Bull. Environ. Contam. Toxicol. (1999) 62:630-637

Bulletin of Environmental Contamination and Toxicology

Use of Macroalgae as Biological Indicators of Heavy Metal Pollution in Thermaikos Gulf, Greece

K. Fytianos,[1] E. Evgenidou,[1] G. Zachariadis[2]

[1] Environmental Pollution Control Laboratory, Chemistry Department, Aristotelian University of Thessaloniki, 540 06 Thessaloniki, Greece
[2] Analytical Chemistry Laboratory, Chemistry Department, Aristotelian University of Thessaloniki, 540 06 Thessaloniki, Greece

Received: 30 January 1999/Accepted: 19 March 1999

Heavy metal concentrations in macroalgae are used to monitor the level of bioavailable metals in estuarine and sea shore areas. Macroalgae correspond to the characteristics for bioindicators, since they provide a time-integrated picture of the bioavailable pollutants (Phillips, 1977). Macroalgae have a relatively long-life span, they can offer an environmental picture over extensive periods of time and occur abundantly along the seashore (Phillips et al. 1986, Levine 1984, Fytianos et al. 1997). Different species are usually used to monitor metal concentrations in various geographical areas, according to heavy taxonomic distribution (Ho, 1990, Stratis et al. 1996). Algae accumulate metals by means of a two-stage process, consisting first of a rapid, reversible physico-chemical process of adsorption on the exterior surface, and then of a slower metabolically regulated intracellular uptake (Garnham et al. 1992). Consequently, metal concentrations are greatly dependent both on external factors which affect metal interactions with the cell wall (pH, salinity, inorganic and organic complexing molecules) and on physico-chemical parameters which control the metabolic rate (temperature, light, oxygen and nutrients). Since metal binding on the external wall is a continuous process during the life-span of algae, older tissues usually contain higher amounts of many metals (Shimshock et al. 1992, Barreiro et al. 1993). Algae bind only free metal ions, the concentrations of which depend on the nature of suspended particulate matter. This, in turn, consists of both inorganic and organic complexes (Martin et al., 1994). Only a few benthic algae satisfy all requirements for successful metal monitoring, due to virtually continuous seasonal and geographical distribution in estuaries, tolerance to large salinity variations and resistance to high pollution concentrations (Edwards, 1972). The macroalgae most extensively used for monitoring heavy metal contamination of water belong to the genera Fucus, Enteromorpha, Laminaria and Ulva. The studies of Shiber and Washburn (1978), Sawidis and Voulgaropoulos (1986) and Ho (1990) support the existence of a direct relationship, between metal concentrations in water and in the tissues of U. lactuca. These macroalgae assimilate dissolved nutrients during their growth and released them almost completely in superficial sediment during their mineralization, this later process follows the almost complete disappearance of macroalgae and continues throughout winter (Sfriso et al., 1988). Few areas in Greece are considered to be polluted by heavy metals and in most of them, studies have been made in order to define the level of metal accumulation in the soft tissues of different organisms (Vasilikiotis et al., 1983; Catsiki et al. 1991).

Correspondence to: K. Fytianos

The present study describes an approach to heavy metal estimation in a particular area of the Northern Aegean Sea, the Thermaikos Gulf. Macroalgae were sampled in four stations representative of three areas of Thermaikos Gulf. The city of Thessaloniki with more than 1.200.000 inhabitants and its surrounding industrial area use Thermaikos Gulf as the final reservoir for their wastewaters. Thermaikos Gulf, which has an overall perimeter of about 70 km, consist of two basins, the inner and the central bay, having a small mean depth of about 20 m. The two basins communicate together and to the opean sea by narrow and shallow mouths, which do not permit the streams to carry off the wastes into the open sea for keeping the pollution on low levels. In Greece, certain coastal areas and especially several closed gulfs such as the Thermaikos Gulf in North Aegean Sea have been in the recent years very rapidly affected by industrialization, which is taking place without the adequate provisions for protective measures to maintain the quality of the marine environment. About 120.000 m^3/day of untreated and partially treated (40.000 m^3/day) sewage water from the city of Thessaloniki and an amount of about 30.000 m^3/day of treated/untreated industrial effluents are discharged on the north western coast of the Gulf.

MATERIALS AND METHODS

The first two stations (St. 1 and 2) are situated on the south-east coast of the gulf and practically are free from any specific source of pollution but densely populated specially during the summer months. The third station (St. 3) is situated at the main harbor of the city of Thessaloniki, the second biggest harbor of Greece. The fourth sampling station (St. 4) is on the north-west coast of the gulf and on the outskirts of the industrial zone. Several oil refineries and chemical industries and one drainage canal render this marine area heavily polluted. Additionally, rice and corn plantations cover the vicinity. Finally, the wide delta of Axios river and the effluents of a waste water treatment plant affect to wide extent the coastal zone.

Each one of the species of the green algae Enteromorpha and Ulva which dominate the sampling stations, covers generally more than 10% of the total biomass.

The sampling scheme covered the period from Sept. 1997 to Oct. 1998 and was conducted every month. At the same time, the heavy metal concentrations of seawater in the same stations were also determined. In some stations and for some periods either of the two species was not existed and so it was not sampled. About 0.25 kg of fresh weight were harvested every month. Samples were not found at all stations in all seasons: only Ulva development was continuous throughout the year, while Enteromorpha was present only for a few seasons during the year. The samples were washed in seawater at the sampling site and transferred to the laboratory in polyethylene boxes in refrigerated conditions. At the laboratory they were washed carefully in seawater to remove sand and particulate matter. Finally, samples were rapidly rinsed in double deionized water to remove salts from the seawater, dried at 80°C pulverized and stored until analysis.

Three subsamples (2 g) of each sample were submitted to acid digestion using conc. HNO_3 and $HClO_4$ in a sand bath (Góren et al., 1993). Digested samples were diluted to 50 ml with double deionized water and were analysed for metals using an atomic absorption spectrophotometer (Perkin-Elmer model 2380). Procedural blanks were run within each batch; the analytical procedure was checked by means of analysis of certified material of algal nature (CRM 279, BCR) (Accuracy: ± 2%). For the total dissolved trace metals in seawater, the filtrate was preconcentrated following the APDC/MIBK (ammonium pyrolidine dithioarbamate / methylo-isobutyl-ketone) extraction procedure (Fytianos et al., 1997; Standard Methods, 1989) and analysed using ET AAS to determine the total dissolved Cu, Cd, Pb, Fe, Zn, Mn and Ni. Analytical results were checked using the seawater standard (CRM 403, BCR). Variability between months and sampling sites was statistically analysed for each metal by two-way ANOVA. Concentration factor (CF) was calculated according to Foster (1976) in which CF is the ratio of metal concentration in the alga (μg/g dry wt.) to the concentration of dissolved metal in seawater (μg/L).

RESULTS AND DISCUSSION

In tables 1 and 2 the results of mean annual metal concentrations together with standard deviations calculated from 12 monthly measurements are given for Enteromorpha and Ulva sp. separately. From these results it is obvious that the bioaccumulation of the examined heavy metals is generally greater in the alga Enteromorpha sp than in Ulva sp. In fig. 1 the fluctuation of each metal concentration at the sampling station 3 and for the two algae is given.

Table 1. Mean metal concentrations (μg/g ± sd) for *Enteromorpha* sp

	St 1	St 2	St 3	St 4
Zn	48.0 ± 36.66	42.4 ± 29.38	47.1 ± 16.79	-
Cd	7.6 ± 2.69	6.7 ± 2.64	7.5 ± 3.80	-
Cu	1.0 ± 0.31	0.8 ± 0.45	14.0 ± 42.11	-
Pb	34.9 ± 56.96	16.8 ± 8.05	16.3 ± 4.34	-
Mn	35.6 ± 27.35	33.7 ± 21.78	12.1 ± 5.66	-
Fe	597.1 ± 316.14	565.0 ± 219.55	439.9 ± 277.18	-
Cr	6.1 ± 4.11	4.4 ± 3.39	5.9 ± 7.02	-

Table 2. Mean metal concentrations (μg/g ± sd) for *Ulva* sp

	St 1	St 2	St 3	St 4
Zn	34.5 ± 10.10	35.8 ± 20.03	49.7 ± 16.12	43.6 ± 31.78
Cd	4.1 ± 0.93	4.8 ± 2.31	6.2 ± 1.93	10.7 ± 4.14
Cu	0.5 ± 0.42	0.5 ± 0.25	0.5 ± 0.14	0.7 ± 0.17
Pb	10.7 ± 3.12	14.5 ± 6.97	12.0 ± 3.32	21.5 ± 10.55
Mn	36.6 ± 25.29	29.1 ± 14.23	16.7 ± 10.55	31.5 ± 32.97
Fe	373.5 ± 110.73	362.5 ± 152.85	479.9 ± 205.73	80.52 ± 262.17
Cr	4.3 ± 2.65	5.3 ± 3.31	5.1 ± 2.69	9.0 ± 262.17

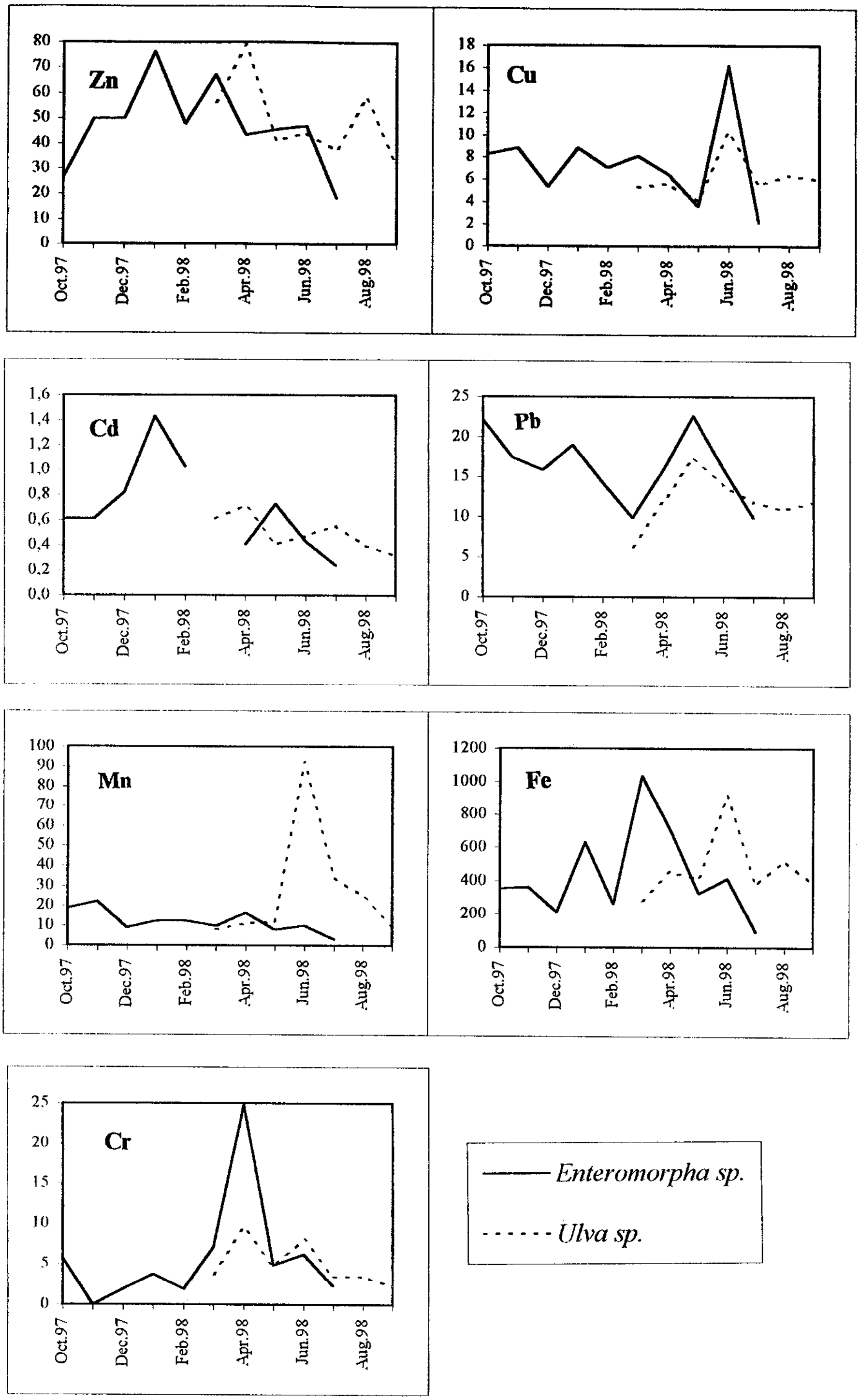

Figure 1. Fluctuations of metal concentrations (μg/g d.w.) in sampling station 3

High concentrations of metals were observed for Ulva in St. 4, which is located near the estuaries of Axios River and near the industrial area of Thessaloniki.

The observed relatively high values in the unpolluted areas (St. 1 and St. 2) are due to the pollution transport by the coastal currents and especially during the north-winds (Fytianos et al., 1983). Following the seasonal fluctuations of bioaccumulation of Zn, it can be seen that maxima of this bioaccumulation for the Enteromorpha occur in winter and for Ulva occur when spring is approaching.

Metals accumulated in Ulva and Enteromorpha sp decreased in the order Fe > Mn > Zn > Pb > Cr > Cu > Cd. Concentration values are rather comparable to those of other authors (Favero et al., 1996).

In Station 1 concentration of Fe was the highest, followed by Mn, Zn, Pb, Cr, Cu and Cd. In this station the Enteromorpha sp showed a tendency to concentrate trace metals in larger quantity than the Ulva sp. At St. 3, Fe was again present at the highest concentration in the two analysed species followed by Zn, Mn, Pb, Cr, Cu and Cd. Enteromorpha has higher concentrations of all the examined metals except Zn, Mn and Fe, which were accumulated more in Ulva sp. In St. 4 only Ulva was sampled and analyzed. All the examined metals showed the highest concentration in comparison with all other stations.

Calculation of the concentration factor (CF) for the analyzed metals was performed for the mean values of the whole year using the mean concentration values in sea water for the same stations (Table 3). The magnitude of the CF (Table 4) differs according to metal and suggests the specific affinity of Enteromorpha towards metals. The higher accumulation occurs for Fe, the metal most concentrated in water.

Table 3. Mean values (μg/L) of the examined metals in sea-water of Thermaikos Gulf

Station	Zn	Cu	Cd	Pb	Mn	Fe	Cr
1	13,2	4,5	0,2	6,5	6,4	39	3,2
2	15,4	3,4	0,35	7,4	4,9	36	3,6
3	14,6	6,3	0,40	12,5	6,8	44	4,4
4	16,2	8,8	0,45	9,6	6,7	56	5,5

The variation of a metal concentration in a kind of species depends mainly on two factors: the sampling site and the sampling time. Two-way analysis of variance is a capable tool to statistically evaluate the significance of each of the above two factors. For this reason, separate matrix tables for each metal and each species were constructed. The sampling site was the column factor while the sampling time was the row factor. The hypothesis tested at a 95% propability level was that neither the sampling site nor the sampling time were caused significant variation. Table 5 summarizes the results of this test. The F_{exper} values obtained are compared with F_{crit} values taken from tables, which are 2.09325 for 11 degrees of freedom and 2.89157 for 3 degrees of freedom respective. Higher F_{exper} values than F_{crit} indicate significant source of variance. In all metals (except Cd) and for both

Table 4. Concentration factor (CF) for metals in *Ulva* Sp and *Enteromorpha* sp

	St 1		St 2		St 3		St 4	
	Enteromorpha	Ulva	Enteromorpha	Ulva	Enteromorpha	Ulva	Enteromorpha	Ulva
Zn	3.6×10^3	2.6×10^3	2.7×10^3	2.3×10^3	3.2×10^3	3.4×10^3	-	2.7×10^3
Cd	4.84×10^3	2.7×10^3	2.3×10^3	1.5×10^3	1.8×10^3	1.3×10^3	-	1.5×10^3
Cu	1.7×10^3	0.9×10^3	2.0×10^3	1.4×10^3	1.2×10^3	1.0×10^3	-	1.2×10^3
Pb	5.3×10^3	1.6×10^3	2.3×10^3	2.0×10^3	1.7×10^3	0.9×10^3	-	2.2×10^3
Mn	5.5×10^3	5.7×10^3	6.9×10^3	5.9×10^3	1.8×10^3	2.5×10^3	-	4.7×10^3
Fe	1.5×10^4	0.9×10^4	1.6×10^4	1×10^4	0.9×10^4	1.1×10^4	-	1.5×10^4
Cr	1.9×10^3	1.4×10^3	1.2×10^3	1.5×10^4	1.3×10^3	1.1×10^3	-	1.6×10^3

species, it is concluded that the sampling site (column factor) is a very significant source of variation, which means that some of the sampling sites give significantly higher values than others. On the other hand, the sampling period caused significant variation only in case of Cr, Cd and Zn in Ulva.

The hypothesis that possible interactions between different metals exist, was tested by means of correlation analysis. All samples collected from different stations and throughout all the sampling periods were statistically evaluated. In the first scheme, the correlation matrix was consisted from Enteromorpha and Ulva species together, in order to obtain a rough profile of possible interactions of metals. The results are listed in Table 6. It can be seen that no significant car-relation appearanced and all correlation coefficients were less than 0.6.

Table 5. Analysis of variance (probability level 95%)

Sampling factors		*Enteromorpha*			*Ulva*		
		F_{exper}	F_{crit}	Significant	F_{exper}	F_{crit}	Significant
Zn	site (column)	2.5655	2.0932	Yes	4.4561	2.0932	Yes
	time (row)	10.7801	2.8916	Yes	3.7166	2.8916	Yes
Cu	site (column)	1.2839	2.0932	Yes	1.9166	2.0932	No
	time (row)	15.7444	2.8916	Yes	20.8674	2.8916	Yes
Cd	site (column)	1.0006	2.0932	No	5.1025	2.0932	Yes
	time (row)	1.0059	2.8916	No	8.1263	2.8916	Yes
Pb	site (column)	1.2397	2.0932	No	0.7723	2.0932	No
	time (row)	3.0988	2.8916	Yes	8.6329	2.8916	Yes
Mn	site (column)	1.3055	2.0932	No	1.2021	2.0932	No
	time (row)	10.9517	2.8916	Yes	18.1227	2.8916	Yes
Fe	site (column)	1.1030	2.0932	No	1.9790	2.0932	No
	time (row)	13.1497	2.8916	Yes	20.9481	2.8916	Yes
Cr	site (column)	1.5276	2.0932	No	2.7819	2.0932	Yes
	time (row)	5.1376	2.8916	Yes	5.4442	2.8916	Yes

Table 6. Correlation matrix for *Enteromorpha* sp

	Zn	Cu	Cd	Pb	Mn	Fe	Cr
Zn	1						
Cu	0.28415	1					
Cd	0.1181	0.03749	1				
Pb	-0.107	0.18103	-0.0422	1			
Mn	0.01377	0.60494	-0.061	0.04328	1		
Fe	0.19579	0.4221	0.21836	0.09578	0.040166	1	
Cr	0.35588	0.21154	0.02821	0.01912	0.28259	0.31617	1

In the second scheme, the correlation matrix was consisted from Enteromorpha species only, and the results are given in Table 7. In this case, all the correlation coefficients were less than 0.4 and obviously not significant.

Table 7. Correlation matrix for *Ulva* sp

	Zn	Cu	Cd	Pb	Mn	Fe	Cr
Zn	1						
Cu	0.29401	1					
Cd	0.13805	0.05242	1				
Pb	-0.194	0.09184	0.0655	1			
Mn	0.23011	0.40014	-0.1418	-0.0554	1		
Fe	0.26185	0.11466	0.32957	-0.0068	0.23443	1	
Cr	-0.0726	-0.099	0.06115	-0.0106	-0.0228	0.3539	1

In the third scheme, the correlation matrix was consisted from Ulva species only and Table 8 summarizes the obtained results. It can be seen that all correlation coefficients are positive, revealing positive interactions between metals in Ulva species. The majority of coefficients were less than 0.6, however two of them ($r \approx 0.8$) indicate a possible but not secure possitive correlation between Zn-Cr and Cu-Mn respectively. This means that e.g. increased zinc concentrations are expected to be accompanied by increased chromium concentrations.

Table 8. Correlation matrix for *Enteromorpha* sp and *Ulva* sp

	Zn	Cu	Cd	Pb	Mn	Fe	Cr
Zn	1						
Cu	0.28801	1					
Cd	0.28287	0.3333	1				
Pb	0.14985	0.66115	0.21868	1			
Mn	0.0428	0.79117	0.13164	0.4668	1		
Fe	0.13704	0.64365	0.35214	0.62733	0.53949	1	
Cr	0.79437	0.39921	0.22628	0.2054	0.34994	0.28574	1

Finally, a different approach followed, in order to test the hypothesis that the two species behave similarly in heavy metal accumulation. The correlation matrix consisted from pairs of concentration values obtained by determination of each metal in Enteromorpha and Ulva samples collected from the same station and the same time. The calculated correlation coefficient was 0.774, thus, indicating a possibility of using either Enteromorpha or Ulva for monitoring the heavy metal profile of coastal seawater.

REFERENCES

Barreiro R, Real C, Carballeira A (1993) Heavy metal accumulation by fucus ceramoides in a small estuary in north-west Spain. Mar Environ Res 36: 39-61

Catsiki V, Papathanassiou E, Bei F (1991) Heavy metal levels in characteristic benthic flora and fauna in the central Aegean Sea. Mar Pollut Bull, 22: 566-569

Edwards P (1972) Benthic algae in polluted estuaries. Mar Pollut Bull 3: 55-60

Favero N, Cattalini F, Bertaggia D, Albergoni V (1996) Metal accumulation in a biological indicator (*Ulva rigida*) from the lagoon of Venice (Italy). Arch Environ Contam Toxicol 31: 9-18

Foster P (1976) Concentrations and concentration factors of heavy metals in brown algae. Environ Pollut 10: 45-53

Fytianos K, Fachantidis P (1997) Cr (III) and Cr(VI) speciation in environmental samples. Toxicological and Environmentgal Chemistry 64: 197-202

Fytianos K, Charitonidis S, Albanis T, Konstantinou I, Seferlis M (1997) Bioaccumulation of PCB congeners in different species of macroalgae from Thermaikos Gulf, Greece. J Environ Sci Health A32: 333-345

Fytianos K, Vasilikiotis G (1983) Concentration of heavy metals in seawater and sediments from the N. Aegean Sea, Greece. Chemosphere 12: 83-91

Garnham G, Codd G, Cadd G (1992) Kinetics of uptake and intracellular location of cobalt, manganese and zinc in the estuares green alga Chlorella saline. Appl Microbiol Biotechnol 37: 270-276

Güren K, Saygi N, Öztürk B (1993) Survey of metal contents of Bosporus algae and sediments. Botanica Marina 36: 175-178

Ho Y (1990) *Ulva lactuca* as bioindicator of metal contamination in intertidal waters in Hong Kong. Hydrobiology 203: 73-81

Levine H (1984) The use of seaweeds for monitoring coastal waters. In: Schubert L (ed) Algae as ecological indicators. Academic Press, N.Y., pp. 189-210

Martin J, Huang W, Yoon Y (1994) Level and fate of trace metals in the lagoon of Venice (Italy). Mar Chem 46: 371-386

Phillips D, Seagar D (1986) Use of bio-indicators in monitoring concervative contaminants programme design imperatives. Mar Pollut Bull 17: 10-17

Phillips D (1977) The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments - a review. Environ Pollut 13: 281-317

Sawidis T, Voulgaropoulos A (1986) Seasonal bioaccumulation of iron, cobalt and copper in marine algae from Thermaikos Gulf of the N. Aegean Sea, Greece. Marine Environ Res 19: 39-58

Sfriso A, Paroni B, Marcomini A, Orio A (1988) Annual variations of nutrients in the lagoon of Venice. Mar Pollut Bull 19: 54-60

Shiber J, Washburn E (1978) Lead, mercury and certain nutrient elements in *Ulva lactuca* from Ras Beirut, Lebanon. Hydrobiology 61: 187-192

Shimshock N, Sennefelder G, Dueker M, Thurberg F, Varish C (1992) Patterns of metal accumulation in Laminaria longicruris from Long Island Sound. Arch Environ Contam Toxicology 22: 305-312

Standard Methods, AWWA, APHA (1989) 16th ed.

Stratis I, Simeonov V, Zachariadis G, Sawidis T, Madjukov P, Tsakovski S (1996) Chemometrical approaches to treat analytical data from aquatic macrophytes and marine algae. Fresenius J Anal Chem 355: 65-70

Vasilikiotis G, Fytianos K, Zotou A (1983) Heavy metals in marine organisms of the N. Aegean Sea, Greece. Chemosphere 12: 75-81