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Seasonal accumulation of metals by red alga *Gracilaria verrucosa* (Huds.) Papens. from Thermaikos Gulf, Greece

P. Malea & S. Haritonidis
University of Thessaloniki, School of Biology, Department of Botany, PO Box 109, Gr 54006 Thessaloniki, Greece

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Abstract

Concentrations of Fe, Pb, Cu, Zn and Cd were determined during one season in the red alga *Gracilaria verrucosa*, sediment and seawater from the Thermaikos Gulf, Greece. This region has been subject to change due to increases in industrial and domestic activities. The relative abundance of metals in *G. verrucosa* and seawater decreased in the order: Fe>Zn>Pb>Cu>Cd and in the sediment: Pb>Fe>Zn>Cu>Cd. Cadmium concentration in the alga correlated positively with that in seawater. There was positive correlation between Fe concentrations in the alga and those of the Zn and Cu. The concentrations of metals in the alga showed no significant differences between the stations. Lead, Zn and Cu concentrations in the alga were slightly higher at Biamyl, whereas Cd was higher at Perea and Fe at Nea Krini. Seasonal variation of metal concentrations in the alga was significant for Cd and Fe. Copper and Fe increased from winter to summer, whereas Cd was the opposite. Zinc concentrations were minimum and Pb concentrations were maximum during spring. These variations are discussed in relation to tissue age, life cycle, ambient concentrations of metals and other environmental conditions. Cd and Pb concentrations in *G. verrucosa* in the Thermaikos Gulf were higher and those of Cu and Zn were lower than in other species of the genus.

Introduction

The benthic algal flora of the Thermaikos Gulf was reduced from 120 to 30 species during the period 1966–1985 as a result of increasing pollution by sewage, agricultural drainage and industrial discharges (Haritonidis, 1978, 1993). This Gulf receives pollutants directly from urban effluents as well as through rivers (Axios, Aliakmon, Loudhias, Gallikos). The effluents from the Greek area come from an industrial zone (e.g. petrochemical distilleries, leather tanning, chrome plating), from domestic sewage from 1,200,000 persons of Thessaloniki Gulf and from agricultural drainage. Effluents also come from activities in the Former Yugoslav Republic of Macedonia via the R. Vardar, which becomes the R. Axios.

There is only limited information concerning the heavy metal content in macroalgae along the eastern Mediterranean coasts (e.g. Shiber & Washburn, 1978; Hornung et al., 1992; Malea et al., 1994 a, c). Little information is published on the levels of metals in algae of the Thermaikos Gulf (North Aegean Sea) (Sawidis & Voulgaropoulos, 1986 a, b; Djingova et al., 1987; Haritonidis & Malea, 1995), whereas studies on metals in the green algae, *Ulva rigida, Enteromorpha linza* have also been reported as part of this project (Haritonidis & Malea, 1999a; Malea & Haritonidis, 1999).

Since metals such as Fe, Cu, Zn, Pb and Cd are associated with the sewage and industrial effluents of Thermaikos Gulf, they were selected for this study (Diamantopoulos et al., 1989; Marcantonatos, 1990). Diamantopoulos et al. (1989) found that Cu, Zn and Fe concentrations in the sewage decreased gradually during 1980–1989. During seasonal samplings samples of red alga *Gracilaria verrucosa* (Huds.) Papens were collected. Heavy metal accumulation by the species of *Gracilaria* has been carried out in several studies (Güven et al., 1992; Karez et al., 1994), including some measurements made in the Thermaikos Gulf for Fe, Co, Cu, Mn and Ni (Sawidis & Voul-

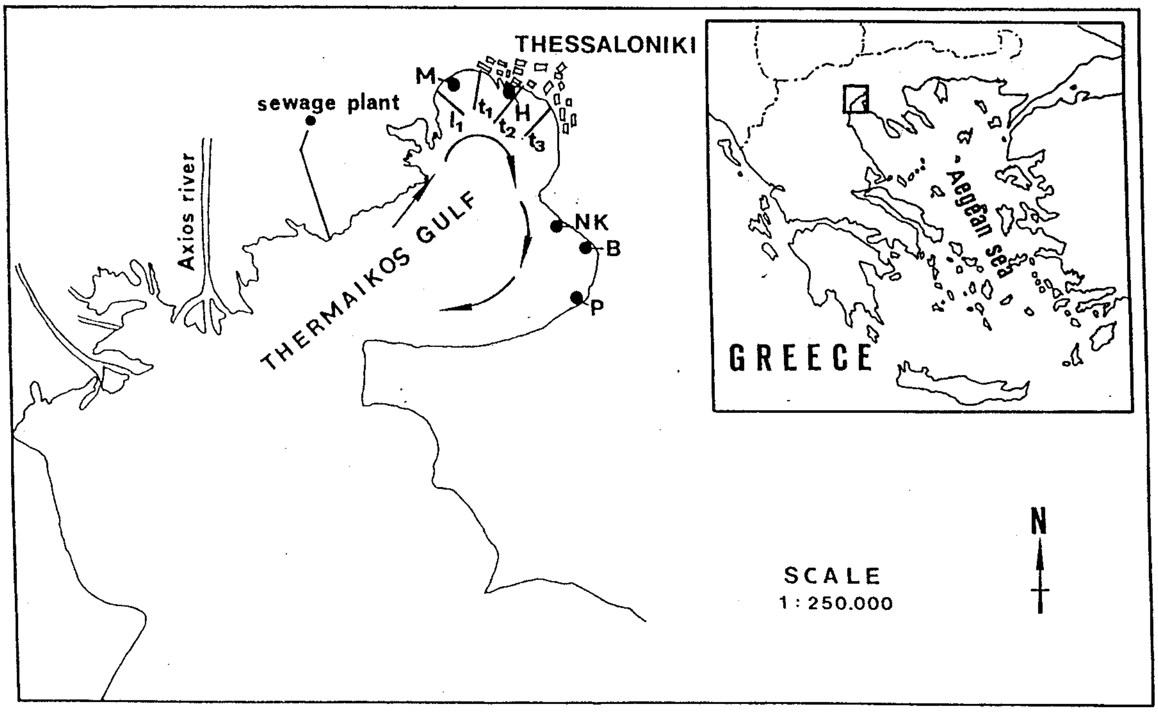

Figure 1. Sampling stations in Thermaikos Gulf: NK = Nea Krini; B = Biamyl; P = Perea. M = the industrial area (Mamidakis); H = Harbour. The direction of sea currents (according to Ganoulis and Krestenitis 1982) is marked with an arrow, while the industrial (I_1) and domestic wastes (t_1, t_2, t_3) discharged are marked with vertical lines.

garopoulos,1986a,b; Djingova et al.,1987); seasonal studies were not included.

There are many reports suggesting the use of macroalgae as indicator species (e.g. Ho, 1990; Favero et al., 1996). According to Phillips (1977) and Brix et al. (1983) the requirements of an ideal indicator may be summarised as follows. All individuals should show a simple correlation between residue content and the average pollutant concentration in their surrounding water or substrate. The species should accumulate the pollutant without being killed, be sedentary, of sufficient size, have a widespread distribution, be long-lived and easy to sample.

The aim of this study was to measure the levels of Fe, Pb, Zn, Cu and Cd in *G. verrucosa*, the sediment and seawater throughout the year at three stations in the Thermaikos Gulf distributed at a gradually increasing distance from the source of pollution. This would provide information on the local and seasonal variation of Fe, Pb, Zn, Cu and Cd concentrations in *G. verrucosa*, on possible intermetal correlations and the effect of metal content in the environment on metal concentration in the alga. This would in turn provide data on metal contamination in the Gulf by comparing the metal content in *G. verrucosa* of Thermaikos Gulf with that in other areas and establish whether *G. verrucosa* can serve as metal bioindicator.

Materials and methods

Study area and sampling locations

The study area (Thermaikos Gulf, Greece) consists of three parts: i) Bay of Thessaloniki (inner part) ii) Gulf of Thessaloniki south of the Bay; iii) outer part of the Gulf (Figure 1). The Bay of Thessaloniki receives the untreated sewage from 1,200,000 persons (tubes t_1, t_2, t_3 of Figure 1); only 30% of the sewage has been treated (since 1992) via the sewage plant. The effluents from the industrial area are discharged into the northwestern part of the Gulf (symbol I_1 of Figure 1). Copper, Fe, Pb and Zn are found in effluents of petrochemical distilleries, Fe from leather tanning, Cu and Zn from chrome plating, Fe, Cd, Cu and Zn from chemical industry, Zn from steel factories, Zn, Cu and

Fe from factories producing non-metal material (for more details see p. 714 of Marcantonatos, 1990)

The currents have a cyclic direction moving towards the inner part of the Gulf (Figure 1) (Ganoulis & Krestenitis, 1982). The physico-chemical features of the water during the sampling period were: temperature, 9.5–26.2 °C, salinity 32.1–36.9‰, pH of the seawater 7.8–8.3, dissolved oxygen in seawater 3.65–8.45 mg L^{-1} (Nikolaidis, 1985).

Analytical procedure

Seasonal samplings (January, April, June, October) of the red alga *Gracilaria verrucosa* (Huds.) Papens. were carried out at three stations (Nea Krini, Biamyl, Perea) distributed along the second part of the Thermaikos Gulf at gradually increased distance from the pollution sources (Figure 1). The algal samples were clipped off at sediment level using a stainless-steel spattle. In each station sediment and seawater samples were also taken. In the laboratory the samples were washed in seawater and in distilled water to remove seawater and any organic debris. Alga and sediment samples were dried to a constant weight (80 °C for the plant and 105 °C for the sediment); the plant samples were ground to powder in a mill and sediment samples were sieved in plastic sieves first with a 2-mm mesh and subsequently with a 0.2-mm mesh. Three subsamples of each sample (250 mg) were ashed in a Teflon PTFE beaker at 420 °C for 24 h, dissolved in 4 mL HNO_3 (65% p.a.) filtered through an acid-washed filter (Whatman GF/C) and adjusted to 20 mL with distilled water (Sperling & Bahr 1980; Malea & Haritonidis, 1995). In the field the seawater was acidified with 1.5 mL conc. HNO_3 L^{-1}. Subsequently the seawater samples were evaporated and the salt was ashed (420 ° C) and then treated with 0.2% HNO_3. (Haritonidis & Malea, 1995). Iron, Pb, Zn, Cu and Cd concentrations were measured by flameless AAS (Perkin-Elmer HGA 72) with deuterium background correction. Accuracy was checked by analysis of standard reference material of Orchard leaves (National Bureau of Standards no. 1571). When 250 mg of reference material was subjected to the same procedure as the algal samples, the results were within 10% for Fe and 3% for Zn of NBS certified values, whereas for Cu, Cd and Pb the results were 6%, 20% and 2%, respectively, lower than the standard. The precision of the analyses for *G. verrucosa* appeared good based on the mean values for coefficient of variation (CV%), which were calculated by measuring at least three independent subsamples of the same sample. The mean percent deviation was for Fe 5.8%, for Cu 17.7%, for Zn 6.8%, for Pb 11.4% and Cd 12.8%.

Table 1. Mean ($\bar{x}$), number of samples (N), range and standard error (SE) of metal concentrations from all locations and seasons in *Gracilaria verrucosa* sediment (μg^{-1} dry weight) and seawater ($\mu g\ L^{-1}$)

	Metal	N	$\bar{x}$	Range	SE
Gracilaria verrucosa	Cd	11	1.8	0.8–3.1	0.2
	Pb	12	14.4	9.5–19.0	0.9
	Cu	12	2.0	1.1–3.2	0.2
	Zn	12	53.4	35.0–67.7	9.8
	Fe	12	95.1	63.0–121.0	5.3
sediment	Cd	10	0.4	0.1–0.9	0.08
	Pb	10	55.4	5.0–130	14.6
	Cu	10	2.8	0.9–6.1	0.5
	Zn	10	26.1	12.3–39.5	2.7
	Fe	10	51.6	22.5–643	3.8
seawater	Cd	12	0.2	0.1–0.3	0.02
	Pb	12	2.1	1.6–2.8	0.09
	Cu	11	1.4	0.9–2.05	0.1
	Zn	12	20.1	15.5–25.2	0.9

Non-parametric statistical analysis (Spearman rank-order correlation coefficient, Kruskal-Wallis one-way analysis of variance by ranks) was performed.

Results

From the mean values (±SE) of metal concentrations in *G. verrucosa*, the sediment and seawater it is obvious that the relative abundance of metals in the alga and seawater decreased in the order: Fe > Zn > Pb > Cu > Cd, whereas in the sediment the order was the same except for Pb > Fe > Zn (Table 1).

No metal concentration in *G. verrucosa* showed significant correlation (Spearman rank-order correlation coefficient) with those in the sediment, but Cd concentrations in the alga correlated positively ($p < 0.001$) with the respective ones dissolved in seawater (Table 2). Correlation matrices of metal concentrations in *G. verrucosa* showed that there was significant positive correlation (Spearman coefficient) between Fe-Zn ($p < 0.05$) and Fe-Cu ($p < 0.01$) (Table 3). Kruskal-Wallis analysis demonstrated that the concentrations of each metal in *G. verrucosa* were distributed uniformly ($p > 0.05$) among the three stations (Table 4). Lead, Zn and Cu contents in *G. verrucosa*

Table 2. Correlation (Spearman coefficient corrected for ties, rs) between metal concentrations in *Gracilaria verrucosa* and the sediment and seawater. The number of samples used is given in brackets

	Cd	Pb	Cu	Zn	Fe
	ns	ns	ns	ns	ns
G.verrucosa	–0.063	0.328	0.255	0.673	0.559
sediment	(9)	(10)	(10)	(10)	(10)
		ns	ns	ns	
G.verrucosa	–0.888**	0.014	0.516	–0.361	
seawater	(11)	(12)	(11)	(12)	

Significance level ** $p < 0.01$, ns = not significant at significance level.

Table 3. Correlation (Spearman coefficient corrected for ties, rs) between concentrations of different metal pairs in *Gracilaria verrucosa*. The number of samples is given in brackets

	Cd	Pb	Cu	Zn	Fe
		ns	ns	ns	ns
Cd	1.000	0.339	–0.502	0.241	–0.383
		(11)	(11)	(11)	(11)
			ns	ns	ns
Pb		1.000	0.077	0.060	–0.063
			(12)	(12)	(12)
				ns	**
Cu			1.000	0.284	0.825
				(12)	(12)
					*
Zn				1.000	0.595
					(12)
Fe					1.000

Significance levels: * $p < 0.05$, ** $p < 0.01$, ns = not significant at significance level 0.05.

Table 4. Mean ± SE of metal concentrations in *Gracilaria verrucosa* (μg g^{-1} dry weight) at 3 sampling stations (independently of seasons) (Kruscal-Wallis one-way analysis of variance, not significant for all metals)

	Cd	Pb	Cu	Zn	Fe
Nea Krini	1.567	11.90	1.95	50.50	97.6
	± 0.271	± 1.00	± 0.306	± 4.83	± 9.71
Biamyl	1.739	17.06	2.14	60.2	94.7
	± 0.455	± 1.30	± 0.444	± 4.61	±11.9
Perea	1.950	14.29	1.929	49.41	92.91
	± 0.422	± 1.68	± 0.332	± 4.90	± 8.52

were slightly higher at Biamyl, whereas those of Cd were higher at Perea and of Fe at Nea Krini (Table 4).

The seasonal variation of metals in *G. verrucosa* was significant for Cd and Fe (Kruskal-Wallis one-way analysis, $p < 0.01$) (Figure 2). Copper and Fe concentrations increased from winter to summer, whereas Cd content followed the opposite pattern. Zinc concentrations were minimum in spring and maximum in the remaining seasons, whereas Pb levels were higher in spring (Figure 2).

Discussion

The relative uptake of metals in *G. verrucosa* in theThermaikos Gulf was the same as that in *Ulva rigida* and *Enteromorpha linza* during the sampling period (Haritonidis & Malea, 1999a; Malea & Haritonidis, 1999) and seemed to reflect the concentrations of dissolved metals in the seawater and the total concentrations in the sediment. Lead in the sediment had higher values than Fe and Zn, but the converse occurred in the alga. This may be due to the fact that Fe and Zn being essential elements, having high bioavailability in the study area or having a high capacity for binding with the anionic sites of the alga (Kabata-Pendias & Pendias, 1985; Karez et al., 1994).

The positive correlations between Fe-Zn and Fe-Cu may indicate that among these metals there are synergistic interactions for the binding sites of the plant or that Fe, Zn and Cu enter the Gulf through the same sources and follow a similar distribution pattern (Karez et al., 1994). The relatively high concentrations of Cu, Zn in the sediment (Haritonidis & Malea, 1999b) and of Cu, Zn and Pb in *G. verrucosa* at Biamyl can probably be attributed to the fact that around this station there are many shipyards. Currents may also transport these metals from the industrial zone of Thessaloniki (Mamidakis station) towards this station (Figure 1) (Ganoulis & Krestenitis, 1982). At this station the concentrations of Cu, Fe, Pb and Zn in *Ulva rigida* and Cu and Zn in *Enteromorpha linza* were higher (Haritonidis & Malea, 1999a; Malea & Haritonidis, 1999).

The seasonal variation of Cd in the alga followed the variation of dissolved Cd in the seawater collected from 7 stations of the Thermaikos Gulf (Haritonidis & Malea, 1999b). There was a significant correlation among Cd concentrations in the alga and those in the seawater. The seasonal variations of Cu, Zn and Fe in *G. verrucosa* were also similar to those of the respective metals in *Ulva rigida* and *Enteromorpha linza* in

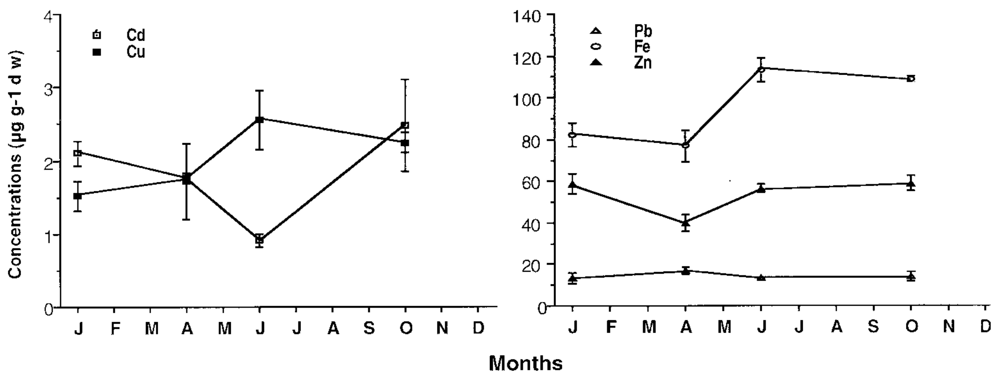

Figure 2. Seasonal variation of metal concentrations in *Gracilaria verrucosa* (μg g^{-1} dry weight). Each point represents the mean of the values recorded in a season at all sampling stations; bars represent the standard error. (Kruscal-Wallis one-way analysis, $p < 0.01$ for Cd and Fe and non-significant for Pb, Cu, Zn).

the study area (Haritonidis & Malea, 1999a; Malea & Haritonidis, 1999).

High levels of Fe and other metals (e.g. Cu, Zn) in macroalgae during summer-fall have been attributed to the fact that during these months photosynthesis and respiration are more intense, thus enhancing algal metal uptake; a similar suggestion has been made for seagrasses (Wahbeh, 1984). High levels of Fe during summer-fall were also noticed in other macrophytes (Malea et al., 1994c; Malea & Haritonidis, 1995).

High levels of Cd and Zn during fall-winter and of Cu and Fe during fall in *G. verrucosa* may occur because of increased rainfall during this period, resulting in fluvial inputs. Rivers such as the Axios carry metals from upstream sites. High levels of these metals, which are weakly bound to the suspended fraction and thus become more biologically available may be expected (Karez et al., 1994). Lacerda et al. (1987) showed that Zn and Cd were totally, but weakly, bound to the suspended particulate fraction and Pb was strongly bound, thus decreasing its availability.

Seasonal variation of Cu and Fe in *G. verrucosa* was associated with growth dynamics. The highest concentrations were reported in summer when biomass was at a minimum and the lowest in winter and spring when biomass had maximum values (Haritonidis, 1978). The increase in biomass resulted in a decrease of the metal content per mass unit due to dilution (e.g. Lyngby & Brix, 1982; Rönnberg et al., 1990; Malea et al., 1994b; Haritonidis & Malea, 1995). The Cu and Fe contents of *G. verrucosa* were low after the appearance of the new individuals (winter) and increased with the increase of the age of the tissue (from winter to summer). An increase with age in content of some metals, such as Fe and Zn, has also been described for many macrophytes (e.g. Försberg et al., 1988; Lyngby & Brix, 1989; Malea et al., 1994c). This is attributed to the fact that these metals are taken up irreversibly by the plants, more binding sites are created with the increase of age and some contamination of the older parts with fine particles (Bryan & Hummerstone, 1973; Brix & Lyngby, 1982). The seasonal variation of Cd is reinforced by the fact that, in contrast to Cu and Fe, Cd concentrations decrease with increasing age of the plant. This decrease with age is the outcome of the re-translocation of metal from the older to the younger thallus and of metal leakage for metal from the older thallus to the surrounding water; these suggestions have been first made in the seagrass *Zostera marina* by Brix and Lyngby (1982). Cadmium concentrations in *G. verrucosa* in Thermaikos Gulf were higher than those in this species in the Black Sea (Güven et al., 1992) and in other species of the genus from other areas (Karez et al., 1994).

It should be noted that the comparison among species in different areas should normally be based on collections made at the same time of year, preferably in late winter or early spring when concentrations are highest in order to ensure that the plants analysed have the same age structure or that the plant age is eliminated as a variable in the inter-site or inter-time comparisons (Ward, 1987). There have been no previous reports on seasonal changes in metal concentrations, so it is impossible to make comparisons. Moreover, it cannot be stated for certain that the species is resistant to the concentrations of Cd found in the area, as no experimental studies were done; however, no necrotic phenomena have been observed in material sampled from the area.

Phillips (1977) and Hellawell (1986) reported on the requirements for an ideal indicator species.*Gracilaria verrucosa* in Thermaikos Gulf generally fits these requirements. *G. verrucosa* fulfils what is probably the most important criterion, that the pollutant contents of the organism and the environment should be correlated (Pergent-Martini, 1998), because Cd concentrations in the plant were correlated significantly with those in the seawater. The choice of *G. verrucosa* as a possible indicator species should be characterized as successful, as it is suggested that in the Thermaikos Gulf the species can be regarded as a 'bioindicator' for Cd. The best season for the species to be used as an indicator for Cd is fall-winter, when Cd concentrations were higher. In this area the two other most abundant macroalgae, *Ulva rigida* and *Enteromorpha linza* can also be regarded as indicators, the first for Pb, Zn and Cd and the second for Pb and Zn (Haritonidis & Malea, 1999a; Malea & Haritonidis, 1999).

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References

Brix H, Lyngby JE (1982) The distribution of cadmium, copper, lead and zinc in eelgrass *Zostera marina* L. Sci. tot. Environ. 24: 51–63.

Brix H, Lyngby JE, Schierup HH (1983) Eelgrass (*Zostera marina* L.) as an indicator organism of trace metals in Limfjord, Denmark. Mar. environ. Res. 8: 165–181.

Bryan GW, Hummerstone LG (1973) Brown seaweed as an indicator of heavy metals in estuaries in south-west England. J. mar. Biol. Assoc. U.K. 53: 705–720.

Diamantopoulos E, Deligiannis A, Ziogou K, Kozlakidou S, Stylianou I, Bagiatis C (1989) Quantitative features of the sewage of Thessaloniki. 6th Seminar for Protection of the Environment. Aquatic recipients, Thessaloniki, Greece: 129–137.

Djingova R, Kuleff I, Arpadjan S, Voulgaropoulos A, Sawidis T, Alexandrov S (1987) Neutron activation and atomic absorption analysis of *Ulva lactuca* and *Gracilaria verrucosa* from Thermaikos Gulf, Greece. Toxicol. environ. Chem. 15: 149–158.

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.

Försberg A, Söderlung S, Frank A, Petersson LR, Pedresén M (1988) Studies of metal content in the brown seaweed *Fucus vesiculosus*, from the Archipelago of Stockhol. Environ. Pollut. 49: 245–263.

Ganoulis J, Krestenitis J (1982) Choix du site optimum d'evacuation des eaux usées dans une région côtière. Societé Hydrotechnique de France, 17 Journeés de l' Hydraulique.

Güven KC, Topcuog̅lu S, Kut D, Esen N, Erenturk N, Saggi N, Ceyher E, Guvener B, Ozturk B (1992) Metal uptake by Black Sea algae. Bot mar. 35: 337–340.

Haritonidis S (1978) Contribution to the research of marine macrophyceae (Chloro-, Phaeo- and Rhodophyceae) of Thermaikos Gulf. Ph D Thesis, Thessaloniki, Greece (in Greek) 175 pp.

Haritonidis S (1993) Introduction into eutrophication problems and metal distribution in Greek lagoons. In Proceedings COST-48 Symposium of Sub Group III. Macroalgae, Eutrophication and Trace Metal Cycling in Estuaries, and Lagoons, Thessaloniki, 24–26 September: pp. 154–163.

Haritonidis S, Malea P (1995) Seasonal and local variation of Cr, Ni and Co concentrations in *Ulva rigida* C. Agardh and *Enteromorpha linza* Linnaeus from Thermaikos Gulf, Greece. Environ. Pollut. 89: 319–327.

Haritonidis S, Malea P (1999a) Bioaccumulation of metals by the green alga *Ulva rigida* from Thermaikos Gulf, Greece. Environ. Pollut. 104: 365–372.

Haritonidis S, Malea P (1999b), Seasonal and local variation of metals in seawater treated by different methods and sediment of Thermaikos Gulf, Greece, Oebalia 25: 89–109.

Hellawell JM (1986) Biological Indicators of Freshwater Pollution and Environmental Management. Elsevier Applied Science Publishers, London, 546 pp.

Ho YB (1990) *Ulva lactuca* as bioindicator of metal contamination in intertidal waters in Hong Kong. Hydrobiologia 203: 73–81.

Hornung H, Kress N, Friedlander M (1992) Trace element concentrations intertidal algae collected along the Mediteranean shore, Israel. Fresenius Envir. Bull. 1: 84–89.

Kabata-Pendias A, Pendias H (1985) Trace Elements in Soils and Plants. CRC Press, 315 pp.

Karez CS, Magalhaes VF, Pfeiffer WC, Amado Filho GM (1994). Trace metal accumulation by algae in Sepetiba Bay, Brazil. Environ. Pollut. 83: 351–356.

Lacerda LD, Pfeiffer WC, Fiszman M (1987) Heavy metal distribution, availability and fate in Sepetiba Bay, S.E. Brazil. Sci. tot. Environ. 65: 163–173.

Lyngby JE, Brix H (1989) Heavy metals in eelgrass (*Zostera marina* L.) during growth and decomposition. Hydrobiologia 176/177: 189–196.

Malea P, Haritonidis S (1995) Local distribution and seasonal variation of Fe, Pb, Zn, Cu, Cd, Na, K, Ca and Mg concentrations in the seagrass *Cymodocea nodosa* (Ucria) Aschers. in the Antikyra Gulf, Greece P.S.Z.N.I. Mar. Ecol. 16(1): 41–56.

Malea P, Haritonidis S (1999) Metal content in *Enteromorpha linza* Linnaeus in Thermaikos Gulf (Greece). Hydrobiologia 394: 103–112.

Malea P, Haritonidis S, Kevrekidis T (1994a) Metal content of some green and brown seaweeds from Antikyra Gulf (Greece). Hydrobiologia 310: 19–31.

Malea P, Haritonidis S, Kevrekidis T (1994b) Seasonal and local variations of metal concentrations in the seagrass *Posidonia oceanica* (L.) Delile in the Antikyra Gulf, Greece. Sci. tot. Environ. 153: 225–235.

Malea P, Haritonidis S, Stratis I (1994c) Bioaccumulation of metals by Rhodophyta species at Antikyra Gulf (Greece) near an aluminium factory. Bot. Mar. 37: 505–513.

Marcantonatos G (1995) Treatment and disposal of aquatic wastes. Sewage, Industrial Effluents, Animal Residue, Athens, pp 738.

Nikolaidis G (1985) Qualitative and quantitative investigation of marine plant (Chlorophyceae, Phaeophyceae and Rhodophyceae) in different polluted (Gulf of Thessaloniki) and non-polluted areas (Chalkidiki). Ph D. Thesis, Thessaloniki, Greece, pp 152.

Phillips DJH (1977) The use of biological indicator organism to monitor trace metal pollution in marine and estuarine environments – A review. Environ. Pollut. 13: 281–317.

Sawidis T, Voulgaropoulos AN (1986a) Determination of the bioaccumulation of Co, Ni and Mn in algae. International Conference of Chemicals in the Environment, Lisbon: 159–164.

Sawidis T, Voulgaropoulos AN (1986b) Seasonal bioaccumulation of iron, cobalt and copper in marine algae from Thermaikos Gulf of the Northern Aegean Sea, Greece. Marine Environmental Research 19: 39–47.

Shiber J, Washburn E (1978) Lead, mercury and certain nutrient element in *Ulva lactuca* Linnaeus from Ras Beirut Lebanon. Hydrobiologia 61: 187–192.

Sperling KR, Bahr B (1980) Determination of extremely low concentrations of Cd in blood and urine by flameless AAS. I. Testing of a micromethod. Z. anal. Chem. 301: 29–31.

Wahben M (1984) Levels of Zn, Mn, Mg, Fe and Cd in three species of seagrasses from Aqaba (Jordan). Aquat. Bot. 20: 179–183.

Ward TJ (1987) Temporal variation of metals in the seagrass *Posidonia australis* and its potential as a sentinel accumulator near a lead smelter. Mar. Biol. 95: 315–321.