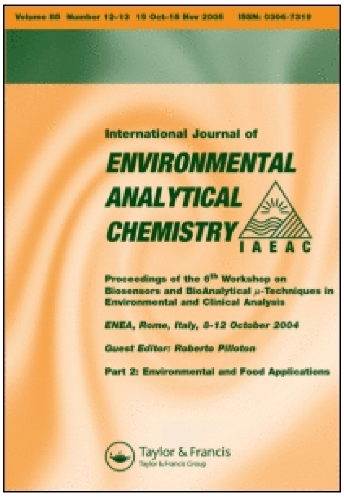

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Monitoring of Fe, Mn, Cu, Pb and Cd levels in two brown macroalgae from the Bulgarian Black Sea coast

Alexander Strezov[a]; Tzvetana Nonova[a]
[a] Bulgarian Academy of Sciences, Institute for Nuclear Research and Nuclear Energy, 1784 Sofia, Bulgaria

Online publication date: 12 May 2010

To cite this Article Strezov, Alexander and Nonova, Tzvetana(2003) 'Monitoring of Fe, Mn, Cu, Pb and Cd levels in two brown macroalgae from the Bulgarian Black Sea coast', International Journal of Environmental Analytical Chemistry, 83: 12, 1045 — 1054

To link to this Article: DOI: 10.1080/03067310310001621042

URL: http://dx.doi.org/10.1080/03067310310001621042

Intern. J. Environ. Anal. Chem.
Vol. 83, No. 12, December 2003, pp. 1045–1054

MONITORING OF Fe, Mn, Cu, Pb AND Cd LEVELS IN TWO BROWN MACROALGAE FROM THE BULGARIAN BLACK SEA COAST

ALEXANDER STREZOV* and TZVETANA NONOVA

Bulgarian Academy of Sciences, Institute for Nuclear Research and Nuclear Energy, 72, Tzarigradsko Shosse, 1784 Sofia, Bulgaria

(Received 7 May 2003; In final form 31 July 2003)

Heavy metal contents (Fe, Mn, Cu, Pb and Cd) were determined in two brown macroalgal species (*Cystoseira crinita* and *Cystoseira barbata*). Samples were collected during six seasons from spring 1996 to summer 2000 from five different sites of the Bulgarian Black Sea coastal zone – Tuzlata, Ravda, Sozopol, Ahtopol and Sinemoretz. The local and seasonal metal distribution and their variations were examined.

The data obtained indicate that the two investigated species demonstrate various degrees of metal accumulation and can be used as indicators for the type and quantity of anthropogenic contamination in marine ecosystems.

Keywords: Brown algae; Black sea; Pollution; Heavy metals

INTRODUCTION

Anthropogenic contamination of marine ecosystems makes systematic monitoring and control essential. Investigations studying the contamination of marine environments are based on descriptions of contaminant sources, pathways, dispersals and concentration changes.

Coastal zone contamination in the Black Sea is a powerful ecological factor that affects the macrophytic species and in many cases can lead to a decrease in species number or total mass in the ecosystem. It can also break the equilibrium between communities so that the weakest disappear.

Trace elements are active components which are of environmental concern and subject to intensive scientific investigation. The reason is their unique ability to influence and in many cases to affect the important metabolic functions of organisms. Pb, Cd,

*Corresponding author. Fax: +359-29753619. E-mail: strezov@inrne.bas.bg

ISSN 0306-7319 print: ISSN 1029-0397 online
DOI: 10.1080/03067310310001621042

Cu, Zn and other toxic elements are among the most studied trace elements in the marine environment. The determination of anthropogenic trace metal pollutants, like Cd and Pb, in natural waters is of great importance because they represent the natural ecological background and affect the biological cycle by their high toxicity [1]. Algae have been widely used as bioindicators for contamination studies in water basins.

Marine macrophytes are a major component of the group of organisms used for analysis and control of marine ecosystems. They are proven indicators for the assessment of dissolved heavy metals in the sea, because of their accumulation capacity [2,3]. The use of bio-monitors eliminates the need for complex studies on the chemical speciation of aquatic contaminants [4]. Algae are organisms widely distributed in various aquatic environments (in salt water from littoral to pelagic zones) and they have several characteristics favorable for metal accumulation. These characteristics, together with their ability to adapt to environmental changes while retaining their life functions and ability to absorb contaminants to a certain extent, make algae suitable as bio-indicators.

Metal concentrations vary in a wide range depending on various environmental conditions – depth, salinity, temperature, sea currents, sources of pollution, etc. Metals can settle in sediments in the process of mixing of fresh (from rivers) with saline waters or be sorbed by phytoplankton cells.

The Black Sea has a variety of macroalgae and one of the widespread brown species is the genus *Cystoseira*. *Cystoseira* brown algae have shown a clear selectivity for some trace elements such as As, Cr, Cd, Cu and Co, which may encourage their use as biological indicators for trace element pollution [5].

It has been shown [6] that more than 90% of *C. barbata* populations have been lost since the 1960s on the Romanian and Ukrainian shelf because of eutrophication and pollution, showing that the genus *Cystoseira* does react towards pollution.

This study presents the results of investigations into the iron, manganese, copper, lead and cadmium contents in two brown macroalgal species (*C. crinita* and *C. barbata*), collected during six seasons from five different sites of the Bulgarian Black Sea coastal zone. The aim of the study was to determine the level of heavy metal accumulation and to give information on the seasonal distribution of metal concentrations in the algae and on the inter-metal correlation. The local distribution of metal contents in brown macrophytes was also investigated.

EXPERIMENTAL

Sampling

The two brown macroalgal species *C. crinita* and *C. barbata* were sampled at five sites (Tuzlata, Ravda, Sozopol, Ahtopol and Sinemoretz), located along the Bulgarian Black Sea coast as shown in Fig. 1. Algae were taken during the warm period of the year: spring (May) and autumn (September) in 1996, spring 1998, spring and autumn 1999 and summer 2000.

The plants were hand-picked in the coastal zone at a depth of about 1–2 m. The samples were washed with seawater to remove any adherent particles and small invertebrates. They were dried to constant weight (85°C) in the laboratory and ground to a fine powder, which was used for chemical analysis.

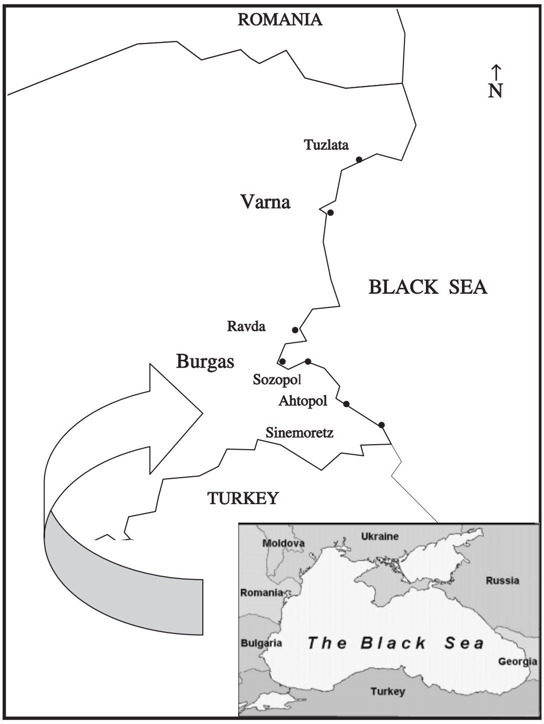

FIGURE 1 Sampling locations.

Determination of Heavy Metals

Algae were digested with concentrated HNO_3 (Merck) and evaporated. Fe, Mn and Cu were determined using a Pye Unicam 1950 atomic absorption spectrophotometer with air-acetylene flame, Pb and Cd with a Perkin-Elmer Zeeman 3030 spectrophotometer with graphite furnace. Quality assurance and accuracy were checked by means of two certified materials: NIES-CRM-3 (*Chlorella*) and IAEA/V–10 Hay powder.

Assessment of Precision and Reproducibility

The precision of the analytical method was determined by the SR[%] values [7] for each element in relation to standard reference materials (see below).

$$SR[\%] = \frac{|C_e - C_c| + 2\sigma}{C_c} \times 100,$$

where C_e is the experimental value; C_c is the certificate value; σ is the standard deviation. $SR < 25\%$ – excellent; $25\% < SR < 50\%$ – acceptable; $SR > 50\%$ – unacceptable. The reproducibility is presented as a relative standard deviation RSD[%].

RESULTS

The data obtained for heavy metal contents in brown algae (in μg/g dry weight) are presented in Table I. The metal concentrations decrease in the order Fe > Mn > Cu > Pb > Cd. Similar orders of decreasing concentration were established in our previous studies for brown as well as for green and red macroalgal species [8,9].

The mean Fe value obtained from all samples is about 230 μg/g. The minimum value of 111 μg/g was measured in *C. barbata* from Tuzlata (spring 1999) and the maximum value – 567 μg/g – in *C. barbata* from Ravda (spring 1996). These concentrations are much lower than those we obtained for *Chlorophyta* macroalgae and have a much narrower range (unpublished data). Guven *et al.* [10] have investigated *C. barbata* species collected along the Turkish Black Sea coast, and report similar concentrations of Fe (124–604 μg/g). The seasonal dependence of Fe accumulation in the studied algal species is presented graphically in Fig. 2.

The results obtained for Mn vary in a more narrow range (6–60 μg/g) with a mean value of 22 μg/g. Mn enters the aquatic environment from waste effluents from industry as well as from rock minerals.

Low concentrations of Cu, ranging from 0.3 to 9 μg/g, have been obtained in the brown algal species. The maximum measured values (9 μg/g dry weight) were in *C. crinita* and *C. barbata* at Ravda (spring 1996), while the lowest (0.3 μg/g) were measured for *C. crinita* from Ahtopol (autumn 1996) and Sinemoretz (summer 2000). The data for Cu for the different seasons and the observed locations are presented in Figs. 3 and 4. Similar Cu values in *Cystoseira* species are cited by Guven *et al.* [10] for the Black Sea and by Al-Masri *et al.* [11] for the eastern Mediterranean coast.

The determination of Pb and Cd contents in marine macroalgae is of major importance for monitoring the marine environment as they are mobile elements of

TABLE I Metal contents in macroalgae (μg/g dry weight)

Location	*Season*	*Algae*	*Fe*	*Cu*	*Mn*	*Pb*	*Cd*
Tuzlata	Spring 1996	*C. crinita*	420 ± 33	3.6 ± 0.1	15 ± 1	6.3 ± 0.6	0.36 ± 0.06
	Autumn1996	*C. crinita*	125 ± 6	0.4 ± 0.1	9 ± 1	1.0 ± 0.3	0.33 ± 0.11
	Spring 1998	*C. crinita*	121 ± 3	1.6 ± 0.1	37 ± 2	0.9 ± 0.1	0.26 ± 0.05
Ravda	Spring 1996	*C. crinita*	510 ± 13	6.0 ± 0.7	34 ± 3	3.2 ± 0.5	0.21 ± 0.06
	Autumn1996	*C. crinita*	142 ± 4	0.5 ± 0.1	12 ± 1	0.7 ± 0.1	0.87 ± 0.18
	Spring 1999	*C. crinita*	206 ± 6	3.3 ± 0.2	60 ± 2	1.0 ± 0.2	0.70 ± 0.14
Sozopol	Autumn1999	*C. crinita*	165 ± 1	3.8 ± 0.1	11 ± 1	2.4 ± 0.3	0.34 ± 0.06
Ahtopol	Spring 1996	*C. crinita*	79 ± 17	5.8 ± 0.9	11 ± 1	3.3 ± 0.4	0.22 ± 0.05
	Autumn 1996	*C. crinita*	66 ± 1	0.3 ± 0.1	17 ± 2	0.7 ± 0.2	0.40 ± 0.14
	Summer 2000	*C. crinita*	95 ± 1	5.3 ± 0.1	22 ± 3	0.6 ± 0.3	1.87 ± 0.18
Sinemoretz	Autumn 1996	*C. crinita*	51 ± 2	1.5 ± 0.1	15 ± 1	0.4 ± 0.1	0.43 ± 0.18
	Summer 2000	*C. crinita*	64 ± 1	0.3 ± 0.1	14 ± 1	0.8 ± 0.2	0.30 ± 0.11
Tuzlata	Spring 1996	*C. barbata*	446 ± 28	6.2 ± 0.6	16 ± 1	2.1 ± 0.2	0.10 ± 0.01
	Autumn 1996	*C. barbata*	250 ± 13	0.6 ± 0.1	10 ± 1	0.7 ± 0.1	0.10 ± 0.01
	Spring 1999	*C. barbata*	111 ± 2	1.8 ± 0.1	14 ± 2	1.3 ± 0.2	0.50 ± 0.07
	Summer 2000	*C. barbata*	323 ± 7	3.7 ± 0.2	9 ± 1	1.8 ± 0.4	1.25 ± 0.05
Ravda	Spring 1996	*C. barbata*	567 ± 1	9.0 ± 0.5	39 ± 1	2.4 ± 0.3	0.12 ± 0.04
	Autumn 1996	*C. barbata*	328 ± 3	5.4 ± 0.2	24 ± 1	1.0 ± 0.4	0.23 ± 0.04
	Spring 1998	*C. barbata*	472 ± 25	5.0 ± 0.3	54 ± 2	2.3 ± 0.3	0.16 ± 0.05
Sozopol	Autumn1999	*C. barbata*	119 ± 1	0.8 ± 0.1	6 ± 1	2.0 ± 0.2	0.15 ± 0.02

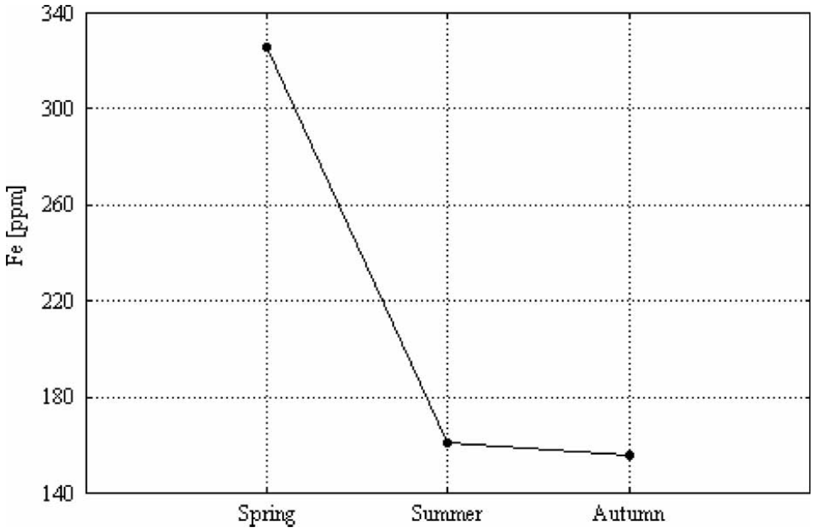

FIGURE 2 Seasonal variation of mean Fe concentration in brown algae.

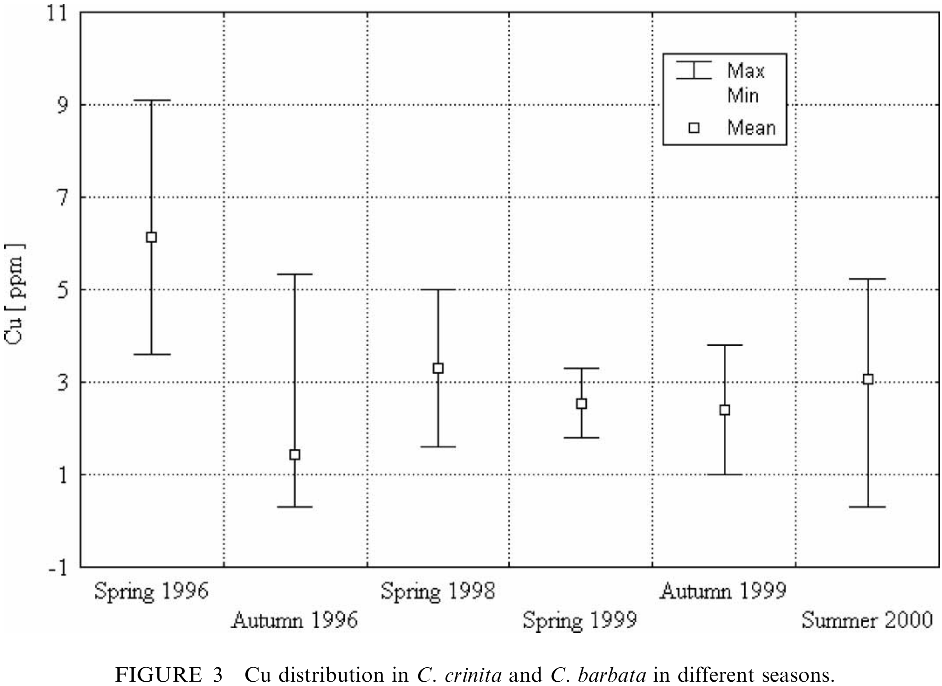

FIGURE 3 Cu distribution in *C. crinita* and *C. barbata* in different seasons.

mainly anthropogenic origin. Pb and Cd are generally characterized as toxic and hazardous for plants [12].

The Pb values obtained are in the range from 0.4 to 3.3 μg/g dry weight (except for *C. crinita* from Tuzlata – spring 1996). The minimum Pb value is measured for the same species in autumn 1996 at Sinemoretz. Maximum Cd values in the studied macroalgae are obtained in *C. crinita* from Tuzlata (1.25 μg/g) and from Ahtopol

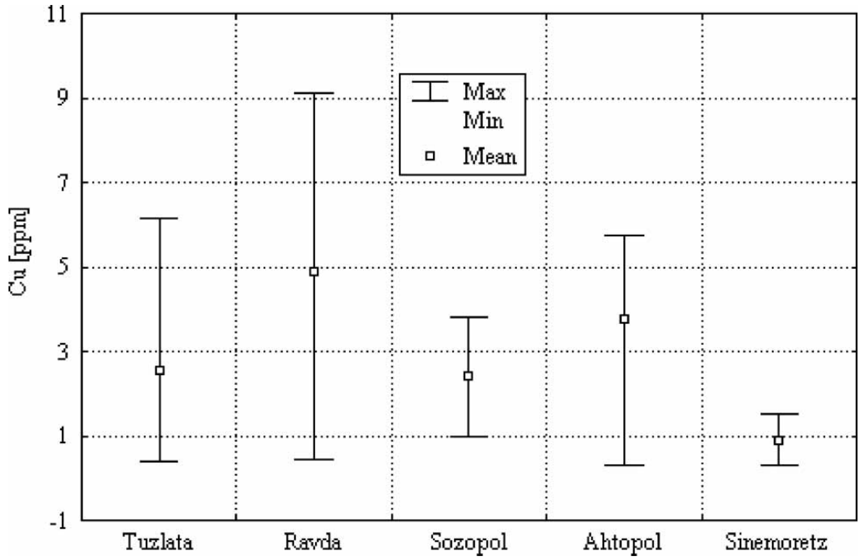

FIGURE 4 Cu distribution in *C. crinita* and *C. barbata* from different locations.

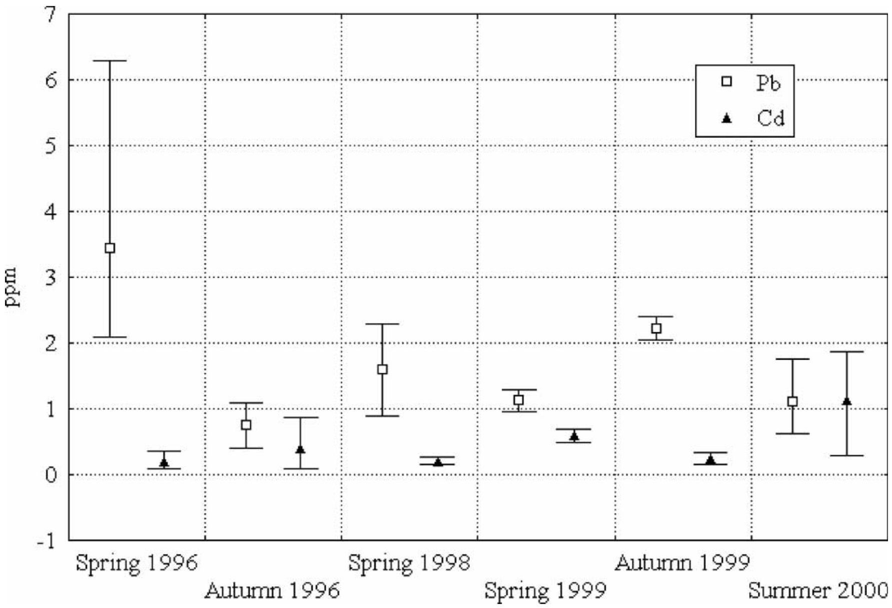

FIGURE 5 Pb and Cd contents in *C. crinita* and *C. barbata* in different seasons.

(1.87 μg/g) collected in summer 2000. The obtained data for Pb and Cd are presented in Figs. 5 and 6.

The precision and reproducibility of the analytical method are presented as SR[%] and RSD[%] in Table II. The data show that Fe, Mn, Pb and Cd results in *Chlorella* species are excellent ($SR < 25\%$) and acceptable ($25\% < SR < 50\%$) for Cu. All the results of Hay powder analysis are excellent ($SR < 25\%$). The RSD for Fe and Mn is

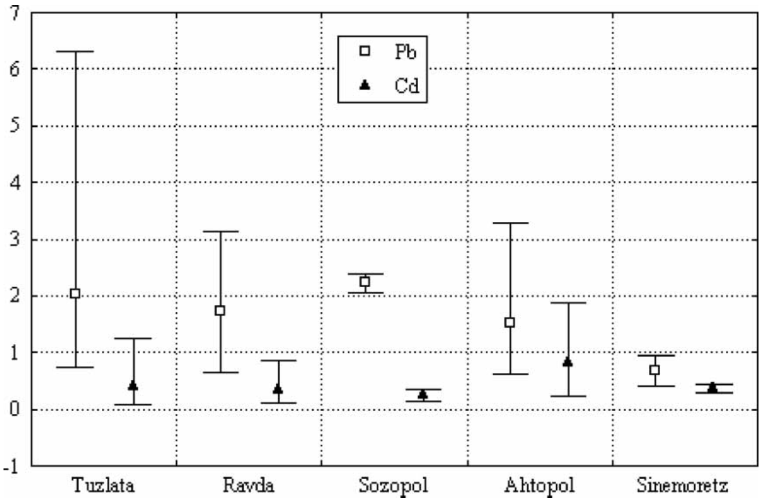

FIGURE 6 Distribution of Pb and Cd in *C. crinita* and *C. barbata* from different locations.

TABLE II Results [μg/g dry weight] from the AAS of standard reference materials

Element	*Chlorella*				*Hay powder*			
	This article	*Certified*	*SR*[%]	*RSD*[%]	*This article*	*Certified*	*SR*[%]	*RSD*[%]
Cd	0.024 ± 0.002	0.026[a]	7	9	0.029 ± 0.001	0.03	8	3
Cu	3.05 ± 0.28	3.5	29	9	9.6 ± 0.8	9.4	15	8
Fe	1825 ± 21	1850	4	1	215 ± 4	185	12	2
Mn	58 ± 3	69	24	5	46 ± 1	32–52[b]	5	2
Pb	0.65 ± 0.08	0.60	5	12	0.63 ± 0.01	0.6	2	2

[a]Information value. [b]Data from laboratories participating in the intercomparison test; no certified value.

TABLE III Correlation between concentrations of different metal pairs in *C. crinita*

Element	*Cd*	*Cu*	*Fe*	*Mn*	*Pb*
Cd	1.000	0.177	−0.218	−0.021	−0.317
Cu		1.000	0.497	0.234	0.518
Fe			1.000	0.292	0.709*
Mn				1.000	−0.142
Pb					1.000

Significance levels: $*p < 0.01$; $N = 12$.

under 5% and for Cd, Cu and Pb under 10% (except for Pb in *Chlorella*). This demonstrates that the analytical procedures performed lead to good precision.

Correlation coefficients were calculated on the basis of obtained results, showing the degree of correlation between the toxic metals accumulated by the two studied brown macroalgae. The correlation coefficients for the metal concentrations in the two species are presented in Tables III and IV.

TABLE IV Correlation between concentrations of different metal pairs in *C. barbata*

Element	*Cd*	*Cu*	*Fe*	*Mn*	*Pb*
Cd	1.000	−0.150	−0.191	−0.330	−0.071
Cu		1.000	0.896*	0.643	0.567
Fe			1.000	0.727**	0.544
Mn				1.000	0.488
Pb					1.000

Significance levels: *$p < 0.01$; **$p < 0.05$; $N = 8$.

DISCUSSION

The algae from the genus *Cystoseira* (*C. crinita* and *C. barbata*) are the largest brown algae in the Black Sea. They belong to the group of perennial species dominant through the whole year but with two characteristic maxima of development – spring and autumn. That is why we investigated the heavy metal contents in the two species mainly during the spring and autumn. Samples were also collected at three locations during summer 2000 – Tuzlata (north) with a higher degree of contamination and Ahtopol and Sinemoretz (south).

Iron and Manganese Distribution

The high Fe and Mn contents compared to the other heavy metals are connected with their function in the organism. It is known that there is a correlation between the biosorption of trace elements and their role in the organism. The major part of heavy metals form relatively stable complexes with proteins, lipids and phosphates, taking part in enzyme synthesis (Fe, Mn, Co) and in the metabolic processes in the organisms. Fe has a great binding capability for algal lipids and was accumulated to the greatest extent in the studied macrophytes.

There seems to be a tendency for the Fe content to decrease in both algal species during the last seasons investigated. The order of Fe concentration is: spring 1996 > spring 1998 > summer 2000 > autumn 1996 > spring 1999 > autumn 1999. There is a pronounced seasonal dependence in Fe accumulation in both brown algal species (Fig. 2). The mean Fe content in the spring is 326 μg/g; in the summer it is 161 μg/g and in the autumn 156 μg/g, which is connected with Fe variation with the light cycle. A similar dependence was established by Haritonidis and Malea [13] for Cr accumulation in the green macroalgae *Ulva rigida* and *Enteromorpha linza*.

The Fe variation depending on the sampling location is in the order: Sinemoretz < Ahtopol < Sozopol < Tuzlata < Ravda. Thus the southern regions have lower Fe contents in macroalgae than the central (Sozopol and Ravda) and northern region (Tuzlata).

The dependence of Fe content on different seasons can be illustrated at Tuzlata. A considerable decrease in Fe content is observed during the period spring 1996 to spring 1999, passing through a maximum (summer 2000). This dependence of Fe accumulation in the Tuzlata region differs from the other locations but coincides with the seasonal dependence in samples taken from Ahtopol.

The lowest Mn value is measured in *C. barbata* from Sozopol – autumn 1999 (6 μg/g), while the highest is in *C. crinita* from Ravda – spring 1999 (60 μg/g). Mn accumulation in brown macrophytes shows a seasonal dependence similar to Fe, but the highest content is in spring 1998 (46 μg/g) – twice as much as in spring 1996 (23 μg/g). The Mn concentration in different regions increases in the order: Sozopol < Sinemoretz < Tuzlata < Ahtopol < Ravda.

Comparing the orders of Fe and Mn it can be concluded that the two elements are accumulated to the highest degree at Ravda and at Tuzlata. Similar Fe and Mn trends are obtained for other locations except Sozopol.

Copper Contents in Brown Algae

Copper is one of the most biologically important metals. The element and its compounds usually exist in the biosphere only in trace quantities and participate in the biological cycle only in very low concentrations so that any increase of Cu content may lead to substantial damage in living organisms. Periodical monitoring of Cu concentrations in different biological species including marine biota is necessary. Muller *et al.* [14] investigated the chemical speciation of copper in the western Black Sea and established that a very large proportion of Cu (93–99.8%) is organically complexed (especially in shelf waters). However, it is known that the biological uptake is a function of the free metal concentration, so it can be assumed that a considerable part of the Cu dissolved in the water phase is not assimilated in practice by the macroalgae.

There is no clear seasonal tendency for the change in Cu concentration from the spring 1996 till summer 2000, but as a whole the Cu content does not vary much except in spring 1996 (Fig. 3). It can be seen from Fig. 4 that the lowest Cu content is measured in the southern site Sinemoretz, while the highest is at Ravda (4.8 μg/g), where the sampling was performed during 1996 and 1998 when there is a decrease in the overall content.

Distribution of Pb and Cd

Low Pb contents (below 1 μg/g) are measured at the southern sites (except Ahtopol, spring 1996), increasing gradually when going north. The highest mean Pb values are measured at Sozopol situated to the south of Burgas.

Temporal changes for Pb concentration follow the order: spring 1996 > autumn 1999 > spring 1998 > spring 1999 > summer 2000 > autumn 1996. The Cd concentration, in contrast to Pb, has a maximum during the summer. The values obtained for the two algal species *C. crinita* and *C. barbata* are in the range 0.1–1.87 μg/g. There is an increase in the last two seasons of the studied period – from spring 1998 to spring 1999 the mean concentration doubles and it also doubles between spring 1999 and summer 2000. Cd is exceptionally toxic even at very low concentrations which necessitates the study of Cd contamination sources as well as its distribution in marine ecosystems.

Mean Pb and Cd concentrations in the brown macroalgae are 1.75 and 0.43 μg/g, respectively. The Pb concentration is several times higher than the Cd concentration, but both elements are present in the macroalgae in concentrations that should not substantially affect the algal life cycle.

Correlation Between Different Metals

The correlation matrices of metal concentrations, independently of seasons and locations, showed a different distribution pattern in the two macroalgal species.

The correlation coefficients obtained for *C. crinita* (Table III) differ substantially from unity and therefore do not demonstrate a clearly expressed dependence between the separate experimental quantities. The data demonstrate a negative correlation between Cd and all the other determined metals except for Cd–Cu in *C. crinita*. This result suggests that there are antagonistic interactions between Cd and these elements.

The positive correlations between Fe–Cu (0.896), Fe–Mn (0.727) and Cu–Mn (0.643) in *C. barbata* show that these elements have a similar distribution pattern (Table IV).

CONCLUSIONS

Fe, Mn, Cu, Pb and Cd contents were determined in two of the brown macroalgal species most widespread along the Bulgarian Black Sea shore. The obtained results show that the highest degree of Fe, Mn, Cu and Pb accumulation is during the spring. The Cd content is lower during the spring and also during the maximum development in autumn. This is probably due to the algae's ability to desorb Cd and the increased intensity of the exchange processes in the two periods.

The fact that the *Cystoseira* macroalgae are proven bio-monitors gives us a reason to assess the contamination of the marine ecosystem at the observed five locations. The region of Ravda can be identified as a place with higher Fe, Mn and Cu contents while the region of Sozopol shows higher contents of Pb. The investigated heavy metals, however, are present in the macroalgae in concentrations that should not significantly affect their development and spreading. The decreasing trend of metal contents in both *Cystoseira* species in the last seasons and the comparatively similar values in the different regions suggest an absence of specific geographically dependent contamination.

References

[1] J.Y. Cabon, *Spectrochim. Acta Part B*, **57**, 513–524 (2002).
[2] A. Forsberg, S. Soderlund, A. Frank, L.R. Petersson and M. Pedersen, *Environ. Pollut.*, **49**, 245–263 (1988).
[3] P. Rainbow, *Marine Pollut. Bull.*, **31**, 183–192 (1995).
[4] D.J. Phillips and D.A. Segar, *Marine Pollut. Bull.*, **17**, 10–17 (1986).
[5] M.S. Al-Masri, In: *Proceedings of the CIESM Workshop* (18–20 April 2002, Marseille, France), 3–6 (2002).
[6] G.G. Polikarpov, In: *Proceedings of a Symposium on Marine Pollution* (5–9 October 1998, Monaco), IAEA-TECDOC-1094 1999, 38–43 (1999).
[7] E. McFarren, R. Lishka and J. Parker. *Anal. Chem.*, **42**, 358–360 (1970).
[8] A.S. Strezov, Tz.P. Nonova, M.I. Ayranov, T.T. Stoilova and K.K. Mitev, In: *Proceedings of the Third Black Sea International Conference* (6–8 June 2001, Varna, Bulgaria), 44–55 (2001).
[9] A.V. Jordanova, A.S. Strezov, M.I. Ayranov and T.T Stoilova, *Water Sci. Technol.*, **39**, 207–212 (1999).
[10] K.C. Guven, E. Okus, S. Topcuoğlu, N. Esen, R. Kucukcezzar, E. Seddigh and D. Kut, *Toxicol. Environ. Chem.*, **67**, 435–440 (1998).
[11] M.S. Al-Masri, S. Mamish, Y. Budier, *J. Environ. Radioactivity*, **67**, 157–168 (2003).
[12] J.M. Wood, *Science*, **183**, 1049–1052 (1974).
[13] S. Haritonidis and P. Malea, *Environ. Pollut.*, **89**, 319–327 (1995).
[14] F.L. Muller, S.B. Gulin and A. Kalvoy, *Marine Chem.*, **76**, 233–251 (2001).