALIZY AKTYWACYJNEJ

Abstract: For the first time the concentration of 26 macro- and microelements (Na, Mg, Al, Cl, K, Ca, Sc, V, Mn, Fe, Co, Ni, Zn, As, Br, Rb, Sr, Sb, I, Cs, Ba, Sm, Nd, Ag, Au, and U) in the thalli of brown algae *Cystoseira barbata* C. Ag. and *Cystoseira crinita* (Desf.) Bory was determined by instrumental neutron activation analysis (INAA), Sevastopol region, south-western Crimea, the Black Sea. The observed peculiarities of the elemental accumulation showed that *Cystoseira* spp. can be used as a biomonitor of coastal waters pollution in the study area.

Keywords: biomonitoring, trace elements, *Cystoseira barbata*, *Cystoseira crinita*, neutron activation analysis, Black Sea

Introduction

Marine macroalgae can accumulate macro- and trace elements in concentrations that are orders of magnitude greater than their content in their environment [1]. This ability is used to indicate the level of coastal waters pollution in different regions of the World Ocean, including the Black Sea [2-5]. Widely spread brown algae are believed to be the most preferable for monitoring purposes because of the presence of alginic acid providing their higher resistance to metals compared to red or green algae [2, 6]. Although the features of macro- and trace element accumulation in the Black Sea macroalgae have been described by a number of authors, the data on the relationship between elemental concentrations and plant age, morphostructural elements of the thalli and seasons are very scarce. To cover this gap the present study is focused on variations of macro- and trace element concentrations during spring - summer season, their relationship with age and different parts of the thalli of the Black Sea brown algae *Cystoseira barbata* C. Ag. and *Cystoseira crinita* (Desf.) Bory from the coastal waters of Sevastopol region (SW Crimea).

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Materials and methods

Study area

The study was carried out in the Sevastopol region (Fig. 1). The coastal zone of Sevastopol region is characterized by a crenellated shoreline, numerous small bays, including semi-enclosed bays with limited water exchange (Sevastopolskaya and Balaklavskaya Bays), bays that are deeply intrusive into the land (Karantinnaya Bay) and bays with free water exchange with the open sea (Kruglaya Bay). The sites in the investigated coastal zone are characterized by different degrees of anthropogenic pollution (Table 1). The most polluted bays, according to the trophic index E-TRIX (the function of concentration of dissolved oxygen, total phosphorus, sum of mineral forms of nitrogen and chlorophyll “a”), ranked in descending order, are Sevastopolskaya Bay → Karantinnaya and Balaklavskaya Bays → Kruglaya Bay [7]. Likewise, according to the content of total petroleum hydrocarbons (TPH) in the bottom sediments, Sevastopolskaya Bay is more polluted than Balaklavskaya and Karantinnaya Bays, and the least polluted is Kruglaya Bay [8, 9]. The coastal waters near the Cape Fiolent are a part of the local hydrological natural monument named “Coastal aquatic complex near Cape Fiolent”, and they are considered to be relatively clean. The sampling sites and data regarding their level of pollution are shown in Figure 1 and Table 1, respectively.

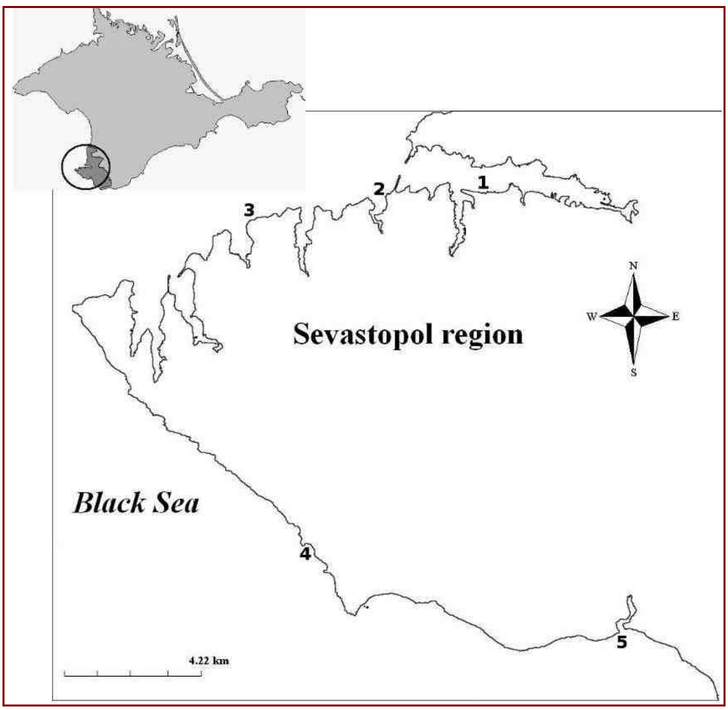


Fig. 1. Study area and sampling sites in the coastal waters of Sevastopol region (south-western Crimea, Black Sea): 1 - Sevastopolskaya Bay (Cape Pavlovskiy); 2 - Karantinnaya Bay (Cape, eastern entrance); 3 - Kruglaya Bay (Cape, eastern entrance); 4 - “Coastal aquatic complex near Cape Fiolent” (near Cape Lermontov); 5 - Balaklavskaya Bay (near Cape Kuron)

Table 1
The coordinates of sampling sites and data on their anthropogenic pollution levels (SW Crimea, Black Sea)

Sampling sites	**Coordinates**		**Pollution data**	
	Northern longitude	**Eastern latitude**	**E-TRIX* [7]**	**TPH** in bottom sediments [mg/100 g] [8, 9]**
Sevastopolskaya Bay (Cape Pavlovskiy)	44°36’58’’	33°32’47’’	3.24	1187
Karantinnaya Bay (Cape, eastern entrance)	44°36’42’’	33°30’7’’	2.99	65
Kruglaya Bay (Cape, eastern entrance)	44°36’28’’	33°26’51’’	2.27	5
“Coastal aquatic complex near Cape Fiolent” (near Lermontov’s Cape)	44°30’31’’	33°28’52’’	–	–
Balaklavskaya Bay (near Kuron Cape)	44°29’34’’	33°35’38’’	2.76	160

* E-TRIX - trophic index for waters; ** TPH - total petroleum hydrocarbons; the line means lack of data

Sampling and sample preparation

The whole plants of *Cystoseira barbata* (stations 1-2, Figure 1) and *C. crinita* (stations 3-5, Figure 1) were sampled during the spring (May) and summer (July) seasons in 2012 at five marine sites in the Sevastopol region with different degree of anthropogenic pollution (Table 1). The sampling was carried out at depths of 0.5-1.5 m; features of macro- and trace elements accumulation were studied separately for “stems” and “branches” of thalli from 6 months to 5 years of age. To determine the age of the plants the method based on the constancy of the average annual growth rate of the “stem” of *Cystoseira barbata* and *C. crinita* was used [10]. Plants were washed with marine water from the sites they grow, carefully cleaned from epiphytes, and then dried to a constant weight at 40°C during 24 hours. Further the samples were manually homogenized in an agate mortar. A total of 52 samples *Cystoseira* spp. were prepared.

Analysis

To determine the elemental concentrations samples were subject to INAA at the reactor IBR-2 of the Frank Laboratory of Neutron Physics of the Joint Institute for Nuclear Research, Dubna, Russia [11].

To determine the short-lived isotopes of Al, Cl, Ca, V, Mg, Mn, and I, samples of 0.3 g weight were packed in polyethylene bags and irradiated for 3 min in the irradiation channel with a neutron flux density of $1.3\cdot 10^{12}$ n/(cm$^2\cdot$s). Gamma spectra of induced activity were measured twice, for 3 min after 5-7 minutes of decay and for 12-15 min after 20 min of decay.

The long-lived isotopes of Na, K, Sc, Fe, Co, Ni, Zn, As, Br, Rb, Sr, Sb, Cs, Ba, Sm, Nd, Ag, Au, and U were determined using epithermal neutrons in a cadmium-screened irradiation channel with a neutron flux density of $1.6\cdot 10^{12}$ n/(cm$^2\cdot$s). Samples were irradiated for 90 h, repacked and then measured twice after 4-5 d of decay during 45 m and after 20 d of decay during 2.5-3 hours.

To process gamma spectra and to calculate concentrations of elements in the samples, software developed at FLNP JINR was used [12]. The errors in the determined concentrations were in the range of 5-15% and 30% or more for those elements (*eg* Ag) which concentrations in the samples were at the level of detection.

Quality control was provided by using standard reference materials SRM Rice Flour-1568a, Pine needles-1575a and Apple Leaves-1515, all NIST production, irradiated in the same conditions together with the samples under investigation. The NAA data and certified values of reference materials are given in Table 2.

Table 2

NAA data and certified values of reference materials [mg/kg]

E*	1568a (determined)	1568a (certified)	1575a (determined)	1575a (certified)	1515 (determined)	1515 (certified)
Na	8.65±0.7	6.6±0.8	68.3±2.7	63±66.8	32.2±4.2	24.4±1.2
Mg	481±24.6	560±20.2	1150±120	1060±170	2610±130	2710±81
Al	4.2±0.3	4.4±0.1	540±25	580±30.2	296±8.3	286±8.87
Cl	265±20	300±90	421±29	421±7.16	530±32	579±23.2
K	1120±84	1280±7.68	4960±347	4170±70	15100±900	16100±193
Ca	128±13	118±6.02	2610±130	2500±100	14700±150	15260±153
Sc	-	-**	0.0112±0.0004	0.0101±0.0003	0.028±0.007	0.03±0.009
V	0.0065±0.002	0.007±0.002	-	-	0.28±0.03	0.26±0.03
Mn	16.4±0.4	20±1.6	468±11.7	488±12.2	50.1±1.5	54±3
Fe	7.8±0.34	7.4±0.9	49±1.3	46±2	80±3.5	83±5
Co	0.017±0.003	0.018±0.005	0.057±0.002	0.061±0.002	0.09±0.02	0.09±0.03
Ni	-	-	1.48±0.08	1.47±0.1	0.93±0.1	0.91±0.12
Zn	20.5±0.78	19.4±0.5	35±2.9	38±2.01	12.8±0.15	12.5±0.3
As	0.26±0.03	0.29±0.03	0.041±0.003	0.039±0.002	0.039±0.004	0.038±0.007
Br	8.0±2.7	8.0±2.4	-	-	1.23±0.5	1.8±0.5
Rb	6.17±0.08	6.14±0.09	17±0.6	16.5±0.9	10.7±1	10.2±1.5
Sr	-	-	-	-	26±1.8	25±2
Sb	0.00048±0.0002	0.0005±0.00015	-	-	0.013±0.006	0.013±0.004
I	0.009±0.008	0.009±0.003	-	-	0.32±0.08	0.3±0.09
Cs	-	-	0.29±0.005	0.283±0.009	-	-
Ba	-	-	5.8±0.2	6.0±0.2	49.6±2.5	49.0±2
Au	-	-	-	-	0.001±0.0005	0.001±0.0003
Nd	-	-	-	-	17.9±6.5	17±5.1
Sm	-	-	-	-	2.67±0.8	3±0.9
U	0.0003±0.00007	0.0003±0.00009	-	-	0.0058±0.002	0.006±0.002

* - elements; ** - element is absent in the passport of the reference material

Statistical analysis was performed with STATISTICA 9.

Result and discussion

The concentrations of 26 elements accumulated in the “stems” and “branches” of *Cystoseira* spp., as sampled in the 2012 of spring and summer in the coastal zone of the Sevastopol region, are given in Table 3.

Table 3

The average concentrations of macro [mg/g] - and trace elements [μg/g] in *Cystoseira barbata* and *C. crinita* (Sevastopol region, Black Sea)

Element	Part of the thalli	2012 year									
		Spring					Summer				
		C. barbata		*C. crinita*			*C. barbata*		*C. crinita*		
		1	2	3	4	5	1	2	3	4	5
Na*	s**	9.8±1.6	10.4±0.9	12.7±0.7	14.5±2.1	18.5±0.9	16.7±1.6	19.7±1.2	19.5±0.8	19.7±1.2	18.5±1.4
	b***	10.9±1.4	11.3±0.4	12.8±0.6	17.6±2.8	20±1.3	18.5±0.9	26.6±1.4	23.5±1.8	21.5±1.4	25.3±1.6
Mg*	s	6.3±0.6	6.1±0.2	8.4±0.1	8±0.3	8.7±0.4	6.5±0.1	6.7±0.2	8.8±0.5	7.8±0.2	8.9±0.7
	b	6.4±0.08	6.9±0.8	7.9±1.1	8.4±0.8	7.8±0.2	7.03±0.04	9.3±1.7	9.5±0.5	11±1.3	12.1±1.7
Al	s	562±187	205±98	76.8±27	124±32	268±18	218±57	436±288	46.5±37	44.3±29	31.8±13
	b	206±40	268±18	82.9±49	246±114	222±48	269±91	1230±170	135±57	49.5±8.02	349±96
Cl*	s	40±3.8	33.2±6.2	20.2±5.8	22.3±3.3	27.7±4.5	24.7±4.3	30±3.1	16.7±5.5	12.4±2.6	24.4±5
	b	40±2	36±0.5	33.9±4.3	39.3±5.2	50±2	29±6.2	60±8.5	34.2±7.7	25±1.6	73.3±13
K*	s	43.4±7.8	33.5±5.4	19.3±6.8	23.7±2.9	26.9±0.9	30.8±3	31.3±2.3	22.2±5.6	21.8±5.9	22.1±3
	b	45.4±6.2	41±8.5	28.2±0.5	34±6.5	53.7±3.8	33.4±3.3	62±4	34.7±14.1	29.4±6.8	46.7±2.2
Ca*	s	14.9±4	15.7±1.2	18.1±1.3	13.6±0.6	17.4±0.8	14.9±0.6	20.9±8.7	14.5±2.2	14.9±3.5	14.1±1.5
	b	12.4±1.6	21±1.6	16.2±4.8	12.6±0.7	12±1.01	13.3±0.2	22.6±3.5	16.7±1.3	34.1±9.2	17.3±2.9
Sc	s	0.1±0.04	0.05±0.007	0.02±0.01	0.07±0.02	0.08±0.03	0.04±0.01	0.04±0.01	0.02±0.008	0.02±0.009	0.02±0.007
	b	0.05±0.01	0.07±0.04	0.04±0.001	0.16±0.02	0.06±0.006	0.02±0.008	0.22±0.01	0.05±0.01	0.09±0.008	0.11±0.03
V	s	0.34±0.2	0.78±0.1	0.69±0.1	0.9±0.1	2.03±0.5	0.9±0.7	1.17±0.8	0.5±0.4	0.23±0.1	0.7±0.6
	b	0.54±0.4	1.06±0.1	0.27±0.06	0.4±0.3	0.7±0.2	1.49±0.15	3.9±1.4	0.44±0.2	0.48±0.23	0.8±0.3
Mn	s	42±4	20±4.5	16.6±4.8	13.4±1.6	19±1.8	23.2±6	32.3±11	54.6±17	15.7±2.9	20.7±2.4
	b	19.3±3.3	25.2±3.96	9.1±1.8	10.2±0.5	8.6±1.1	15.9±4.8	43.2±3.7	41.4±12	19.3±3.2	33.4±9.1
Fe	s	376±170	156±45	81.2±51	116±39	206±91.2	157±58	193±64	78±23	51.3±35	21.7±18
	b	189±22	209±74	75.2±7.1	259±46	148±18	218±83	722±13.4	153±27	90.9±22.7	255±43
Co	s	0.5±0.09	0.26±0.08	0.28±0.03	0.51±0.1	0.48±0.1	0.4±0.04	0.7±0.18	0.78±0.2	0.63±0.1	0.47±0.06
	b	0.24±0.02	0.29±0.1	0.1±0.03	0.3±0.04	0.16±0.02	0.35±0.04	0.9±0.04	0.65±0.17	0.63±0.2	0.68±0.05
Ni	s	3.9±1.7	3.9±0.9	2.7±0.9	3.6±1.4	5.23±1.06	2.6±0.1	3.7±0.35	1.38±1.2	1.94±0.5	1.98±0.4
	b	-	3.45±1.45	2.7±1.9	3.2±0.2	2.76±0.8	2.7±0.8	4.3±0.31	2.9±0.2	2.9±1.1	6.08±0.6

Element	Part of the thalli	2012 year									
		Spring					Summer				
		C. barbata		*C. crinita*			*C. barbata*		*C. crinita*		
		1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Zn	s	52.5± 11	25.4± 5.6	40.7± 4.5	51± 11	79.1± 15.8	37.8± 3.2	35.5± 0.8	42± 5.7	34.3± 4.7	61.7± 11
	b	30.1± 0.9	17.3± 5.5	12.2± 2.6	14.1± 1.3	22.7± 1.3	35± 3.4	33.5± 4.7	20± 2.6	18.6± 3.6	25.3± 4.2
As	s	22± 3.3	30± 1.8	34± 10	38± 9.9	28.7± 2.02	9.4± 1.5	19.7± 0.5	40± 13.5	28.6± 9.2	30.3± 6.8
	b	16.3± 0.7	17.9± 0.3	35.4± 13	41.6± 8.3	38.1± 4.1	11.2± 0.7	24.7± 1.4	42.3± 4.8	31.3± 11	54.5± 7.1
Br	s	194± 43	153± 6.3	119± 28	130± 18	223± 17.3	149± 21	197± 44,5	150± 38	152± 19	165± 19
	b	178± 17.7	191± 34.6	162± 48	167± 21	166± 20	140± 18	286± 10.6	337± 120	249± 69	373± 28
Rb	s	15.6± 3.2	12.5± 1.1	8.5± 2.1	8.84± 0.96	10.2± 0.34	11.3± 0.8	10± 0.9	6.8± 2	6.7± 1.7	8.61± 0.7
	b	18.2± 4.1	18± 2.1	12.5± 2	14.1± 1.7	21.9± 1.8	10.9± 0.9	19± 0.42	11.7± 2.7	8.9± 1.7	17.3± 0.8
Sr	s	591± 110	616± 11.3	955± 37	942± 62	974± 6.6	643± 110	694± 12,7	953± 67	894± 91	744± 34
	b	1066± 134	1056± 91	1184± 203	1230± 44	1203± 5.8	1175± 92	1135± 35	1357± 85	1386± 45	1043± 23
Ag	s	0.02± 0.004	0.06± 0.03	0.08± 0.02	0.12± 0.09	0.07± 0.04	-	0.15± 0.008	0.14± 0.08	0.1± 0.04	-
	b	-	-	-	-	-	0.09± 0.04	0.13± 0.007	0.13± 0.08	0.11± 0.01	-
Sb	s	0.06± 0.02	0.09± 0.07	0.05± 0.006	0.06± 0.004	0.1± 0.01	0.03± 0.01	0.03± 0.009	0.04± 0.002	0.04± 0.01	0.04± 0.004
	b	0.05± 0.01	0.05± 0.02	0.06± 0.007	0.06± 0.007	0.07± 0.04	0.05± 0.01	0.06± 0.0004	0.04± 0.006	0.05± 0.01	0.05± 0.01
I	s	85.2± 6	98± 38	83.2± 7.8	84.2± 5.1	135± 7.6	37.7± 2.2	102± 91	59.5± 9.4	87.8± 35	136± 1
	b	140± 7.78	117± 17.7	89.6± 17	96± 11	121± 9.1	52.5± 7	70.4± 6.2	89± 5.1	123± 27	231± 19
Cs	s	0.06± 0.03	0.04± 0.01	0.02± 0.05	0.017± 0.005	0.04± 0.01	0.03± 0.01	0.05± 0.01	0.02± 0.007	0.02± 0.002	0.04± 0.01
	b	0.04± 0.01	0.04± 0.01	0.02± 0.002	0.02± 0.01	0.04± 0.004	0.04± 0.005	0.13± 0.02	0.03± 0.002	0.02± 0.003	0.05± 0.01
Ba	s	30.5± 9.8	28.8± 4.6	40.8± 1.6	40.8± 3.23	44.2± 1.3	31.2± 3.3	33.3± 3.7	43.6± 4.6	30± 6	34± 2.6
	b	53.7± 9.5	48.3± 10.5	45.3± 6.9	50.9± 2.4	52.9± 3.1	68± 1.5	61.4± 5.1	61.7± 7.1	37.8± 2.9	56.1± 0.7
Au	s	0.07± 0.04	-	-	-	-	-	0.004± 0.02	0.007± 0.001	0.008± 0.001	0.007± 0.001
	b	-	-	-	-	0.03± 0.02	0.002± 0.001	0.01± 0.001	0.008± 0.0003	0.01± 0.001	0,01± 0.001
Nd	s	3.1± 1.3	17.7± 9	9.9± 5.3	5.63± 2.6	4.76± 1.9	10.2± 1.8	14.2± 3.5	7.4± 2.3	6.9± 1.3	9.6± 2
	b	2.16± 0.1	3.8± 2.9	9.9± 7.3	9.1± 7.8	4.54± 3.1	9.3± 1.2	20.8± 0.9	16.4± 2.9	11± 4.1	187± 32
Sm	s	0.05± 0.02	-	0.001± 0.0007	0.004± 0.002	0.03± 0.004	0.02± 0.01	0.02± 0.08	-	-	-

Element	Part of the thalli	2012 year									
		Spring					Summer				
		C. barbata		*C. crinita*			*C. barbata*		*C. crinita*		
		1	2	3	4	5	1	2	3	4	5
	b	-	0.0015± 0.001	0.03± 0.01	0.02± 0.01	0.01± 0.008	0.017± 0.007	0.05± 0,04	0.01± 0.008	0.006± 0.005	0.005± 0.008
U	s	-	0.2± 0.09	0.64± 0.2	0.48± 0.07	0.8± 0.1	0.2± 0.04	0.37± 0.02	0.64± 0.2	0.5± 0.3	0.5± 0.05
	b	0.2± 0.08	0.31± 0.09	0.09± 0.1	0.2± 0.1	0.15± 0.09	0.5± 0.1	0.2± 0.18	0.29± 0.2	0.35± 0.06	0.25± 0.1

* - macroelements [mg/g]; ** - “stems” of *Cystoseira barbata* and *C. crinita*; *** - “branches” of *Cystoseira barbata* and *C. crinita*; line means that element was not determined; numbers of stations 1-5 are given as in Figure 1

Concentrations of trace elements within macroalgae *Cystoseira* spp. and the Black Sea water were compared to determine the bioconcentration factor (BCF) for each trace element. In Table 4 the bioconcentration factors are given calculated as BCF = Cc/Cw, where Cc is an average concentration of trace element in *Cystoseira* spp. from the coastal waters of the Sevastopol region [mg/kg]; Cw is the concentration of trace element in the Black Sea water near South coast of Crimea [mg/dm^3].

Table 4

Average concentration of microelements in *Cystoseira* spp. (*Cystoseira barbata* and *C. crinita*) [μg/g], Black Sea water [mg/dm^3], and their bioconcentration factors (Sevastopol region, Black Sea)

Element	Average concentration in *Cystoseira* spp.*	Concentration in the Black Sea water near South coast of Crimea [13]	BCF for *Cystoseira* spp.
Al	253	100×10^{-4} **	10^4
Sc	0.07	0.014×10^{-4}	10^4
V	0.9	3×10^{-3} **	10^3
Mn	24	19×10^{-4}	10^4
Fe	188	300×10^{-4}	10^4
Co	0.42	0.34×10^{-3}	10^3
Ni	4.98	1.8×10^{-3}	10^3
Zn	35	70×10^{-3}	10^3
As	30	19×10^{-4}	10^4
Rb	13	32×10^{-2} **	10^2
Sr	992	1800×10^{-3}	10^3
Ag	0.09	0.013×10^{-2}	10^2
Sb	0.06	0.18×10^{-2}	10^2
Cs	0.04	0.01×10^{-3}	10^3
Ba	45	47×10^{-4}	10^4
Nd	10	5×10^{-6}	10^6
Sm	0.02	0.02×10^{-5}	10^5
U	0.37	0.24×10^{-2}	10^2

* - our data; ** - concentrations in the World Ocean

According to our results, the brown algae *Cystoseira barbata* and *C. crinita* can accumulate trace elements in concentrations that exceed their concentrations in the Black Sea water by 3-4 orders. It is well-known that the rate of trace element accumulation by marine macroalgae depends on many factors. The most important abiotic factors are the concentration of elements in the environment and their speciation, salinity, intensity of water exchange, water temperature and light. Among biotic factors, the rate of metabolism of plants, their morphological characteristics, taxonomic identity, stage of ontogeny and physiological state are considered to be the important ones [13-15].

Accumulation of elements in different morphostructural parts of the Cystoseira spp. *thalli*

The distribution of some elements over the morphostructural parts of the thalli has common features for all stations depend on *Cystoseira* spp. The concentration factors for "branches" of *Cystoseira barbata* and *C. crinita* (CF_b) were determined for each element (Table 5) using the following equation:

$$CF_b = C_b / C_s$$

where C_b and C_s are elemental concentrations in "branches" and "stems" of *Cystoseira* spp., respectively [mg/kg].

Table 5

Concentration factors for "branches" of *Cystoseira barbata* and *C. crinita* (CF_b) in spring and summer of 2012 (Sevastopol region, Black Sea)

CF_b	Spring, 2012		Summer, 2012	
	C. barbata	*C. crinita*	*C. barbata*	*C. crinita*
0.1-0.4	Sb	Sb, Zn, U		
0.4-0.9	V, Zn, U	V		Zn, U
≈1	Na, Mg, Ca, Cl, K	Na, Mg, Ca	Zn	
1.2-1.5	Rb, Sr, I, Ba	Rb, Sr, I, Ba	Na, Mg, Ca	Na, Mg, Ca
1.5-2.5		Cl, K	Cl, K	Cl, K
1.5-4			All trace elements (excluding Zn and U)	

It should be noted that Zn concentration in "stems" of *C. crinita* is 3.5-4 times higher than in "branches", while in "stems" of *C. barbata* it is only 1.4-1.7 higher (Fig. 2). Similar regularity is observed for U.

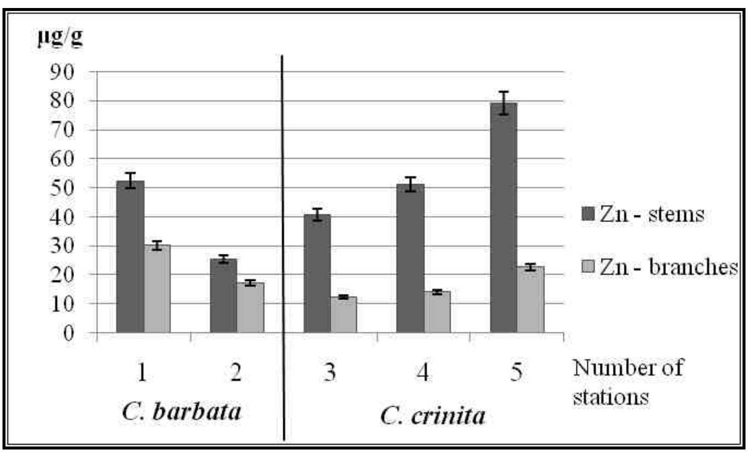


Fig. 2. The content of Zn [μg/g] in "stems" and "branches" of *Cystoseira crinita* and *C. barbata* in spring of 2012 (Sevastopol region, Black Sea)

It might be due to peculiarities of the “stem” structure of *C. barbata*, which is harsher and bigger in diameter compared with the thin and smooth “stem” of *C. crinita* [10] that may influence the sorption processes [5]. The data obtained for Zn concentration in the thalli of *Cystoseira* spp. are in agreement with the previous investigations in other regions of the Black Sea (coastal zone of Crimea and Novorossiysk region) [5, 6] where Zn concentration in the “stems” of *C. crinita* was found 2-fold higher than in “branches”. As for the other trace elements, *C. barbata* and *C. crinita* show pronounced differences in elemental accumulation in the morphostructural parts of the thalli in spring at different stations (Table 6).

Table 6

Concentration factors for “branches” of *Cystoseira barbata* and *C. crinita* (CF_b) in spring of 2012 (Sevastopol region, Black Sea)

Element	***C. barbata***		***C. crinita***		
	1*	**2**	**3**	**4**	**5**
Al, Sc, Fe	0.5	1.5	1.5	2-2.5	≈1
Ni, As, Cs, Nd			≈1		
Ni, As					≈1
Ni, Cs		≈1			
Mn, Co	0.5	1.2	0.7	0.7	0.5

* - numbers of stations 1-5 are given as in Figure 1

<u>In summer</u>, the concentrations of the majority of elements (except Zn and U) in the “branches” of both *Cystoseira* spp. at all stations are 1.5-4 times higher than in the “stems”. A possible reason for this phenomenon is the abundance of epiphytic macroalgae on the “branches” of *Cystoseira* which are an additional source of elemental sorption from the aquatic environment.

Therefore, the distribution of macro- and trace elements over the morphostructural parts varies by seasons and it is different for *Cystoseira barbata* and *C. crinita*. In spring certain elements have different patterns of accumulation, while during the summer time the concentrations of all elements (except Zn and U) are higher in “branches” than in the “stems”.

The relationship between accumulation of elements in Cystoseira spp. *thalli and their age*

The concentration of elements in “stems” of *C. barbata* and *C. crinita* of different age (less than 1 year old, 1-3 years old and 3-5 years old) are shown in Table 7.

In spring and summer the highest concentrations of all macroelements in the “stems” of *C. barbata* and *C. crinita* were found in plants that were younger than one year old (except Mg and Ca in the spring season) at all stations. The highest concentrations of macroelements in “branches” of the youngest plants of *C. crinita* (stations 3-5) were observed only in summer.

Maximal concentrations of trace elements in spring and summer were also determined in the “stems” of *C. barbata* and *C. crinita* that were younger than one year old at all stations (Fig. 3). In spring the content of some elements (Sc, Ni, Ag and Al, V, Fe, Ag and Sm) at all stations in the “stems” of *C. barbata* and *C. crinita* at the age of 1-3 years is 1.5 and 2 times lower, respectively, than in the plants younger than one year old. The similar regularity for Zn, Fe, Mn, and Pb accumulation in the thalli of *C. crinita* was previously found by other researches who noted the age period of 0.5-2 year as a period of rapid changes of these elemental concentrations [6]. The high accumulation of elements in the young plants of *Cystoseira* spp. seems is due to their more intense growth compared to the old ones [15].

Table 7

Average concentration of macro [mg/g] - and trace elements [µg/g] in "stems" of *Cystoseira barbata* and *C. crinita* of different ages in spring of 2012 (Sevastopol region, Black Sea)

S*	Age	Na**	Mg**	Al	Cl**	K**	Ca**	Sc	V	Mn	Fe	Co	Ni
C. crinita	**<1**	16.4	8.43	166	27.9	27.3	16.7	0.06	1.33	18.1	127	0.45	5
	1-3	15.2	8.53	164	23	21.5	17	0.04	1.30	14.8	120	0.36	2.82
	3-5	14.1	8.16	139	19.2	21	15.4	0.07	1.00	16.3	156	0.45	3.69
C. barbata	**<1**	11	6.5	485	40.2	39.3	17.2	0.09	0.78	34.1	312	0.38	4.2
	1-3	9.21	5.93	282	33	37.7	13.5	0.06	0.34	28.1	224	0.37	3.62

	Age	Zn	As	Br	Rb	Sr	Ag	Sb	I	Cs	Ba	Nd	U
C. crinita	**<1**	55.4	42.1	178	10.4	981	0.20	0.08	107	0.02	42.6	8.15	0.6
	1-3	52.5	28.1	150	8.42	924	0.05	0.07	99.9	0.02	41.9	4.86	0.57
	3-5	63.0	30.5	143	8.67	966	0.03	0.06	95	0.03	41.3	7.28	0.7
C. barbata	**<1**	40.9	26.7	192	14.8	641	0.06	0.05	103	0.05	31.5	16.7	0.19
	1-3	37.0	25.6	157	13.3	567	0.03	0.10	80.3	0.05	27.8	4.1	0

* - *Cystoseira* species; ** - macroelements [mg/g]

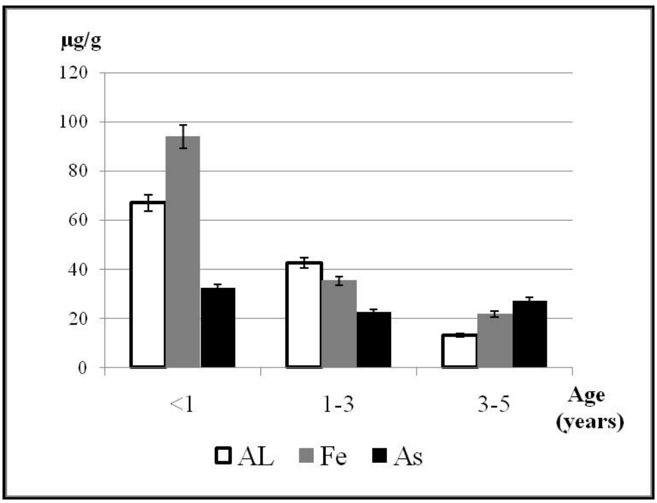
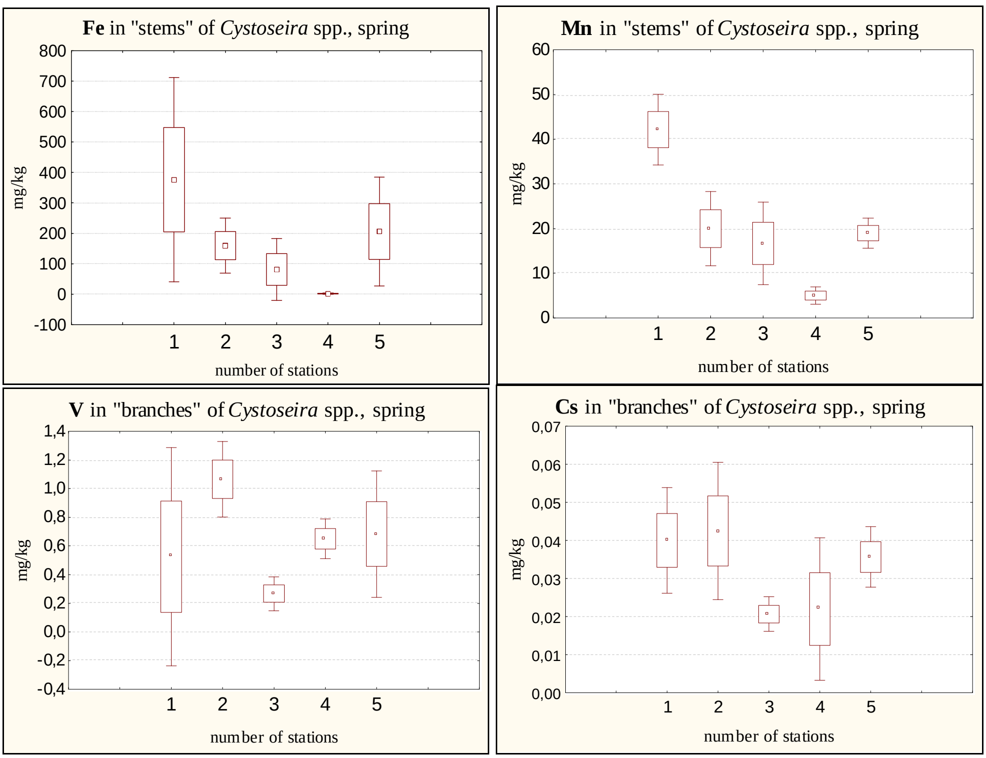


Fig. 3. Average concentration of Al, Fe and As [µg/g] in *Cystoseira crinita* "stems" at various ages in summer of 2012 (stations 3-5, Sevastopol region, Black Sea)

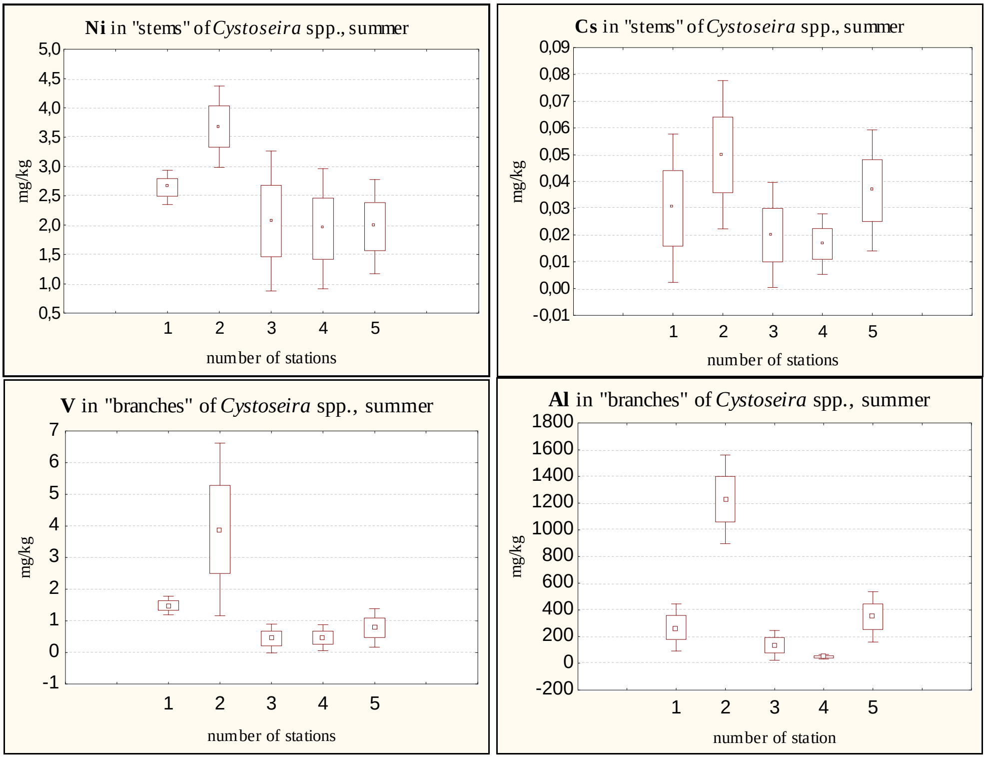
In spring and summer, the concentration of elements in the "branches" of *C. crinita* (stations 3-5, Fig. 1), that are younger than one year old, is usually higher than in the plants of 1-3 years old, but this was not as definite as for the "stems". It can be explained by the fact that "branches" of *C. crinita* and *C. barbata* shed and change after several months because their age does not exceed 5-7 months [10]. Thus, the maximal concentrations of all trace elements (except Al, Sm, and Nd) were found in the 1-2 years old "branches" of *C. barbata* (stations 1 and 2, Fig. 1).

Accumulation of elements in the "stems" and "branches" of Cystoseira spp. *in areas with different level of water pollution*

In more polluted waters the elemental concentration in the "stems" and "branches" of *C. barbata* and *C. crinita* obviously tends to increase (Table 3). The concentration of some elements in *Cystoseira* spp. from different stations is represented as the Box-and-Whisker plots (Fig. 4). They show the mean value and standard deviation of the data for each station and differences in concentration of elements between stations.



A



B

Fig. 4. Box-and-Whisker plots of concentration of elements (□ Mean, □ Mean±Standard deviation and ⌶ Mean ±1.96*Standard deviation; [mg/kg]) in “stems” and “branches” of *C. crinita* and *C. barbata* (stations 1-5 as in Fig. 1) in spring (A) and summer (B) of 2012 (Sevastopol region, Black Sea)

For the biomonitoring purposes some authors recommend to use only “stems” of 3-4 years old plants of *C. crinita* [6] and others - both “stems” and “branches” of 2-5 years old plants of *C. crinita* [5]. The results of our study showed that the elemental concentrations in 1-3 years old “stems” of *C. crinita* and *C. barbata* sampled in summer are in a good agreement with the level of anthropogenic pollution of the studied water areas.

In spring, the maximal concentrations of the majority of trace elements in the “stems” of *Cystoseira* spp. were found in the Sevastopolskaya Bay, then, in descending order, in Balaklavskaya and Karantinnaya Bays, whereas the minimal concentrations were observed in Kruglaya Bay and, for some elements, in the aquatorium near Cape Fiolent. In summer, the maximal concentrations of trace elements were found in the “stems” of *Cystoseira* spp. from Karantinnaya Bay, then - from Sevastopolskaya and Balaklavskaya Bays. In summer, some trace elements (Mn, As, Sr, Ba, and U) were found in maximal concentrations in the thalli of *Cystoseira* spp. from Kruglaya Bay.

The maximal elemental concentrations in the “stems” of *C. crinita* and *C. barbata* from Sevastopolskaya Bay correlate with high concentrations of petroleum hydrocarbons and heavy metals in the bottom sediments determined in the centre of this bay [16, 17]. High concentration of elements in *Cystoseira* spp. from Balaklavskaya Bay and Karantinnaya Bay appears to be due to the high level of integrated pollution of their aquatorium which is worsened due to the poor water exchange of these bays with the open sea [18]. Minimal concentrations of trace elements in the “stems” of *Cystoseira* spp. from Kruglaya Bay in spring are due to lower anthropogenic pressure, as compared to the other bays, and intense water exchange with the deep sea [17]. Elevated concentrations of some elements in the thalli of *Cystoseira* spp. from Kruglaya Bay in summer could be related to the high recreational load (beaches and sewage waters from tourist infrastructure located on the coast), which is confirmed by the increase in the biochemical oxygen demand (BOD_5) [17].

In spring, in the “stems” of *Cystoseira crinita* near Cape Fiolent where the coastal area is considered to be relatively clean, the concentrations of such elements as Co, Sc, Zn, and V turned out to be similar in the “stems” of *Cystoseira* spp. from the polluted areas. It is known that Cape Fiolent is an isolated volcano-tectonic block and the adjacent area near Cape Lermontov consists of volcanic formations of the Middle Jurassic, which are overlapped with organogenic-detrital and clay limestone of the Neogene [19]. Elevated concentrations of Co, Sc, Zn, and V in the thalli of *Cystoseira* spp. can be explained by their high concentrations in mafics and medium rocks [20], which are typical for this area [19]. The concentration of the mentioned elements in such rocks is up to 2-fold higher than in limestone [19, 20] which is typical for the Sevastopol region.

Conclusions

For the first time 26 elements, including macroelements, heavy metals and arsenic were found in brown algae *Cystoseira barbata* C. Ag. and *Cystoseira crinita* (Desf.) Bory from the coastal waters of the Sevastopol region (SW Crimea, the Black Sea).

The peculiarities and differences in accumulation of macro- and trace elements were revealed in morphostructures of the thalli of *C. barbata* and *C. crinita* of different ages. In the summer period the concentrations of all elements (except Zn and U) are higher in the “branches” than in the “stems” whereas in spring some elements behave themselves differently.

The most intense accumulation of elements in the “stems” of *Cystoseira* spp. was observed in spring during the growth period and in the “branches” in summer; it can be related to an abundance of epiphytic macroalgae, which are an additional source of elemental sorption from the environment.

The highest concentrations of the majority of elements were found in the plants younger than one year old at all stations in the spring and summer seasons, regardless of their degree of pollution; it can be related to peculiarities of their ontogenesis.

The results evidence for increased trace element concentrations in the thalli of *Cystoseira* spp. in more polluted waters. In spring the higher concentrations of trace elements were found in the “stems” of *Cystoseira* spp. from Sevastopolskaya Bay than near the entrance capes of Balaklavskaya and Karantinnaya Bays. The lowest concentrations of the majority of elements were found in the “stems” of *Cystoseira crinita* from the waters near the entrance cape of Kruglaya Bay which is considered to be a relatively clean aquatorium. In summer, the higher concentrations of trace elements were found in the “stems” of *Cystoseira* spp. from Karantinnaya Bay than from Sevastopolskaya and Balaklavskaya Bays. The maximum content of some trace elements were also found in the thalli of *Cystoseira* spp. from Kruglaya Bay; it can be related to the increase of the seasonal recreational load.

In the “stems” of *Cystoseira crinita* growing in the relatively clean waters near Cape Fiolent, the elevated concentrations were found of such elements as Co, Sc, Zn, and V; it can be explained by the geological peculiarities of this study area rather than by the factor of anthropogenic pollution.

The results of our study showed that the “stems” of 1-3 years old plants of *Cystoseira barbata* and *C. crinita* sampled in the summer season could be used as biomonitors of anthropogenic water pollution in the coastal zone of the Black Sea.

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OCENA AKUMULACJI PIERWIASTKÓW W CZARNOMORSKICH GLONACH BRUNATNYCH *Cystoseira* Z WYKORZYSTANIEM NEUTRONOWEJ ANALIZY AKTYWACYJNEJ

Abstrakt: Po raz pierwszy oznaczono stężenia 26 makro- i mikroelementów (Na, Mg, Al, Cl, K, Ca, Sc, V, Mn, Fe, Co, Ni, Zn, As, Br, Rb, Sr, Sb, I, Cs, Ba, Sm, Nd, Ag, Au i U) w plechach brunatnic *Cystoseira barbata* C. Ag. i *Cystoseira crinita* (Desf.) Bory. Stężenia pierwiastków w próbkach czarnomorskich glonów, zebranych w okolicy Sewastopola (południowo-zachodni Krym), oznaczono z wykorzystaniem instrumentalnej neutronowej analizy aktywacyjnej (INAA). Szczególny charakter akumulacji pierwiastków wskazuje, że glony *Cystoseira* spp. mogą być używane jako biomonitor zanieczyszczenia wód przybrzeżnych na badanym obszarze.

Słowa kluczowe: biomonitoring, pierwiastki śladowe, *Cystoseira barbata*, *Cystoseira crinita*, neutronowa analiza aktywacyjna, Morze Czarne