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COMPARISON OF METAL UPTAKE CAPACITIES OF THE BROWN ALGAE *CYSTOSEIRA BARBATA* AND *CYSTOSEIRA CRINITA* (PHAEOPHYCEAE) COLLECTED IN SINOP, TURKEY

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ABSTRACT: *Cystoseira barbata* (Stackhouse) C. Agardh, 1820 and *Cystoseira crinita* Duby, 1830 widely distributed and dominant in the Black Sea collected from Sinop coastal zone to monitor the current situation of regional metal pollution during the 2015-2016.

Macro-algae, sediments and seawater samples were analyzed for metal contents (Al, As, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn) using inductively coupled plasma mass spectrometry (ICP-MS). The methodology was provided by using standard reference material BCR 279 – *Ulva lactuca*.

The general metal uptake of *Cystoseira* spp. displayed a tendency in the order of Hg<Cd<Co<Pb<Ni<Cu<As<Mn<Zn<Fe=Al. Accumulation of arsenic showed the highest levels in both seawater and sediments (BSAF>2 and BCF>5000) that was a good indicator for *Cystoseira* species. As a result of measured values, it is seen that Sinop region is below the pollution level. The observed results of the elemental accumulation noticed that *Cystoseira* spp. could be used regularly as a bio-monitor of coastal pollution in the study area.

KEYWORDS: Metals, macro-algae, monitoring, *Cystoseira barbata*, *Cystoseira crinita*, Black Sea

INTRODUCTION

The Black Sea is semi-enclosed sea and is the biggest anoxic basin in the world. Eutrophication or over-fertilization is one of the most important pollution facing the Black Sea and it also has the greatest impact. Many rivers are delivering steadily higher quantities of contaminants to the Black Sea. Sewage discharges into the sea, which become particularly heavy during the tourist season, are accused to be the cause of this catastrophe. The rapid growths of coastal towns and cities in the Black Sea, coupled with shortage of funds for proper urban development, have been disturbed on the coasts. The most important of these contaminants are heavy metals. Many heavy metals are discharged into the Black Sea from industry and mining. The impact they have ranges from gradual changes in plankton species, to lethal effects on other biota. Marine algae are the most affected organisms from these contaminants in the marine coastal environment, because they cannot move and are directly exposed to contaminants including heavy metals. Marine algae grow using dissolved minerals and energy from the sun. Herbivores feed on these algae and themselves fall prey to other animals. The metals are easily absorbed with food, but they are not readily excreted, and even organisms low in the chain can be affected by these contaminants.

Marine macro-algae are progressively used as suitable bio-monitors to describe eco-toxicological significant of costal metal pollution (García-Seoane *et al.* 2018). Although metals exist in either the water column or get deposited on the sediment beds, they do not estimate the toxicity to biota. It is known that benthic seaweeds are able to concentrate free metal ions from seawater and sediments, reflect indirectly the average levels and temporal variations of contaminants (Phillips and Rainbow 1994). The brown alga is the most preferable for pollution biomonitoring to assess environmental situation in coastal areas (Kravtsova *et al.* 2014). Their higher resistance to metals and responsiveness to environmental or anthropogenic changes make them to be useful indicators of water and ecosystem quality according to EU- Marine Strategy Framework Directive (MSFD 2008/56/EC).

Cystoseira is widely spread and used to monitor the pollution of the Black Sea coastal ecosystems by Zn, Cu, Fe, Pb, Ni, Cd, Mn and Co (Güven *et al.* 1992, 2007; Topçuoğlu *et al.* 1998, 2001, 2003; Altuğ *et al.* 2005; Arıcı and Bat 2016; Bat and Arıcı 2016; Arıcı 2017; Tüzen *et al.* 2009; Türk Çulha *et al.* 2010, 2013).

Sinop Province is faced to an increasing anthropogenic pressure owing to the growing population and domestic wastewater discharges (Bat and Baki 2014), alarming high levels of some heavy metals. The aim of this study was to identify metal uptake capacities and to compare the variabilities of metal levels in *Cystoseira* spp. distributed along Sinop coast of the Black Sea.

MATERIALS AND METHOD

Study area:

Sinop Province is located in the northeast point of the Turkish Black Sea exposed to domestic sewage, local fishing activities and tourism facilities (Bat *et al.* 2018). Sediments, seawater and *Cystoseira* species were collected in the upper littoral zone of Sinop during September 2015 and July 2016. Sampling was carried out from a total eight different contamination degrees sites (Fig. 1).

Sampling technique:

The brown macro-algae *Cystoseira* species were collected in the selected areas by hand, rinsed with seawater, kept in the polyethylene bags, labelled and transported to the laboratory. In the laboratory, algae were washed again with bi-distilled water and dried at 70^{o}C- 48 hours. Dried samples digested using a microwave digestion system (Milestone Systems, Start D 260) with Suprapur® HNO_3 (nitric acid) according to Aquatic plant HPR-FO-08 method (Milestone).

Sediment samples were taken with PVC cores from the vicinity of the collected macro-algae, dried at 105^{o}C for 24 hours. Grain sizes less than 63 µm (Förstner and Wittman 1983) were digested with Seawater Sediment HPR-EN-33 methodology (Milestone Systems, Start D 260).

250 cc of surface water samples were acidified with 1 ml of concentrated ultrapure HNO_3 and stored in polyethylene bottles until analysis.

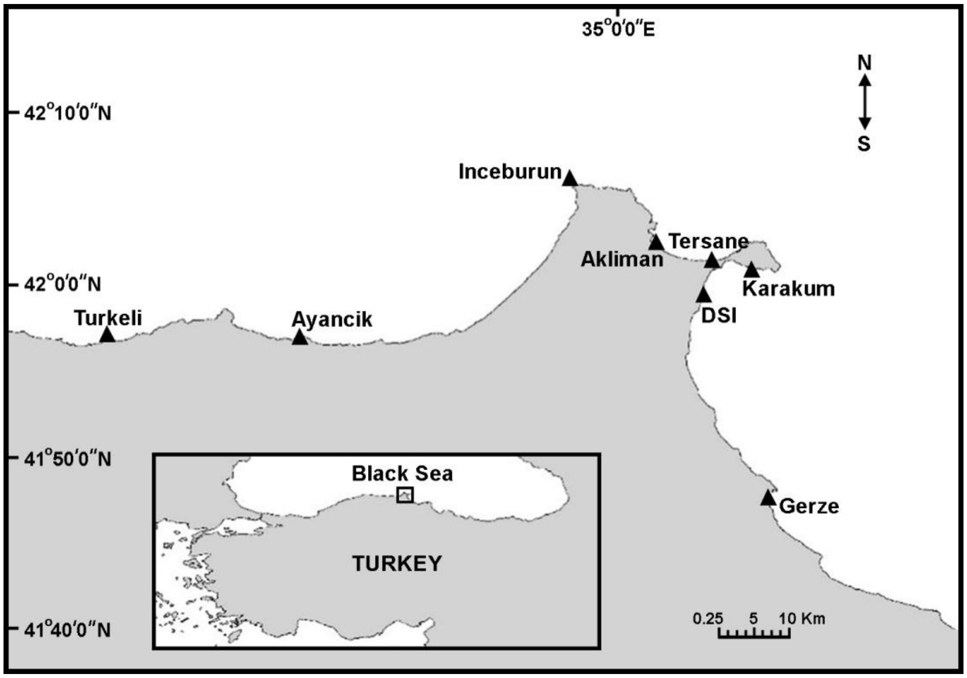

Fig. 1. Study area.

Analytical procedure:

Element analysis of samples were performed using ICP-MS (Inductively Coupled Plasma- Mass Spectrometer) (Agilent Technologies, 7700X) method by accredited SEM laboratory (SEM, 2016). The selected elements (Al, As, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn) in samples were determined, used three replicates and results of mean concentrations were detected as mg/kg dry wt. in algae and sediments, and ppb in water samples. The recovery study was validated by using a Certified Reference Materials (CRMs) (BCR 279- *Ulva lactuca*).

Parameters:

Interactions of metal concentrations between *Cystoseira* spp., seawater and sediments were compared measuring the Bio-Concentration Factor (BCF) and Biota-Sediment Accumulation Factor (BSAF) for each elements.

BCF is used to determine chemical concentration in the water as follows: C_{biota} / C_{water}, where C_{biota} is an average concentration of trace element in *Cystoseira* spp. (mg/kg); C_{water} is the concentration of metals from the coastal waters of Sinop shores (mg/l).

BSAF parameter describe bioaccumulation of metals into the ecological receptors (Kleinov *et al.* 2008) that is calculated according to following formulas: $C_{biota}/C_{sediment}$, where $C_{sediment}$ is the concentrations of elements in sediments.

Statistical analysis:

IBM_SPSS ver. 21.0 was used for statistical calculations. Compare tests were used for determining differences in metals between species, seasons and stations. One way analysis of variance (ANOVA) was used to evaluate inter specific significance between metal levels in different seasons and sites. Differences of metals between species were determined by used *t*-test and correlations of elements were determined with Spearman Correlation test. P-values of less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

The mean concentrations of eleven elements in the species, seawater and sediments were given in Table 1 for the study period. The brown algae *Cystoseira* species accumulated the elements in ascending order Hg < Cd < Co < Pb < Ni < Cu < As < Mn < Zn < Fe = Al; levels of elements in seawater followed the order of Hg < Cd < Co < As < Cu< Pb < Ni < Mn < Zn < Al < Fe; and elements levels of sediments were increased in the order of Hg < Cd < Co < Pb < As < Cu < Ni < Zn < Mn < Al < Fe. The essential element of Fe was observed higher in all samples (1162.9 mg/kg in *C. barbata*, 919.3 in C. *crinita*, 0.4732 mg/l. in seawater, 6251.7 mg/kg in sediment). Concentrations of Co, Ni and Pb exhibited variations depending on seasons in seawater; and Mn, Cd and Hg levels displayed significance depending on locations ($p \leq 0.05$).

Table 1. The mean±standard deviation for elements concentration in samples.

	***C. barbata* (ppm)**	***C. crinita* (ppm)**	**Seawater (ppb)**	**Sediment (ppm)**
Al	316.5±183.7	327.4±265.7	360.9±578.6	3528.8±2066.4
Mn	21.3±10.9	32.8±31.6	23.0±35.2	236.4±152.6
Fe	481.4±376	322.5±226.7	473.2±833.2	6251.7±3651.8
Co	0.50±0.4	0.57±0.4	1.57±0.6	2.72±1.6
Ni	4.44±2.6	4.11±2.4	4.71±2.6	10.03±8.4
Cu	10.2±7.3	4.27±3.9	2.78±2.7	4.85±5.1
Zn	59.5±24.9	47.89±20.9	288.4±203.8	12.8±9.6
As	18.17±9.9	19.09±16.9	1.82±1.3	4.23±1.63
Cd	0.20±0.1	0.23±0.1	0.42±0.2	0.05±0.03
Hg	0.01±0.0	0.01±0.0	0.06±0.2	0.02±0.02
Pb	1.44±0.7	0.87±0.8	2.96±4.2	2.92±1.9

The results for uptake of elements indicated statistically notable differences in levels between different seasons and stations (Table 2). Zinc and cadmium collapse over time to sea floor and accumulate higher amounts in sediments. It is pointed out that levels exceeding 100 mg/kg for Zn in macro-algae indicate anthropogenic contamination (Storelli *et al.* 2001). *C. crinite* showed statistically regional variation for Cu, and seasonal differences for Zn, As and Cd values ($p \leq 0.05$, ANOVA) depending upon human impact. There was no significantly difference between species (p: 0.82, Independent samples *t*-test).

Table 2. Comparison of statistical differences of species (α: 0.05, ANOVA).

	Cystoseira barbata		*Cystoseira crinita*	
	Seasonal variation	**Regional variation**	**Seasonal variation**	**Regional variation**
Al	0.61	0.90	0.25	0.77
Mn	0.18	0.96	0.11	0.40
Fe	0.33	0.96	0.13	0.04
Co	0.21	0.99	0.12	0.63
Ni	0.26	0.99	0.17	0.29
Cu	0.40	0.92	0.77	0.00*
Zn	0.11	0.94	0.02*	0.90
As	0.47	0.65	0.02*	0.74
Cd	0.54	0.92	0.02*	0.90
Hg	0.69	0.96	0.18	0.74
Pb	0.56	0.88	0.85	0.45

*p-value is less than 0.05 that there is difference between the means.

Table 3. BSAF and BCF values of *Cystoseira* species.

	C. barbata	***C. crinita***	***C. barbata***	***C. crinita***
	BCF	**BCF**	**BSAF**	**BSAF**
Al	876.9	907.2	0.08	0.09
Mn	926.1	**1426.1**	0.09	0.13
Fe	**1017.3**	681.5	0.07	0.05
Co	318.5	363.1	0.18	0.20
Ni	942.7	872.6	0.44	0.40
Cu	**3669.1**	**1535.9**	**2.10**	0.88
Zn	206.3	166.1	**4.64**	**3.74**
As	**9983.5**	**10489**	**4.29**	**4.51**
Cd	476.2	547.6	**4.00**	**4.60**
Hg	166.7	166.7	0.50	0.50
Pb	486.5	293.9	0.50	0.29

BCF > 1000 (bio-accumulative) and BSAF > 2 (macro-concentrator) values are shown in bold.

Table 4. Element studies of *Cystoseira* sp. along the Turkish coast.

Region	Al	As	Cd	Cr	Co	Cu	Fe	Hg	Mn	Ni	Sb	Se	Pb	Zn	Ref.
							Aegean Sea								
[a]İzmir			1.35			30.60	7644.00						15.00	90.60	1
[a]Çanakkale			0.05			3.23	239.20		35.71				0.01	41.02	2
[a]İzmir			0.10			3.02	154.90		13.18				0.01	19.00	2
[b]Marmaris			0.63			8.12	462.30		95.32					49.10	2
[a]Çanakkale			0.06			3.78	314.01		50.34				0.01	56.19	2
[a]Çanakkale			0.04			2.67	164.35		21.08					25.84	2
[a]İzmir			0.01			3.78	97.62		17.93					10.81	2
[a]İzmir			0.19			2.25	212.14		8.43				0.01	27.17	2
[b]Marmaris			0.10			4.95	398.16		59.27					35.64	2
[b]Marmaris			0.17			11.28	526.38		131.37					62.48	2
[a]Çanakkale			0.02			0.11							0.09	0.64	3
[a]Çanakkale			0.02			0.15							0.10	0.70	3
[a]Çanakkale			0.03			0.16							0.12	0.77	3
[a]Çanakkale			0.01			0.07							0.08	0.54	3
[a]Çanakkale			0.01			0.08							0.05	0.39	3
[a]Çanakkale			0.01			0.12							0.16	0.78	3
[a]Çanakkale			0.02			0.07							0.03	0.67	3
[a]Çanakkale			0.09	2.13		6.73	302.30	0.12					0.01	75.50	4
[a]Çanakkale			0.28	4.76		3.13	186.60	0.05						36.90	4
[a]Çanakkale			0.02	3.29		5.29	96.80	0.06						23.80	4
[a]Çanakkale			0.31	2.65		2.37	230.00	0.08						40.50	4
[a]Çanakkale			0.11	1.36		5.69	328.70	0.09						51.50	4

Continued………

Region	Al	As	Cd	Cr	Co	Cu	Fe	Hg	Mn	Ni	Sb	Se	Pb	Zn	Ref.
[a]Çanakkal			0.27	44.80		16.10	484.10	0.09						79.30	4
Black Sea															
[a]İstanbul			1.20			4.20	348.00		17.40				5.80	55.10	5
[a]Sinop			2.40			7.90	3414.00		73.30				12.80	85.80	5
[a]İstanbul			2.00			8.79	474.00		81.60				8.70	69.10	5
[a]Sinop			1.40			5.70	654.00		21.70				6.80	64.40	5
[a]İstanbul			2.20			3.20	317.00		14.40				5.30	36.90	5
[a]Sinop			1.30			4.20	446.00			25.00			5.30	12.10	5
[a]İstanbul			1.00			11.80	124.00			35.00			5.50	18.10	5
[a]Kırklareli							100.00		21.45					7.00	5
[a]İstanbul							164.00							49.10	6
[a]İstanbul			0.75	0.60	0.95	5.10	230.50		21.45	6.20	7.50		7.50	97.20	7
[a]İstanbul			0.70		0.90	6.20	166.60		23.00	5.90	1.00		1.00	59.30	7
[a]İstanbul			0.35	0.75	0.60	4.80	526.60		22.15	2.20	1.00		1.00	50.40	7
[a]İstanbul			0.40	0.95	0.65	6.85	1066.00		24.85	4.50	14.00		14.00	65.10	7
[a]İstanbul			0.02	0.06	0.05	5.70	427.00		32.10	9.10			0.10	35.10	8
[a]İstanbul			0.02	0.06	0.05	2.20	130.00		6.70	0.10			0.10	13.90	8
[a]İstanbul			0.78	0.06	0.05	3.43	133.00		12.00	5.70			1.40	21.70	8
[a]Sinop			0.02	0.06	0.05	5.70			27.30	7.20			0.10	43.90	8
[a]Sinop			0.20	0.06	0.05	6.00			22.70	5.70			0.10	191.50	8
[a]Sinop			0.09	1.20	1.78	1.70			33.50	4.70			3.50	6.50	8
[a]Sinop			1.02			16.40	560.00						2.10	48.00	9
[a]Sinop			1.03			5.70	540.00						2.30	46.00	9

Continued………

Region	Al	As	Cd	Cr	Co	Cu	Fe	Hg	Mn	Ni	Sb	Se	Pb	Zn	Ref.
aKırklareli			0.13	2.50		6.90			57.20				0.20	8.30	10
[a]Sinop				0.99	9.05	2.47	242.00		14.90	2.05		0.09		6.62	11
aSinop					2.72	3.91	373.00			5.88			0.01	10.97	12
aSinop					1.02	5.33	81.00			0.95			0.01	20.47	12
aSinop					2.53	2.01	455.00			0.79			0.01	0.08	12
aSinop					0.01	4.42	184.00			9.26			0.01	13.22	12
aSinop					0.61	5.87	991.00			7.47			0.01	6.84	12
aSinop					0.99	6.03	272.00			2.17			0.01	4.65	12
aSamsun			0.01		0.38	6.53	534.43			4.60			0.01	0.20	13
aKastamonu			0.01		0.01	16.33	283.67			14.30			0.01	15.30	13
aOrdu			0.01		1.28	5.06	632.55			3.37			0.01	5.83	13
aSinop						4.02	536.00			5.03			0.01	10.28	13
aSinop							327.00								14
aSamsun														65.00	14
aSinop										0.80					14
aSinop						5.00									14
aInebolu						37.00									14
aSinop					0.20										14
aInebolu					1.50										14
aKırklareli			0.11			10.00	878.00		33.00	1.00			1.20	21.00	14
aKastamonu			0.23			37.00	1151.00		55.00	2.10			1.40	58.00	14
aSinop			0.13			5.00	327.00		11.00	0.80			1.00	44.00	14
aSamsun			0.32			25.00	1250.00		43.00	0.90			1.30	65.00	14

Continued………

Region	Al	As	Cd	Cr	Co	Cu	Fe	Hg	Mn	Ni	Sb	Se	Pb	Zn	Ref.
[a]Sinop			1.20		2.40	4.80	748.00		33.00	3.80			8.00	65.00	15
[a]Sinop						3.10	308.00		2.40	1.74				13.00	15
[a]Sinop			1.30		0.30	7.00	2143.00		64.00	15.00			10.00	76.00	15
[a]Sinop			0.09		1.65	1.30	261.00		19.00	2.30			1.50	5.00	15
[a]Sinop			0.86			4.05	865.00		29.60	5.71			6.50	39.75	15
[a]Sinop	316.51	18.17	0.20		0.50	10.19	481.41	0.01	21.26	4.44			1.44	59.53	16
[a]Sinop	339.29	18.66	0.23		0.56	4.43	305.34	0.01	34.33	4.16			0.96	47.40	16
Marmara Sea															
[a]Bosphorus		78.90	0.90	1.10	0.90	9.00	472.00						9.50	15.00	17
[a]Bosphorus		69.10	0.55	1.00	3.59	5.15	210.00						4.20	35.60	17
[a]Bosphorus		52.60	1.38	1.00	2.47	4.60	216.00						7.30	12.40	17
[a]Bosphorus		83.50	0.58	0.30	2.00	6.13	615.00						7.50	27.90	17
[a]İstanbul			0.02			84.75	812.50		83.70	6.23			1.90	77.70	18
[a]Tekirdağ			0.02	8.63	1.76	5.20	1511.00		143.60	12.36			3.70	113.50	18
[a]Tekirdağ			0.02	0.06	0.05	164.30	114.00		23.80	0.10			0.10	41.80	18
[a]Tekirdağ			0.02			5.20	1511.00		143.60	12.36			3.70	113.50	19
Mediterranean Sea															
[d]İskenderun			0.39			2.05	170.81						2.21		20
[d]İskenderun			0.42			2.23	186.30						6.64		20
[d]İskenderun			0.42			2.23	186.34						6.64	15.87	21
[a]Antalya			0.01		0.50	7.27	310.75			0.01			0.01	21.12	13

[a]*Cystoseira barbata*, [b]*Cystoseira* sp.,[c]*Cystoseira crinita*, [4]*Cystoseira corniculata*,[1]Çetingül and Aysel 1998, [2]Akçalı and Küçüksezgin 2009, [3]Üstünada *et al.* 2011,[4]Akçalı and Küçüksezgin 2011,[5]Güven *et al.* 1992, [6]Topçuoğlu *et al.* 1998, [7]Topçuoğlu *et al.* 2001, [8]Topçuoğlu *et al.* 2003, [9]Altuğ *et al.* 2005, [10]Güven *et al.* 2007,[11]Tüzen *et al.* 2009,[12]Türk Çulha *et al.* 2010,[13]Türk Çulha *et al.* 2013,[14]Arıcı and Bat 2016, [15]Bat and Arıcı 2016,[16]Arıcı 2017,[17]Kut *et al.* 2000,[18]Topçuoğluı *et al.* 2004, [19]Topçuoğlu *et al.* 2010,[20]Piner Olgunoğlu and Polat 2007, [21]Olgunoğlu 2008.

The uptake concentration ratios of heavy metals from seawater and sediments were also shown in Table 3. The BCF is a parameter to define environmental assessment between an organism and the surrounding water. United States Environmental Protection Agency under the Toxic Substances Control Act (TSCA) and REACH framework (1907/2006 EC) are considered very bio-accumulative, if it has a BCF greater than 5000. High level of As contamination was detected (9983.5 for *C. barbata* and 10489.0 for *C. cirinita*). The BSAF is also a reference measurement for describing degrees of uptake elements from sediments. It is in the range of 1 to 2; BSAF>2 macro-concentrator, 1<BSAF<2 micro-concentrator and BSAF<1 de-concentrator are named as (Nenciu *et al.* 2016). Both the *C. barbata* and *C. crinite* were found macro-concentrator (BSAF>2) for Zn, As and Cd. Besides, Cu accumulation from sediment was determined by *C. barbata*.

The significant negative correlation were found between As-Co, As-Cu, As-Pb and As-Fe (r<0.2, Spearman correlation). The level of arsenic was noticed generally higher in sampled *Cystoseira* species. Arsenic is accumulated more, due to high phosphate concentrations in brown macro-algae (Phillips, 1990) that make *Cystoseira* sp. a good indicator especially for As.

Heavy metal content in *Cystoseira* species is mostly more than seawater. Kravtsova *et al.* (2014) also observed 3-4 orders of higher concentrations of trace elements in *C. barbata* and *C. crinita*. Thus, it could be used as bio-monitor to assess environmental situation. Due to their sensitivity to pollutants and anthropogenic impacts, *Cystoseira* is the most preferred macro-algae to monitor coastal areas as useful indicators of environmental health within EU-MSFD (MSFD, 2008/56/EC).

The rate of element accumulation by marine macro-algae depends on abiotic (element concentration in the environment, speciation, salinity, temperature, light *etc*.) and biotic (metabolism of plants, morphology, taxonomic identity, physiological state *etc*.) factors (Burdin and Zolotuhina 1998). Differences in metal concentrations in macro-algae depend on sampling location, plant age, physicochemical properties and other interactions between species (Sawidis *et al.* 2001). Compared to previous studies along coastline of Turkey (Table 4), *Cystoseira* species showed different affinities of metal uptake.

The data on the metal concentrations noticed in *Cystoseira* species are used for evaluating the environmental quality as well as for the comparison between the various waters. Comparing our results with those of others, it could be said that concentrations of elements correspond to the levels found below the pollution levels. Although, concentrations are below the limit levels, the study area must be regularly monitored by predominant *Cystoseira* species due to high anthropogenic inputs.

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