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Research Article

Study of Multielement Concentration in Bulgarian Black Sea Green Macroalgae

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Abstract

The ecological status at eight coastal locations (Shabla, Kaliakra, Tuzlata, Ravda, Ahtopol, Sinemoretz and Rezovo) along the Bulgarian Black Sea coast was studied by determining of 32 elements in 121 samples of eight green Macroalgae species *Ulva rigida*, *Ulva lactuca*, *Enteromorpha, Enteromorpha compressa*, *Cladophora vagabunda*, *Cladophora coleotrix*, *Chaetomorpha gracilis* and *Bryopsis plumosa* during the period 1996-2011. The element concentrations were determined by Energy Dispersive X Ray Fluorescence Analysis (EDXRFA).

Results for 19 elements in the three most widespread green algae *Ulva rigida*, *Enteromorpha intestinalis, Cladophora vagabunda*, are compared in all the locations along the Bulgarian coast to evaluate the level of accumulated elements. It was shown that *Ulva rigida* and *Bryopsis plumosa* species accumulate studied elements less than the other species and the heavy metal concentration in green Macrophytes from Tuzlata, Ravda and Sinemoretz is higher compared to the other locations.

The obtained data show that no serious contamination by hazardous elements in green algae along the Black Sea shore during the studied period was found and some of the studied green algae species are suitable for heavy metal assessment in Black Sea marine ecosystems. The obtained results establish a database for element concentration in green Macroalgae and their ecological impact on marine ecosystems along the Bulgarian Black Sea coast.

Keywords: Black Sea; Green Macroalgae; Trace Metals; XRFA Method

Introduction

The ecological monitoring of marine ecosystems can be performed by controlling marine organisms that have been successfully used as bio-indicators. The bio monitors are important indicators for evaluation of various contaminants impact on marine ecosystems. Macroalgae have been used as indicators for trace elements, because of their accumulation capacity and influence along the trophic chaining the marine environment [1-3]. Algae can bio concentrate amounts of biologically significant elements in their tissues. Some elements are essential for aquatic plant growth and their biological uptake is a function of the free element concentration in seawater. However, increase in element concentrations above certain values can be toxic to marine biota. Some algae have mutation capability so they can survive the ecological stress of the contamination. The determination of widespread bio monitors will allow comparison of accumulated element concentrations over large areas [4-5].

The Black Sea is a low salinity basin, half-isolated from the Mediterranean and its hydrology and phytobentos concentration is different from the other seas in the same area. Toxic elements (and nuclides) are introduced by airborne contaminants, rivers, oil pollution or by direct discharge of industrial wastes into the sea. Trace elements are transported via the seawater currents, coming from the Danube and other rivers. The ecological conditions at the coastal zone are diverse which results in element concentration variations in algae depending on many factors - species type, geographical location, season etc.

Green Macroalgae have been extensively used to monitor marine pollution in various geographical areas [6-9]. Green algae are among the widespread at the Bulgarian Black Sea coast, some of them (*Ulva rigida, Enteromorpha intestinalis, Cladophora* *vagabunda*) can be met at almost all areas, so they are appropriate for comparative assessment of contaminants concentration in different geographical areas. Green Macroalgae species have been used as bio-indicators in the Black Sea and other marine environments in neighboring seas [10-15]. Trace element concentrations in Black Sea green alga species have been determined by other authors [16-19] while data about heavy and toxic metals at the Bulgarian Black Sea coastal zone is scarce and insufficient. The purpose of this paper is to study the level and accumulation of 32 elements in eight macrophytes, collected from nine locations along the Bulgarian Black Sea coast during the period 1996 - 2011. It is important to determine whether these algae can be used as bio-indicators for some element pollution and evaluate the impact on the marine ecosystem habitat. Energy-dispersive X-Ray fluorescence method (EDXRFA) was used for trace element determination.[20-21]. This method was applied because of its Multielement capability, nondestructive analysis, simple sample preparation, relative little time consuming, sufficient sensitivity, accuracy and good reproducibility. Compared to ICP, the amount of analyzed sample is bigger (30 g), which reduces the influence of inhomogeneity error.

Materials and Methods

Sampling sites in this study were selected to cover the whole Bulgarian coastline from north to south - Shabla, Kaliakra, Tuzlata, Rossenetz, Ravda, Sozopol, Ahtopol, Sinemoretz and Rezovo and to possess abundant algae species. Eight green Macroalgae species (*Ulva rigida, Ulva Lactuca*, *Enteromorpha Enteromorpha compressa, Cladophora vagabunda, Cladophora coleotrix, Chaetomorpha gracilis,* and *Bryopsis plumosa*) were collected during the period between spring 1996 and spring 2011.The algae were collected in the subtidal zone at 1 - 4 m depth and those abundantly covered with epiphyte were ejected. The samples were rinsed in clean seawater, followed by distilled water, dried to constant weight (85°C) and the grinded 0.5 grams of three subsamples.

Three spectrometric systems are available in EDXRFA Lab. The first one is equipped with Si(Li) detector with 12 μm Be window and 170 eV energy resolution at 5.9 KevMN-$K_\alpha$line (PGT). The data are acquired with a multichannel analyzer (AX-1), interfaced to a personal computer, that applies specialized software for data acquisition and spectra processing. This system is combined with an exciting head, based on an annular source ^{241}Am (3.7 GBq) (AMERSHAM) and three types of secondary targets (Mo, Dy, Sn) in two excitation modes - transition and reflection in order to select the desired exciting energy. For determination of heavy elements, which are essential for biology the system uses secondary target of Dy, that allows detection of elements down to the Z = 50. This construction permits the measurement of great number of elements with a high sensitivity by achieving the desirable experimental conditions of low background level and at a high count rate.

The second system is equipped especially for light element analysis with Si pin diode detector, Peltie cooling, with 7 μm Be window and 160 eV (KETEK). The determination is performed with three radionuclide sources ^{55}Fe(AMERSHAM) by means of an exciting head, especially designed for low Z elements. In this case the construction, the distances between the source and sample and sample-detector are greatly reduced and optimized, which allows elements with atomic number Z = 13 to 25 to be analyzed.

The third system is equipped with SDD detector with 0.7 μm Be window and 170 eV energy resolution at 5.9 KevMN-Kline (KETEK). An exciting head with source of ^{238}Pu is specially designed for analyzing of elements with Z between 17 and 35. This head is very suitable for analyzing very low concentrations of these elements. The calibration procedures for each spectrometer are based on set of international standards like Bowen's kale, orchard leaves, grass (IAEA - V10), fish flesh (IAEA), copepod (IAEA), Nettle etc. The results were checked with IAEA standard - lichen. Lichen was chosen because of the similar matrix with the green algae due to their similar biochemistry. The Lab has also taken part in two International Inter compression Projects - Worldwide Proficiency Test for X-Ray Laboratories PTXRFIAEA/05 and 06 IAEA (2009). The LLD for elements with Z between 13 and 14 is about 1000 μg/g, for Z between 15 and 20 - about 500 μg/g, for Z between 22 and 56 about 1 to 2 μg/g. The LLD for elements Hg, Pb and U is about 5 μg/g.

Results and Discussion

There is a dependence between microelement bio sorption and their function in the organism. Most intensively accumulated are those microelements that play a major part in the metabolism. A considerable number of elements form stable complexes with proteins, phosphates and lipids. Fe, MN, and Co take part in enzyme formation and as complex compounds participate in exchange reactions. The enriched in elements lipids from algae play a major biogeochemical role for the processes of fixation, transport and sedimentation in the hydrosphere.

The selected green Macroalgae species are suitable for application because they possess potential qualities to be used as bio-indicators, the algae are sufficiently widespread, characteristic for the studied areas, possess enough biomass and are easily collected; and they react to the environmental changes. 32 elements have been measured using EDXRFA in 121 green algae samples - 25 for *Ulva rigida*, 20 for E17 for *C. vagabunda*, 14 for *Ulva lactuca* etc. in the period 1996 - 2011. Data for 19 elements are presented in this paper. The concentrations of some elements are below the LLD limits of the method and the specific spectrometric systems. The concentrations of the most interesting macro and trace elements are presented in tables and graphs.

It is interesting to evaluate the impact of elements accumulation in a certain algae species in all locations along the Black Sea coast. This evaluation can be done at sites where multiple sampling has been performed during the years and where most of most abundant algae were collected. For this reason, we have chosen

Ulva rigida, E. intestinalis and *C. vagabunda* species to assess the ecological status of the coast. The results for 19 detected elements (Figure 1) concentrations in the three most widespread green Macroalgae species from the Bulgarian Black Sea coast, are presented in (Table 1) for *Ulva rigida* (Figure 2-6) (Table 2) for *E. intestinalis* (Figure 7-11) and in (Table 3) for *C. vagabunda* (Figure 12 -16). The measured high element concentrations in algae (2000 - 50000 µg/g) are those for the microelements - Al, Si, P, S Cl, K, Ca, the highest of which are for Cl and S. Cl is essential for the algae metabolism and these high concentrations are understandable. The amount of S in marine algae is much higher, then the terrestrialplants, which is probably due to the dissolved sulfates in the sea water which are sorted by the algae. Fe, Br and Sr concentrations are with one or two orders of magnitude higher than the other elements in all algae species.

Figure 1: Map of sampling locations.

		Si	P	S	Cl	K	Ca	Ti	Cr	Mn	Fe	Cu	Zn	Br	Rb	Sr	Sn	I	Ba	Pb
Site	Season	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]
	Spring	10250	2900	21750	45100	18800	14100	30	31	73	710	2	39	380	21	140	12	47	23	13
Tuzlata	1996	±500	±300	±500	±1600	±400	±500	±3	±3	±2	±25	±1	±2	±15	±2	±3	±2	±2	±2	±2
	Autumn	7700	2750	21800	29400	13700	12300	28		71	500	2		290	14	110	10	26	29	23
Tuzlata	1996	±500	±250	±500	±1300	±400	±300	±2		±2	±20	±1		±11	±2	±8	±1	±1	±1	±2
	Autumn	7600	2650	23500	18700	11000	11100	35		72	415			210	6	100		28	12	3
Ahtopol	1996	±1500	±300	±800	±1200	±850	±500	±3		±3	±20			±15	±2	±8		±3	±2	±2
	Autumn	8000	2500	26800	14300	11500	10300	27	34	74	460	5	27	258	20	121	12	52	22	20
Ravda	1996	±800	±300	±1000	±900	±700	±350	±2	±2	±2	±10	±1	±3	±4	±2	±5	±3	±3	±2	±5
	Autumn	10500	2100	13300	36850	14700	13300	32		65	730	10	33	336	10	152		70	112	6
Sinemorez	1996	±1200	±250	±850	±1200	±850	±160	±2		±5	±35	±2	±2	±8	±2	±9		±4	±10	±3
	Summer	6700	2000	23200	11600	13200	10900	30	35	71	500	4	31	288	23	156	9	44	32	24
Ahtopol	1997	±500	±200	±700	±200	±450	±200	±3	±3	±2	±20	±2	±2	±12	±2	±6	±2	±2	±3	±3
Maslen	Spring	7900	2200	17300	21300	17000	11100	23	55	73	450	5	34	372	22	109	12	48	28	30
nos	1998	±900	±500	±800	±850	±350	±120	±3	±5	±2	±30	±2	±3	±5	±3	±9	±2	±2	±3	±6
	Spring	7000	2200	23200	21200	11400	12300	24		73	440	3		371	15	160	16	56	40	10
Ahtopol	1998	±850	±500	±1200	±1200	±400	±400	±1		±2	±5	±1		±15	±2	±7	±1	±4	±4	±2
	Spring	11000	2300	25000	14600	14200	11300	40	24	73	460		23	228	14	103	8	27	19	10
Ravda	1999	±2000	±500	±600	±700	±800	±300	±5	±2	±2	±10		±2	±10	±3	±8	±2	±2	±3	±3
	Summer	11200	1470	23200	20600	12000	12400	47		71	650	1	11	453	22	106	6	39	14	9
Shabla	2000	±1500	±200	±1200	±600	±120	±120	±3		±3	±30	±1	±2	±10	±2	±8	±2	±3	±2	±3
	Summer	8400	2600	27200	19000	11860	11070	31	30	71	480	3	48	464	20	116	12	79	26	6
Kaliakra	2000	±800	±250	±2000	±1500	±1000	±600	±3	±3	±2	±20	±1	±4	±8	±2	±4	±2	±7	±3	±3
	Summer	15000	2500	30000	27120	13500	14000	64	30	73	460	2	33	378	16	156	11	48	21	7
Ahtopol	2000	±1500	±250	±1000	±1000	±500	±500	±6	±3	±3	±25	±2	±2	±15	±2	±12	±2	±2	±2	±2
	Summer	11800	1700	26700	16600	15400	12150	57	32	73	610	5	20	225	12	153	8	30	28	16
Sinemorez	2000	±1000	±220	±1000	±200	±600	±600	±5	±3	±2	±15	±2	±2	±5	±2	±7	±3	±3	±2	±4
	Autumn	9800	3400	28500	17300	16300	14800	37	40	69	600	3	26	254	9	303	10	43	20	10
Varvara	1996	±850	±200	±1000	±600	±800	±850	±3	±4	±3	±40	±1	±2	±12	±2	±6	±3	±2	±3	±3
	Spring	12700	3000	29000	40200	11000	14200	44		68	560	6		400	16	114	13	41	45	5
Shabla	2003	±1200	±250	±1500	±850	±750	±600	±4		±5	±30	±2		±7	±2	±10	±2	±2	±3	±5
	Spring	12500	3500	31250	24600	11800	12250	70	35	63	470	4		340	11	104	9	45	78	7
Kaliakra	2003	±1000	250	±850	±1300	±800	±450	±5	±2	±6	±10	±1		±20	±2	±5	±1	±2	±5	±3
	Spring	13300	2600	30000	21400	11900	14200	37	34	54	360	1	10	383	9	305	13	40	60	10
Tuzlata	2003	±400	±300	±650	±1000	±400	±700	±4	±4	±4	±20	±1	±2	±15	±2	±10	±2	±2	±4	±3
	Spring	16200	4100	16300	26200	23000	12400	50		72	950	22	81	820	20	92	28	66	120	
Bjala	2004	±1000	±400	±500	±1000	±1000	±200	±2		±5	±45	±2	±5	±20	±2	±5	±3	±5	±5	
	Spring	10300	4100	26800	20100	15100	12200	50			300		24	390	11	131	12	57	27	12
Ravda	2005	±1000	±400	±1200	±600	±400	±600	±5			±50		±3	±20	±2	±12	±3	±3	±3	±3
	Spring	23000	1850	20700	19900	10300	15400	57		73	1100	23	30	284	10	262	14	51	72	3
Pomorie	2005	±2000	±150	±350	±750	±250	±1000	±5		±3	±100	±3	±2	±6	±2	±8	±2	±5	±4	±3
	Autumn	11700	2450	22800	17400	15800	10900	56	23	61	480	6	25	262	10	140	8	37	18	5
Perla	2005	±500	±150	±1000	±1500	±1000	±600	±2	±2	±6	±10	±1	±2	±10	±2	±12	±2	±1	±2	±2
	Spring	8500	1800	26000	16600	11000	10800	27		70	435	5		450	12	86	6	40	5	
Varvara	2005	±500	±200	±1200	±300	±250	±150	±3		±5	±45	±2		±25	±2	±8	±2	±3	±2	
	Spring	9500	2600	31800	21600	12000	11500	53			400			464	9	126		60	21	
Ahtopol	2005	±500	±250	±1500	±1100	±750	±500	±5			±20			±10	±1	±3		±6	±3	
	Spring	12500	2500	28600	15200	12600	11300	43		72	450	5	30	404	8	113	11	30	8	4
Rezovo	2005	±500	±200	±1000	±500	±500	±200	±2		±2	±25	±2	±3	±14	±2	±2	±2	±2	±3	±2
	Autumn	9800	1900	28300	22000	13000	14500	16	32	67	470	9	30	311	12	195	13	68	10	5
Sozopol	2011	±800	±200	±800	±300	±500	±300	±3	±5	±5	±25	±3	±2	±5	±2	±8	±2	±3	±2	±5

Table 1: Element concentratioin in *Ulva rigida*.

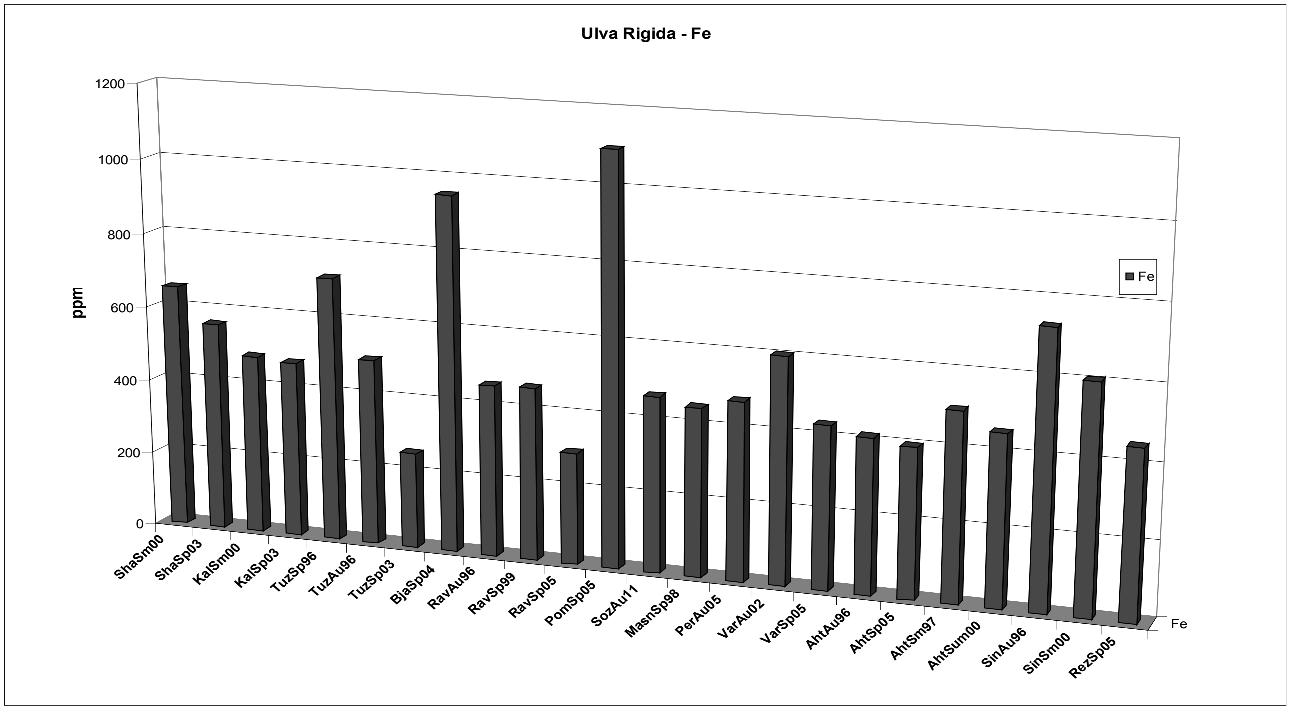

Figure 2: Fe concentration in *Ulva* species.

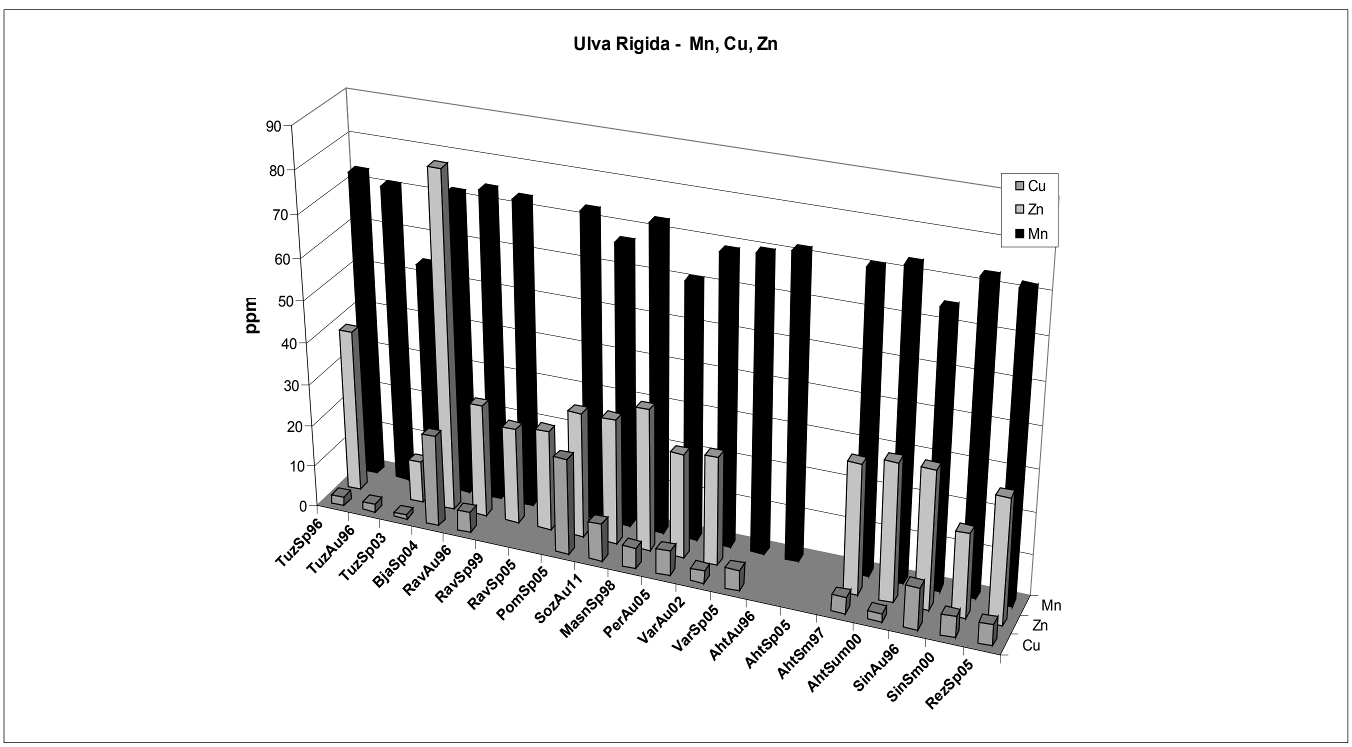

Figure 3: MN, Cu, Zn concentration in *Ulva* species.

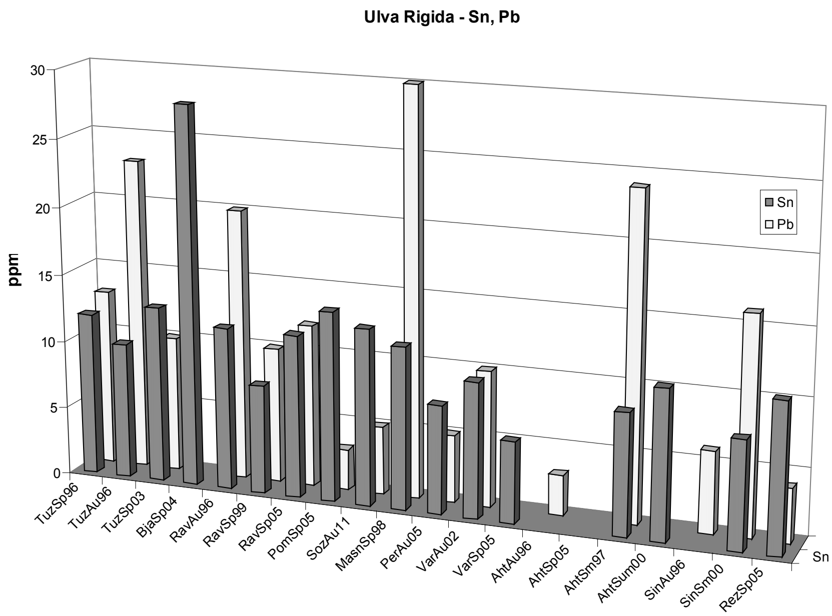

Figure 4: Sn and Pb concentration in *Ulva* species.

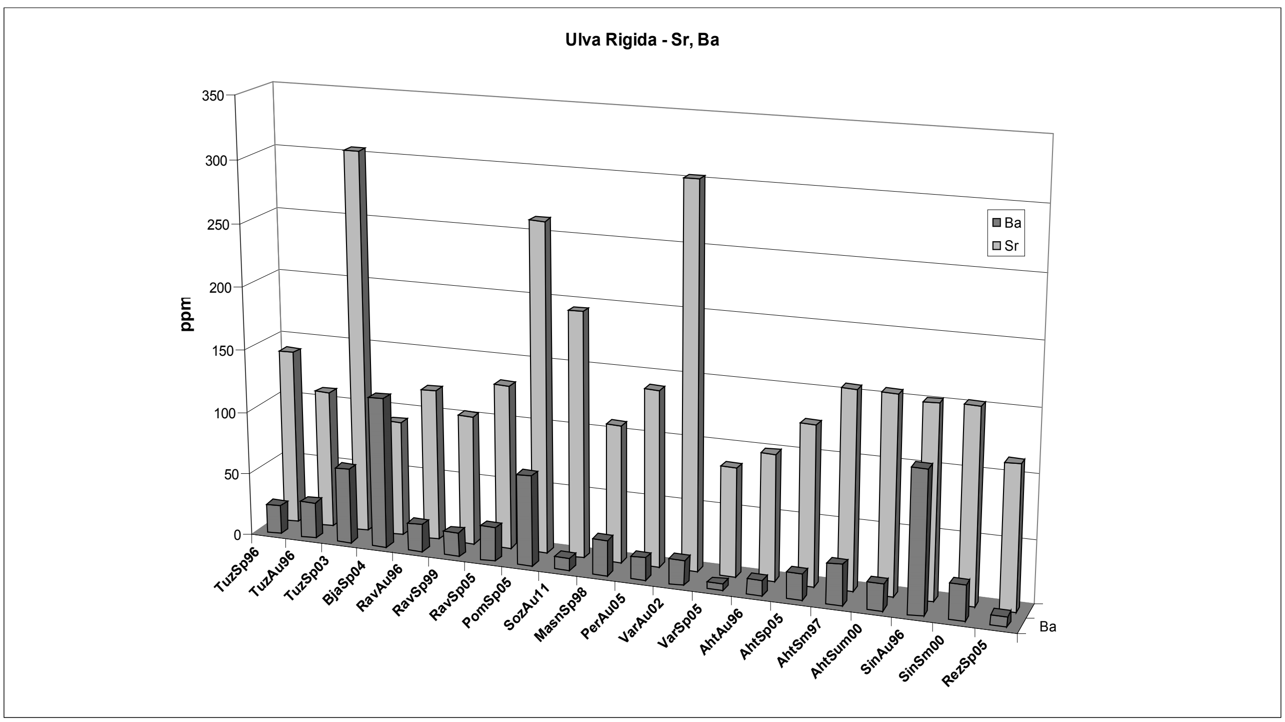

Figure 5: Sr and Ba concentration in *Ulva* species.

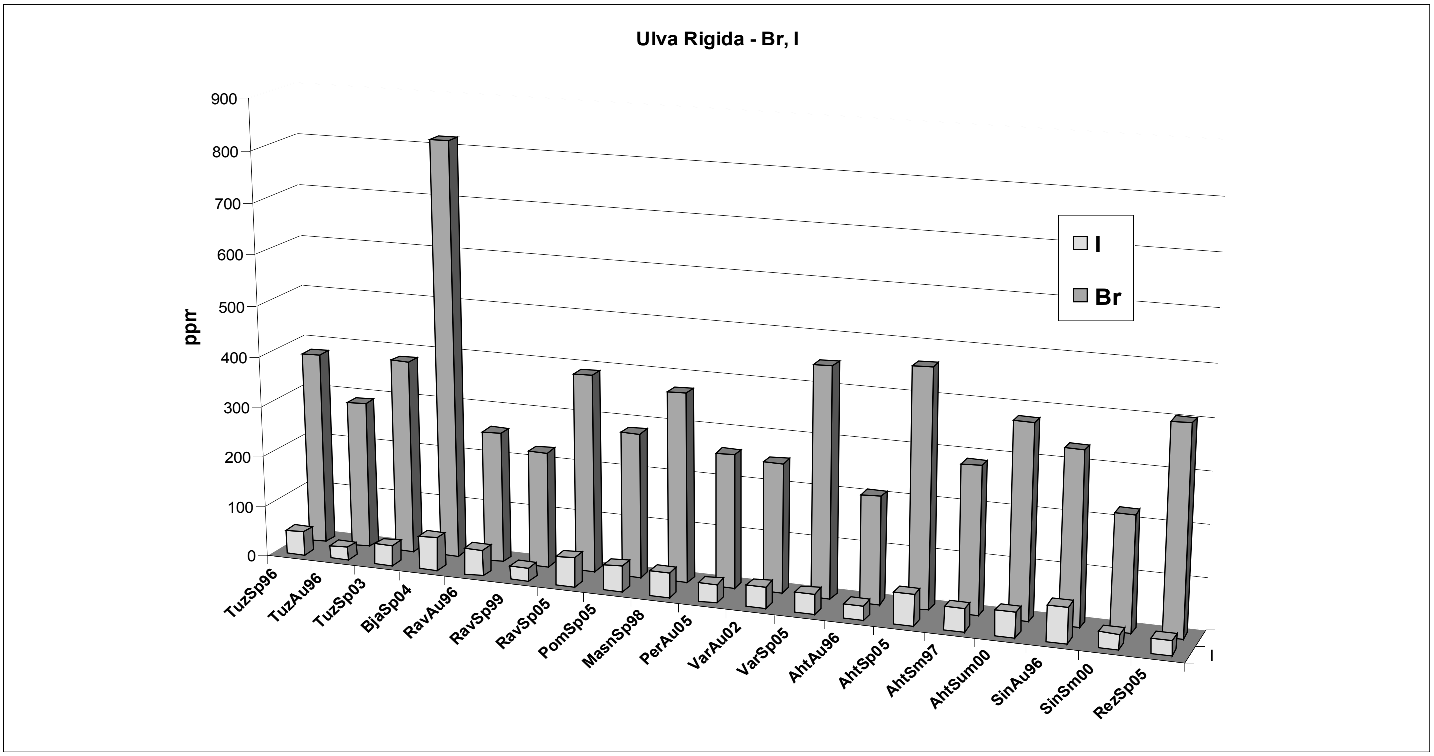

Figure 6: Br and I concentration in *Ulva* species.

Site	Season	Si	P	S	Cl	K	Ca	Ti	Cr	Mn	Fe	Cu	Zn	Br	Rb	Sr	Sn	I	Ba	Pb
		[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]
Ravda	Spring	10000	2200	18500	44200	15700	14400	35		72	822	5		336	7	143	7	38	30	11
	1996	±2000	±250	±1000	±1200	±800	±300	±1		±2	±20	±1		±10	±2	±10	±2	±3	±3	±2
Sinemorez	Autumn	9000	1500	14800	25500	8600	17400	51		73	1840	1	33	233	16	576	8	51	148	7
	1996	±900	±200	±1200	±1500	±500	±1100	±5		±2	±120	±1	±2	±12	±2	±6	±2	±3	±12	±3
Tuzlata	Spring	9400	2270	14400	27100	16600	12600	36	40	72	800	3	34	290	13	137	12	48	40	34
	1998	±900	±400	±400	±750	±500	±300	±4	±5	±1	±30	±2	±4	±10	±2	±10	±2	±5	±2	±5
Shabla	Spring	10700	3300	20100	33600	8500	8700	32		73	480	8	50	540	16	327	7	64	56	5
	1999	±2000	±300	±1400	±1200	±250	±500	±2		±1	±10	±2	±2	±8	±2	±10	±2	±3	±5	±2
Kaliakra	Spring	9700	2600	17600	32100	13800	13300	35		73	687		44	460	16	115	6	42	17	6
	1999	±1500	±200	±200	±700	±300	±200	±2		±2	±23		±1	±2	±2	±4	±2	±4	±3	±3
Tuzlata	Spring	25800	2000	17600	23300	8000	24700	76	41	70	1070	10	31	346	21	735	7	48	50	7
	1999	±2800	±300	±400	±600	±200	±1400	±7	±4	±3	±70	±2	±2	±16	±2	±25	±2	±4	±3	±3
Tuzlata	Summer	22000	2500	13300	23700	15100	14100	51		67	973	5	24	303	13	218	8	22	20	15
	2000	±1000	±200	±300	±1200	±600	±800	±5		±6	±55	±2	±2	±10	±1	±10	±2	±3	±2	±5
Rezovo	Summer	13500	2300	15300	32000	12400	18100	67	35	38	1356	5	2	471	24	371	14	63	64	14
	2000	±1000	±300	±1200	±2200	±750	±600	±2	±3	±4	±40	±48	±3	±13	±3	±15	±2	±2	±3	±4
st Konstan	Spring	12600	2400	26400	36600	17600	13500	51		74	470	3		406	28	123	17	56	20	8
tin,st Elena	2001	±1200	±180	±500	±1200	±800	±150	±5		±1	±6	±1		±24	±2	±2	±2	±2	±3	±3
Sinemorez	Autumn	17200	2800	27200	21000	26900	14700	40		58	870	6	32	183	18	193	12	18	50	20
	2002	±2000	±200	±500	±1600	±1800	±300	±5		±3	±50	±31	±3	±11	±2	±10	±2	±2	±4	±3
Kaliakra	Spring	16000	4300	26000	49200	15500	15300	34	53	73	745	5	37	444	17	127	11	50	30	
	2003	±1000	±350	±1000	±2100	±500	±500	±3	±5	±3	±17	±2	±3	±22	±3	±5	±2	±1	±3	

	2003	±1000	±350	±1000	±2100	±500	±500	±3	±5	±3	±17	±2	±3	±22	±3	±5	±2	±1	±3	
Tuzlata	Spring	22400	2700	21500	37600	16400	13400	52		71	1128	5	34	385	18	555	10	71	131	12
	2003	±2000	±200	±750	±1000	±1200	±1100	±5		±2	±40	±2	±3	±16	±2	±25	±2	±3	±10	±2
Ahtopol	Spring	7300	1750	19900	19500	9800	11800	43	43	72	530	5	39	381	4	178	12	52	17	5
	2003	±750	±150	±1200	±500	±200	±200	±3	±2	±1	±20	±1	±3	±13	±1	±5	±2	±5	±2	±2
Sinemorez	Spring	17600	2740	20600	9000	10000	5600	50	28	140	1415	2	33	2	33	212	12	20	48	10
	2003	±2000	±200	±400	±800	±600	±250	±5	±3	±50	±120	±2	±2	±2	±2	±12	±2	±2	±4	±3
Rezovo	Spring	16600	1680	18200	10400	14500	16600	57	42	62	1470	10	45	375	9	325	5	30	19	
	2003	±1000	±200	±1000	±1000	±1200	±500	±3	±4	±6	±80	±2	±2	±15	±2	±15	±2	±2	±2	
Tuzlata	Spring	16700	2800	19800	35500	10800	25250	47	34	71	820	10	35	301	14	910	13	34	62	
	2004	±1000	±200	±700	±500	±500	±750	±5	±3	±2	±20	±2	±2	±10	±1	±30	±2	±2	±3	
Tuzlata	Spring	10400	3100	22800	54600	11200	15300	33	36	74	480	10	43	356	15	91		25	22	5
	2004	±1000	±300	±1000	±1000	±500	±500	±2	±2	±2	±20	±2	±2	±5	±2	±7		±2	±2	±5
Rosenez	Spring	15500	2500	15600	26800	13700	14000	67		67	2885	111	35	434	19	203	14	86	65	
	2005	±1000	±300	±1000	±2000	±500	±400	±4		±5	±140	±4	±3	±20	±2	±10	±2	±2	±5	
Ahtopol	Spring	13000	3800	21700	36300	12700	20100	52	30	72	510	8	33	360	15	910	10	88	32	13
	2005	±1200	±200	±1100	±1100	±300	±1200	±5	±2	±2	±20	±2	±1	±20	±2	±20	±2	±2	±2	±2
Tuzlata	Autumn	16700	3550	17500	29500	20000	19100	43	51	70	854	5	22	215	22	565	11	29	41	9
	2005	±1100	±150	±1000	±1000	±500	±700	±5	±5	±2	±25	±2	±2	±10	±2	±12	±2	±2	±2	±3

Table 2: Element concentration in *Enteromorpha intenstinalis*.

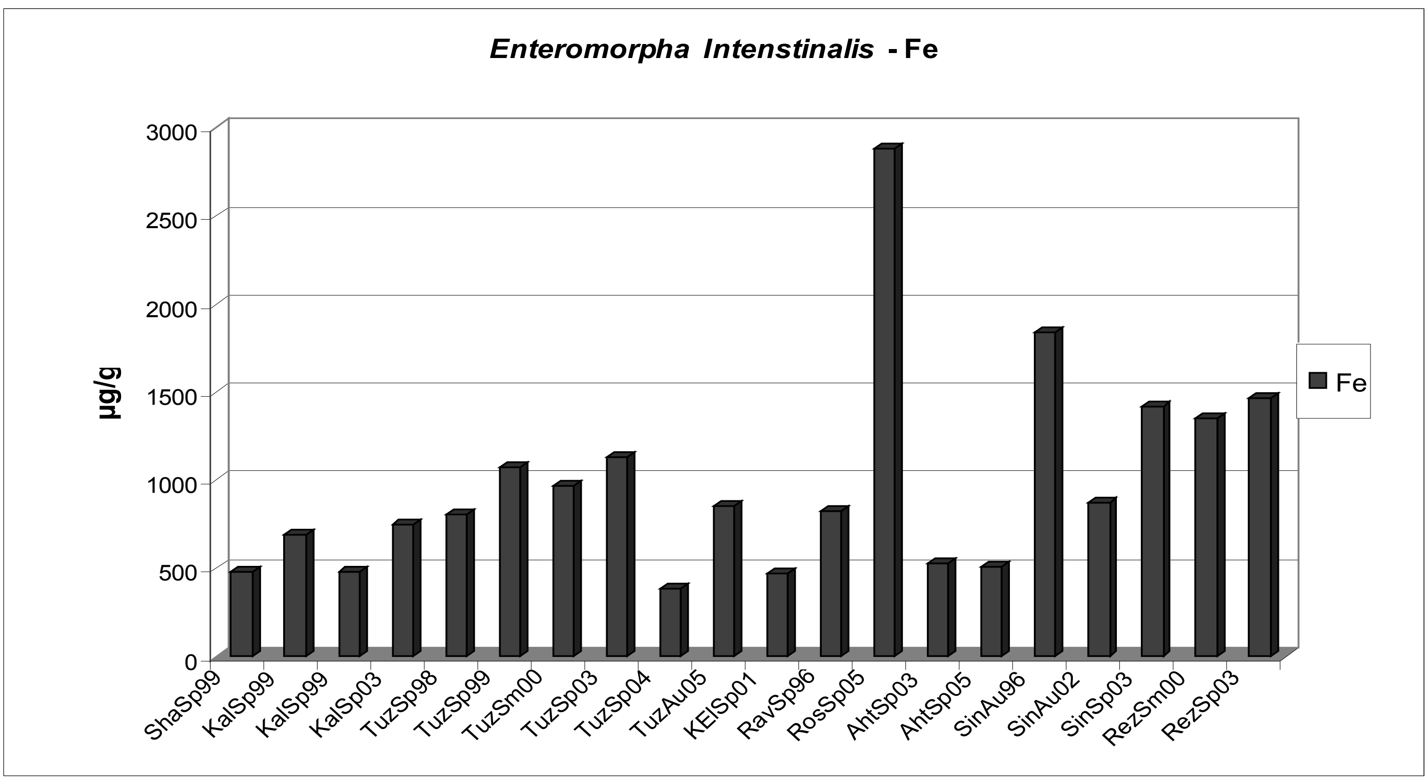

Figure 7: Fe concentration in *Enteromorpha* species.

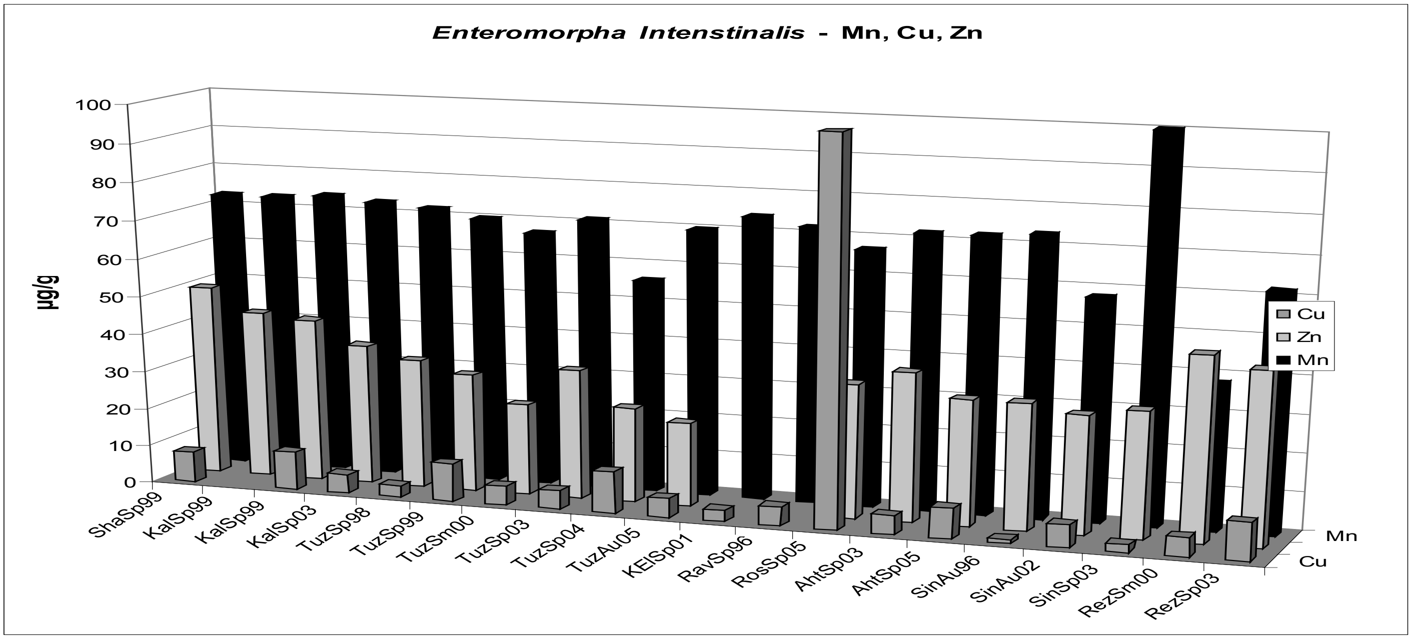

Figure 8: MN, Cu and Zn concentration in *Enteromorpha* species.

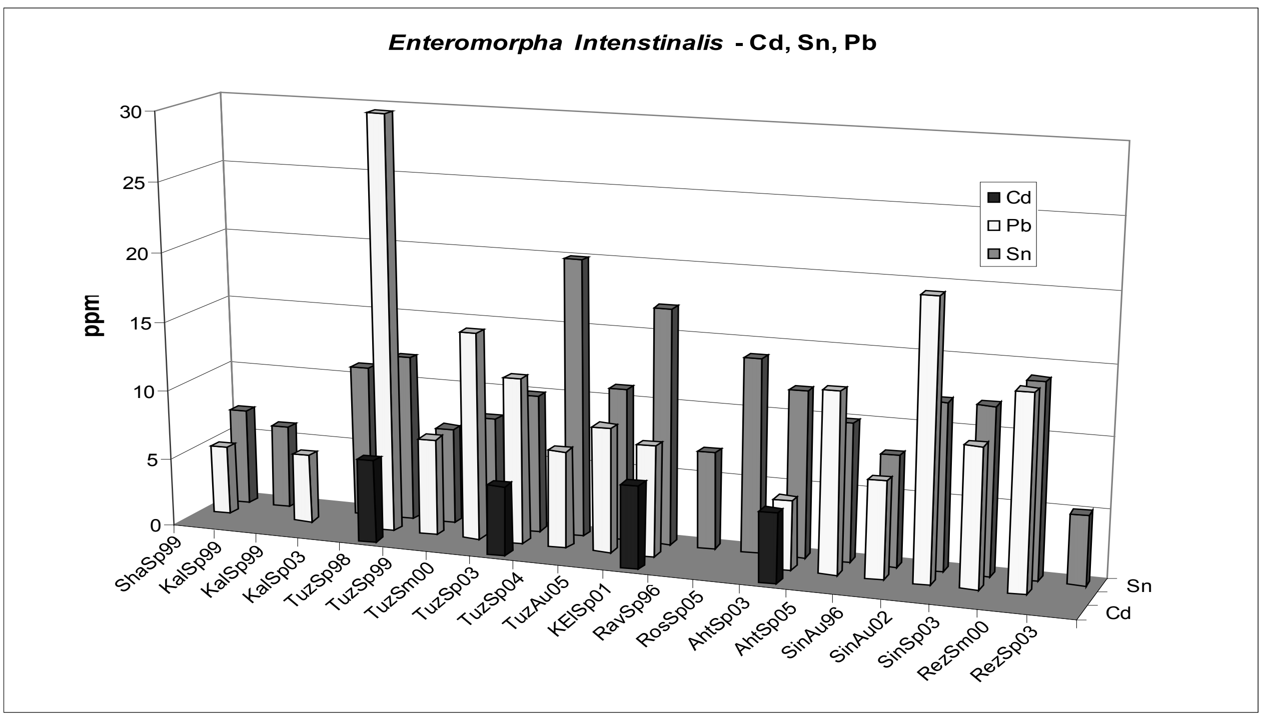

Figure 9: Cd, Sn and Pb concentration in *Enteromorpha* species.

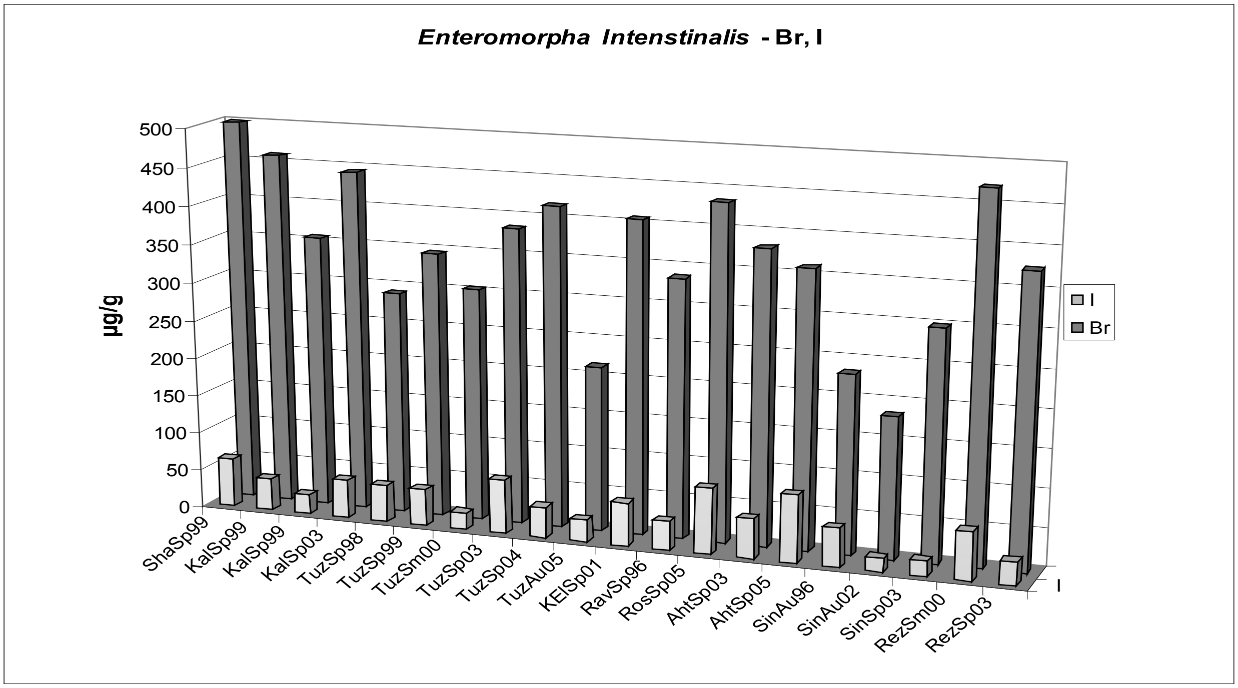

Figure 10: Br and I concentration in *Enteromorpha* species.

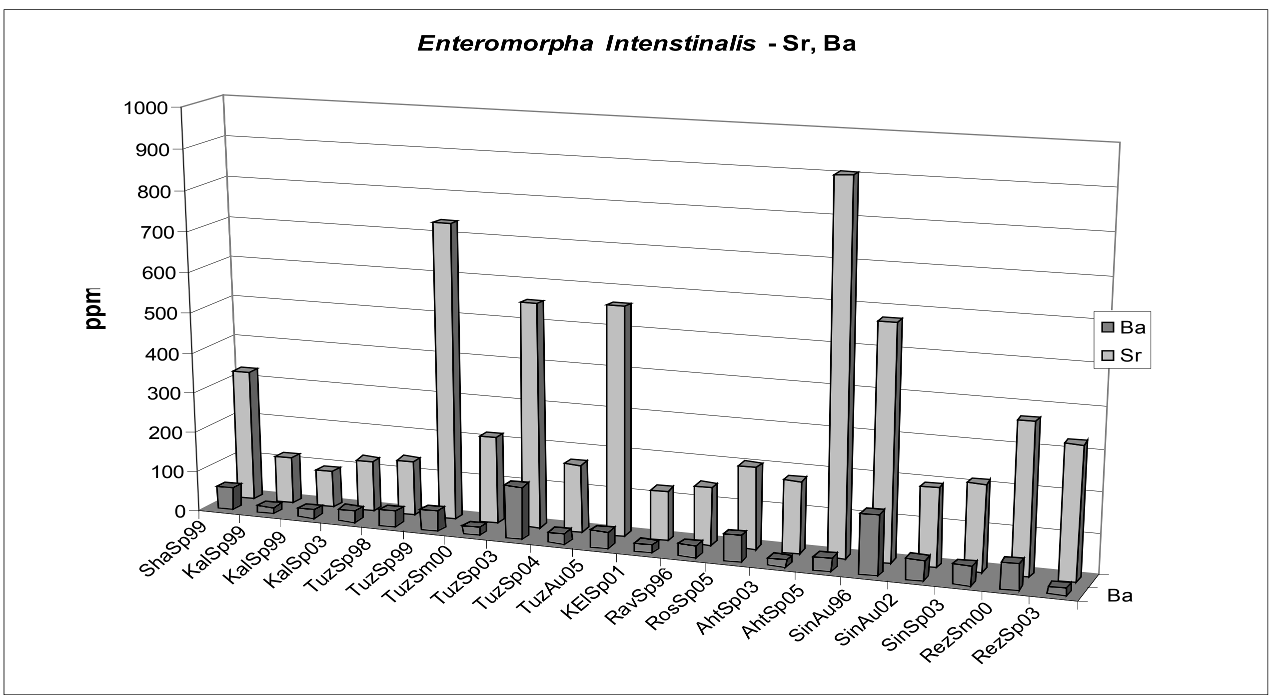

Figure 11: Cd, Sn and Pb concentration in *Enteromorpha* species.

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		Si	**P**	**S**	**Cl**	**K**	**Ca**	**Ti**	**Cr**	**Mn**	**Fe**	**Cu**	**Zn**	**Br**	**Rb**	**Sr**	**Sn**	**I**	**Ba**	**Pb**
Site	Season	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]	[ppm]
	Summer	18600	2550	13200	31700	28400	20000	38		256	1280	9	25	150	16	458		40	91	5
Tuzlata	1992	±1500	±100	±800	±3500	±1200	±2000	±3		±10	±10	±1	±2	±10	±2	±15		±3	±3	±3
	Summer	13500	2100	15900	31200	28400	17800	32	37	217	1035	15	22	150	12	443		33	50	25
Tuzlata	1993	±1500	±200	±500	±1000	±500	±600	±3	±3	±5	±20	±2	±2	±6	±2	±15		±2	±3	±3
	Spring	14600	3600	15600	27000	27100	17600	40	31	73	1800	7	32	197	15	300	5	40	37	9
Tuzlata	1996	±1500	±400	±1000	±800	±250	±1500	±2	±3	±2	±60	±2	±4	±10	±2	±12	±1	±2	±3	±3
	Spring	37800	2670	12800	25600	14000	15300	195		290	6680	17	62	400	41	224	12	160	300	6
Tsarevo	1996	±2500	±250	±300	±800	±500	±200	±10		±15	±330	±2	±5	±20	±3	±10	±2	±5	±20	±2
	Spring	17300	3120	19200	30100	23000	15000	42	27	211	1070	4	23	200	12	230		31	20	7
Tuzlata	1998	±2000	±320	±500	±1000	±500	±500	±3	±2	±15	±30	±2	±2	±7	±3	±10		±2	±2	±2
	Spring	20600	3000	13400	52750	37300	17500	37		315	985	3	29	259	15	165		147	65	15
Ravda	1998	±1500	±300	±500	±2500	±2500	±800	±5		±15	±10	±2	±2	±15	±1	±5		±5	±5	±3
	Spring	23400	2650	12600	55400	41800	17700	40	27	280	1070	4	28	244	13	140	5	37	53	8
Sinemoretz	1998	±1500	±200	±200	±2000	±2500	±800	±2	±3	±30	±70	±2	±3	±15	±2	±10	±2	±2	±5	±2
Maslen	Spring	7200	3300	14200	43800	36900	13400	22		205	645	10	31	283	15	61		87	16	6
nos	1998	±1000	±400	±400	±600	±500	±300	±2		±6	±10	±2	±1	±10	±2	±5		±2	±2	±2
	Spring	21000	3020	17850	28700	25400	16200	80		56	920	6	20	178	17	161		37	14	8
Kaliakra	1999	±1000	±300	±1000	±1000	±800	±800	±4		±5	±20	±1	±1	±12	±2	±4		±3	±2	±2
	Spring	27300	3080	17300	23900	21200	13200	57		371	1054	6	16	150	10	197		20	9	7
Tuzlata	1999	±3000	±400	±500	±500	±350	±200	±3		±15	±50	±2	±2	±10	±2	±10		±2	±2	±3
	Autumn	22500	3000	16100	25300	25400	29300	81		171	1290		25	180	12	311	8	161	32	15
Rezovo	2001	±2000	±400	±800	±1000	±500	±1500	±3		±12	±50		±2	±10	±2	±20	±2	±6	±5	±3
	Autumn	25800	4600	17000	46600	27600	22100	42	34	70	953			246	8	268		188	35	10
Sinemoretz	2001	±2000	±500	±500	±3000	±1500	±1500	±2	±4	±3	±35			±10	±2	±10		±2	±4	±3
	Spring	30000	4600	17150	44500	23000	17700	91		220	3180	3	27	324	7	156		105	248	
Shabla	2003	±3000	±450	±500	±700	±300	±800	±9		±20	±30	±1	±2	±10	±3	±2		±6	±6	
	Spring	16300	3900	21400	29500	32500	13700	36		72	1114	2	24	218	5	133	6	35	20	12
Kaliakra	2003	±400	±500	±200	±600	±1500	±400	±2		±2	±50	±2	±2	±10	±2	±10	±2	±3	±2	±3
	Spring	55100	3470	16350	32000	24450	14200	45	30	430	954	12	34	321	7	144	7	90	18	7
Ahtopol	2005	±2500	±100	±500	±500	±500	±200	±3	±3	±20	±10	±2	±3	±6	±2	±3	±2	±4	±2	±2
	Spring	28350	2600	15800	30900	27500	17430	86		70	2340	5	18	132	16	345	7	106	113	10
Sinemoretz	2005	±2500	±300	±500	±1500	±2500	±1000	±6		±3	±200	±2	±2	±17	±2	±33	±2	±1	±6	±5
	Autumn	45200	3400	14240	35600	36500	22100	104		70	2040	3	25	185	17	395	6	143	93	
Tuzlata	2005	±1000	±400	±500	±1200	±1000	±2000	±4		±3	±100	±2	±2	±5	±2	±22	±1	±5	±7	

Table 3: Elemennt concentratioin in *Cladophora vagabunda*.

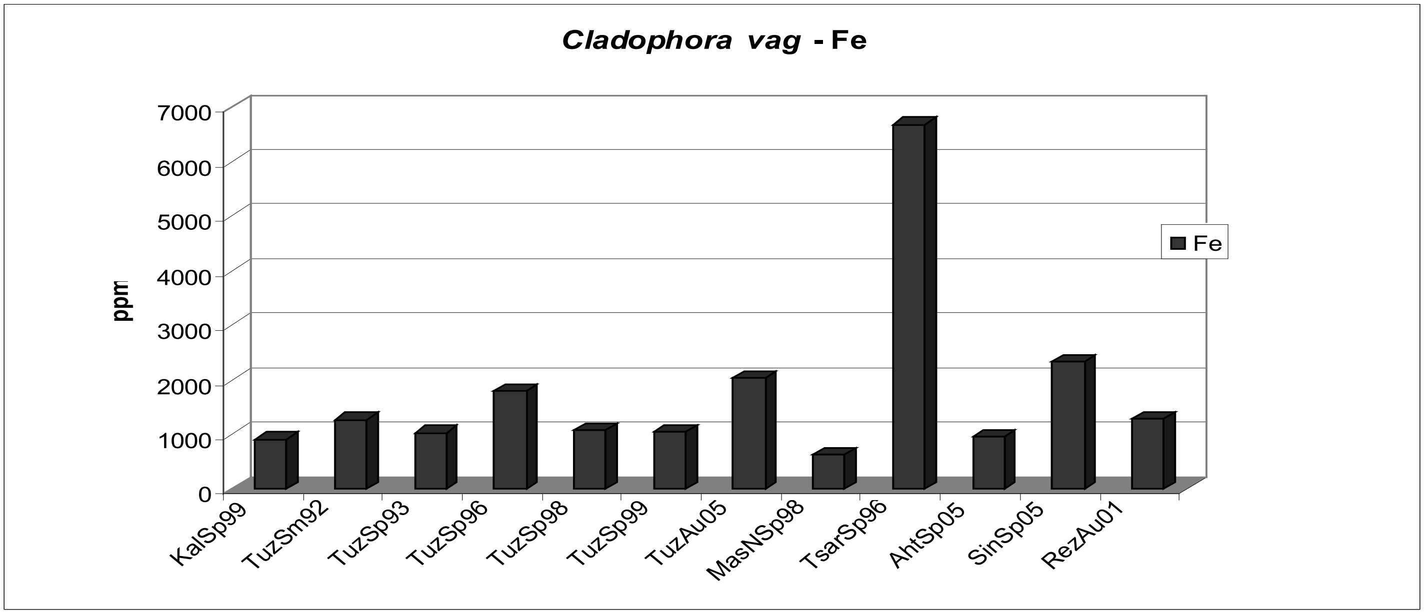

Figure 12: Fe concentration in *Cladophora* species.

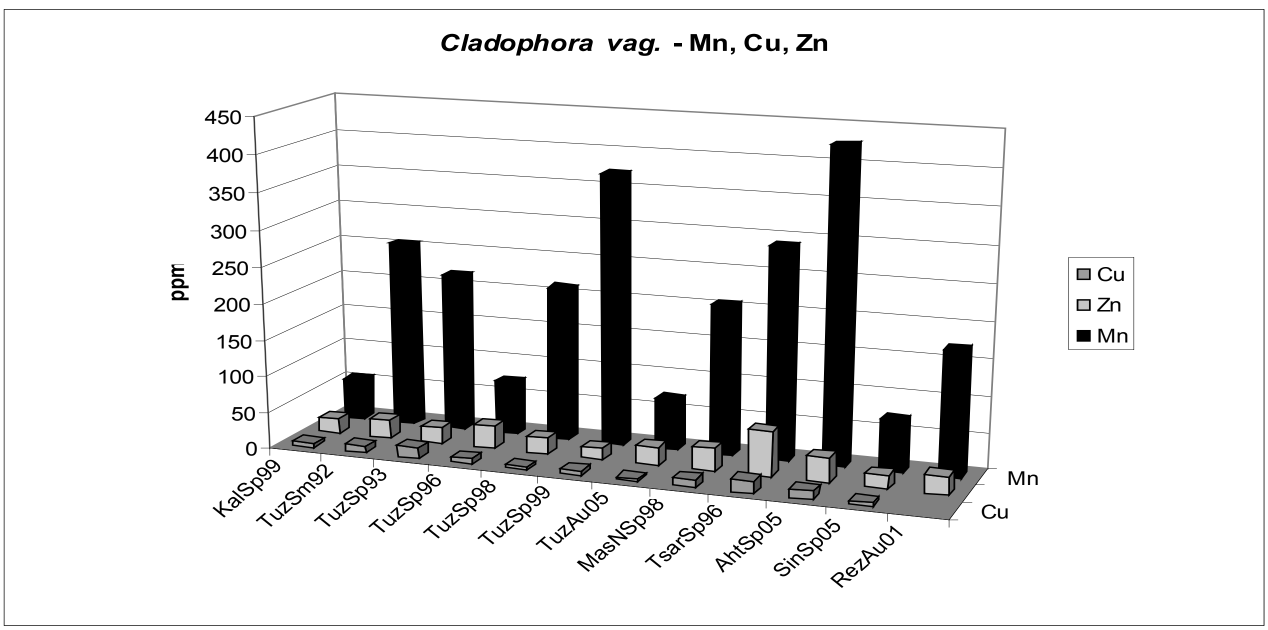

Figure 13: MN, Cu and Cu concentration in *Cladophora* species.

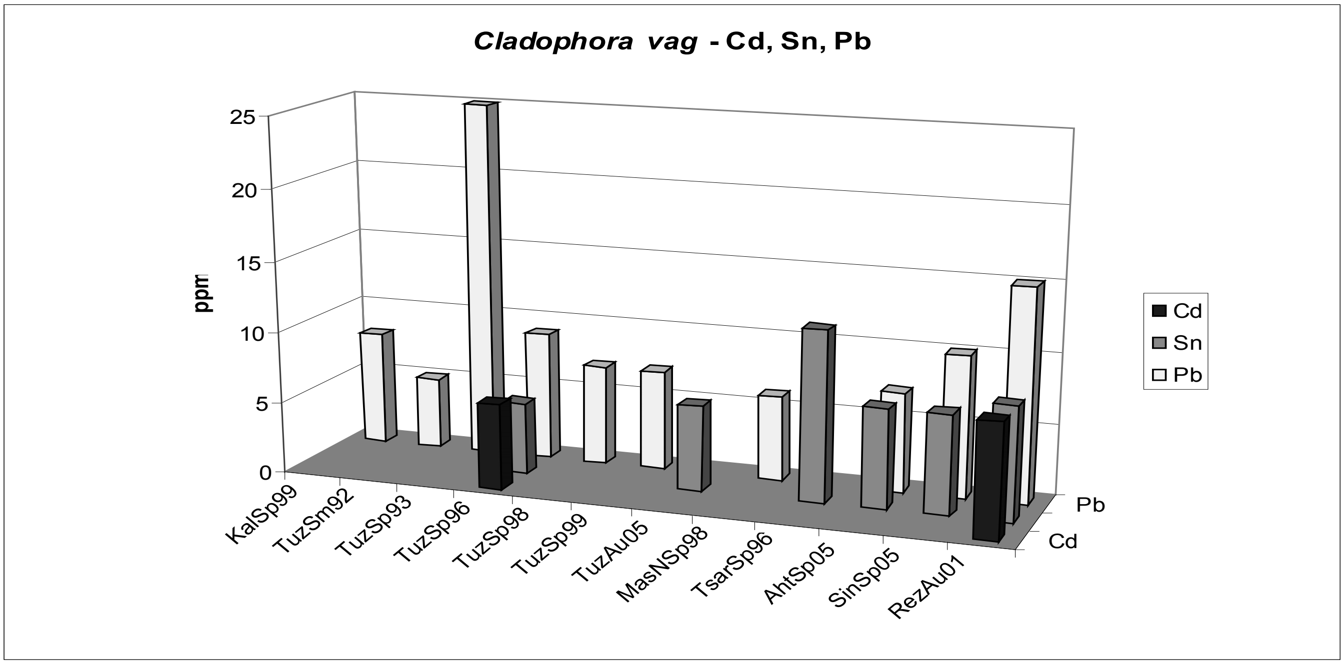

Figure 14: Cd, Sn and Pb concentration in *Cladophora* species.

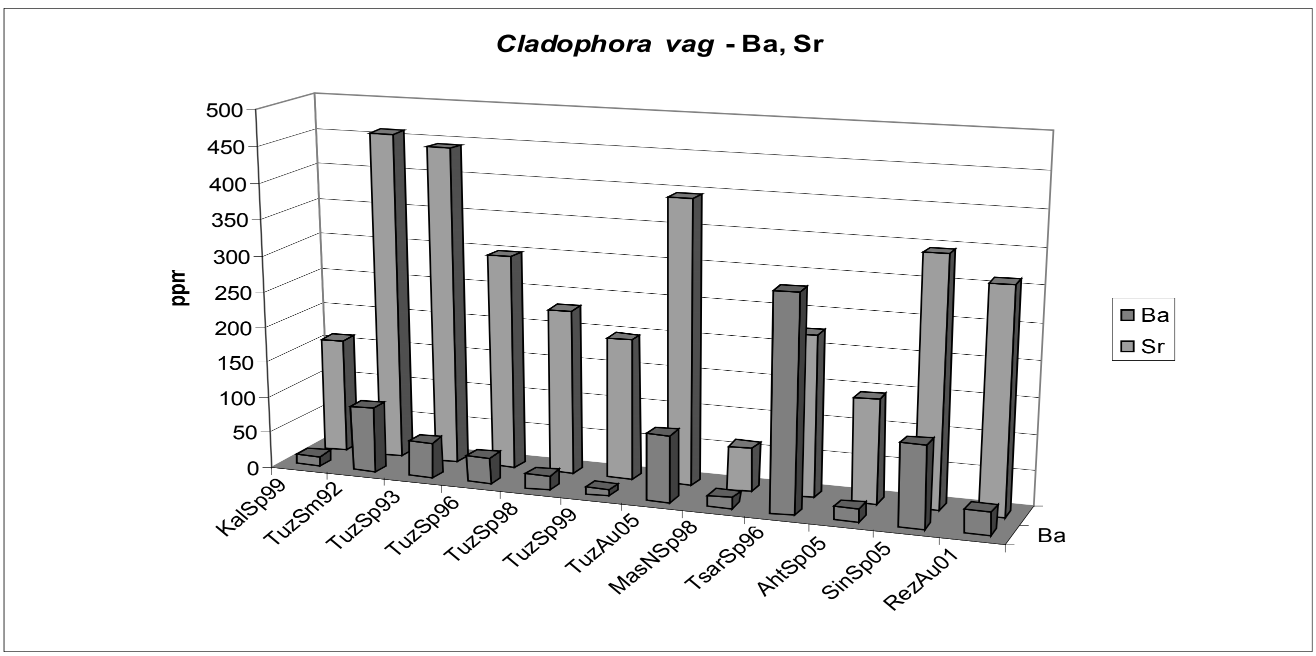

Figure 15: Ba and Sr concentration in *Cladophora* species.

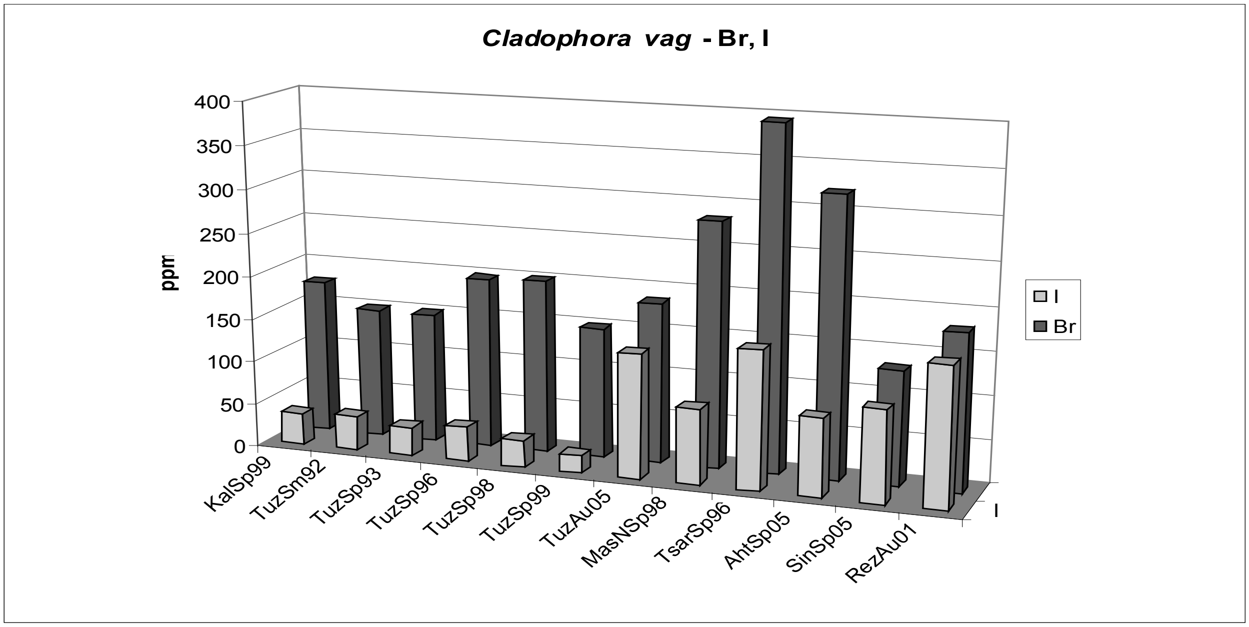

Figure 16: Br and I concentration in *Cladophora* species.

The overall Fe mean value obtained for *Ulva rigidia* 510 µg/g and the variation range is wide (300 – 1100 µg/g). The concentration of Fe in *E. intestinalis* is twice higher, compared to *Ulva rigida*, the mean value being about 1100 µg/g (except Sizemore Autumn'96 - 1840 µg/g and Rossenetz Spring'05 - 2885 µg/g). The mean Fe concentration in *Cladophora vagabunda* is close to *E. intestinalis* with exception of Tzarevo Spring`96 - 6680 µg/g. It is clear that the two green *Cladophora* and *Enteromorpha* species accumulate twice higher Fe concentration than *Ulva* species.

The results for MN in *Ulva* vary in very narrow limit (the mean value 70 ± 3µg/g). The same is true for the results MN in *E. intestinalis* which are also close (the mean value 70 ± 3µg/g) like in *Ulva rig*. except two samples, (Rezovo Summer'00 – 38 µg/g, Sizemore Spring'03 - 140 µg/g) which means that MN concentration is almost constant, independent of sampling time and location. The situation for MN in *Cladophora* is quite different – in one third of the samples the concentration is about 50 µg/g, while in the other samples the concentration is four times higher except Tuzlata Spring`99 - 371 µg/g and Ahtopol Spring`05 - 430 µg/g. The element Cu and Zn belong to the group of biologically important metal ions and their compounds can be found as trace metals in the biosphere. Cu and Zn are essential for organisms' life and its toxicity is connected with the concentrations of other essential elements and some marine algae show an ability to accumulate Cu and Zn. Trace metals should be monitored because they play an important role in metabolism and their high or low concentrations can be equally harmful to the living organisms.

Cu and Zn concentration in *Ulva rigida*, are presented in The Cu values in *Ulva*, *Enteromorpha* and *Cladophora* are close – many of the samples are in the range of 5 - 7 µg/g (while the mean Zn value is 38 ±3 µg/g) except several samples where the concentrations are three or four times higher. Cu and Zn concentrations are almost constant in the uncertainty limit of the method and are also independent of sampling time and location. Two sampling sites (Bjala and Pomorie) have considerably higher Cu and Zn concentration. The case with the Rossenetz location (Sping'05 - 111 µg/g) is completely different – there is a copper mine in the vicinity and the concentration of all elements is completely different than all other sites due to the human mining activity.

Copper concentration determined in algae during the present investigation is comparable with those reported by for algae collected from Crimean coast at the Black Sea, while for lead (nonessential element, but it is present in all biomaterials) the concentrations are lower than those found by Burdin. Pb is found in seawaters mainly in the form of different organic compounds. As it is seen from Pb and Sn data for the three green algae in the highest lead concentration is measured in spring 1996 - 1998, while the concentrations decrease during the next sampling period. The measured concentration for Pb is close to typical Pb obtained for the terrestrial plants.

The concentration of Sn in Ulva are relatively constant (mean 11 ± 3 µg/g), except Bjala (28 µg/g). The Sn values for *E. intestinalis* vary more and are higher than the other two green algae samples and are close to those measured in marine sediments. Cd concentration in Ulva (~4 µg/g) was measured only in four samples from Tuzlata (2), St. Constantine & Elena and Ahtopol. Cd is not measured in all samples for the other green algae (in

Enteromorpha - 4, and *C. vagabunda* only two sites). The overall concentration of Pb, Sn and Cd in the sample Tuzlata Spring 98 is three times higher than all other measured algae samples.

It should be pointed out that some samples differ substantially from the others, which can be attributed to the various factors affecting elements accumulation and the specific conditions of studied locations especially depending on the sampling time (mainly spring). Another example for great deviation of elements values is the data (µg/g) for *Ulva rigida* in Bjala location Spring 2004 and *C. vagabunda* in Tzarevo Spring`1996. The concentration of halogen elements Cl, Br and I, which are biologically important for the algae and marine biota, were measured also in Ulva rigida, *E. intestinalis* and *C. vagabunda*. The obtained average value for Br and I are close for both green algae *Ulva rigida*, *E. intestinalis* (360 µg/g - for Br and 47 µg/g for I. while the Br value in *Cladophora* is twice lower. Lower values were measured for *E. intestinalis* from Tuzlata Autumn 2005, Sinemorez Autumn 1996, Sinemorez Autumn 2002, suggesting seasonal dependence. The measured Cl values in *E. intestinalis* and *C. vagabunda* are higher (mean value 30800 µg/g) compared to those of *Ulva r.* (22400 µg/g). The values in half of the samples in *Cladophora* are twice higher than the other samples.

The concentration of alkaline earth elements Ca, Sr and Ba was obtained for *Ulva rigida*, *E.intestinalis* and *C. vagabunda* The data for *Ulva* and *Enteromorpha* showed no specific dependence on location or time of sampling while the *Cladofora* values varied to a greater extent. The average value for Sr in *Ulva rigida* is lower (~150 µg/g) than those for *Enteromorpha* (~340 µg/g) and *Cladophora*(~280 µg/g) with several samples with much higher values. The average value for the barium concentration for the three algae is close (~40 µg/g) also with few exceptions. The Ba value in Tzarevo Sping`96 is exceptionally high 300 µg/g but the sampling was performed close to the area of the port. We have investigated several other green algae - *Ulva lactuca, Bryopsis plumosa, Enteromorpha compressa, Cladophora coleotrix, Chaetomorpha gracilis*. The data obtained for *Ulva lactuca* species were from locations Rusalka, Bjala, Tzarevo and Sizemore at the Bulgarian Black Sea coast. The determined element concentrations are similar to those, obtained for *Ulva rigida*. The concentrations of the most of elements in Bjala location are higher compared to the other sites (like *Ulva rigida*) – for Fe – 1125 ± 50 µg/g, Cu – 14 ± 2 µg/g, Zn – 78 ± 5 µg/g, Br - 807 ± 20 µg/g, Ba - 84 ± 5 µg/gSn - 28 ± 2 µg/g, Rb – 23 ± 2 µg/g. MN concentration is twice higher in two locations (Sinemorez Spring`98, Tzarevo Autumn`02) than the rest sites. Unlike *Ulva rigida* species, in *Ulva lactuca* Cd was measured only in two locations - Tzarevo Autumn`02 - 4 ± 1 µg/g, Sinemorez Spring`05 - 6 ± 1 µg/g.

The data for *Enteromorpha compressa* are similar to those of *E. intestinalis*. The same is true for both the *Cladophora* algae species (e.g. higher concentration of I). The measured values for the different elements in *Chaetomorphagracilis* are closer to *C. vagabunda*, than to the two green *Ulva rigida* and *Enteromorphavaga*. Special attention should be about the green alga *Bryopsis plumosa*. The elements from alkaline earth group -Sr and Ba are accumulated to much higher extent – two to four orders of magnitude (~30000 µg/g for Ba (~50 µg/g for the other green algae); ~4000 µg/g for Sr (~200 for the others). The concentrations of I is ~2000 µg/g (~50 µg/g for the others). We have measured [13] radio metrically the Ra daughter isotope concentrations and also found three orders of magnitude higher concentration compared to all other algae. This comes to confirm the specific accumulation of alkaline earth elements by the green alga *Bryopsis plumosa*.

Conclusion

The concentrations of 32 elements have been determined in eight Black Sea green Macroalgae from nine locations along the Bulgarian Black sea coast during the period 1996-2011. If Fe data during the monitoring period are compared, no clear seasonal or location dependence can be observed. It has been shown that *Ulva rigida* and *Bryopsis plumosa* species accumulate studied elements less than the other species and the element concentration in green macrophytes from Tuzlata, Ravda and Sinemoretz is higher compared to the other locations.

The obtained data show that no serious artificial pollution along our shore and element concentrations in algae during the studied period demonstrate considerably constant accumulation pattern. The studied green algae species are suitable for assessment of element behavior in Black Sea marine ecosystems (especially *Ulva, Enteromorpha* and *Cladophora*) and can be used as bio-indicators species for the ecological status of the ecosystem, together with the abiotic element concentration in the ecosystem (water, sediment). This work contributes to the evaluation of for heavy metal concentration in green Macroalgae and their ecological impact on marine ecosystems along the Bulgarian Black Sea coast.

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