THE RED MACROALGA *Gracilaria bursa-pastoris* AS A BIOINDICATOR OF METALS (Fe, Zn, Cu, Pb, Cd) IN OLIGOHALINE COASTAL ENVIRONMENTS

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ABSTRACT

The bioaccumulation of iron, zinc, copper, lead and cadmium by *Gracilaria bursa-pastoris* in the coastal section of the Evros River Delta, Aegean Sea was investigated to provide information on the capacity of the red macroalga to act as a bioindicator of these metals in oligohaline coastal environments. Metal concentrations in the macroalga, water and sediments and several water parameters were measured seasonally at three stations located at increasing distance from the river's estuary. Mean iron, zinc, copper, lead and cadmium concentrations in *G. bursa-pastoris* were 817, 44.1, 12.3, 8.6 and 0.5 μg g^{-1} dry wt respectively. Local patterns of zinc, lead and copper in the macroalga were characterized by maximum concentrations at the station located nearest to the estuary, just as their sediment contents; moreover, zinc showed a positive correlation with its sediment loads, indicating that *G. bursa-pastoris* efficiently reflects the ambient zinc abundances, i.e. satisfies the basic pre-requisite for its use as a bioindicator of zinc. Zinc in *G. bursa-pastoris* correlated negatively with water salinity, possibly due to both a higher metal burden in the freshwater flowing in the study area and an increase in zinc uptake with decreasing salinity. Cadmium in the macroalga displayed a local pattern reverse to that of its sediment contents and to those of zinc, copper and lead both in the sediments and in the plant; it also correlated negatively with zinc in the macroalga, suggesting that an antagonistic interaction between these metals markedly interferes with the use of *G. bursa-pastoris* as a bioindicator of cadmium.

KEYWORDS: metals, seaweed, accumulation, salinity, estuary, Mediterranean Sea

INTRODUCTION

Macroalgae can accumulate large amounts of trace metals from ambient waters; additionally, metal contents in the tissues of these plants often indicate their ambient bioavailability [1]. Therefore, several investigations of the accumulation of metals by macroalgae have been carried out (e.g. [2-4]). Nevertheless, to our knowledge, no study investigated in detail the accumulation of metals by the widespread *Gracilaria bursa-pastoris* (Gmelin) Silva.

Gracilaria bursa-pastoris, apart from the fact that it exhibits a broad geographical distribution, is available throughout the annual cycle in coastal environments, is easy to sample and tolerant to fluctuations in salinity and turbidity [5-7], namely, it conforms to several of the pre-requisites for a suitable bioindicator [1]. Additionally, this red macroalga displays a year-round availability in oligohaline coastal environments [8], where only a few seaweed species thrive. Thereby, the analysis of *G. bursa-pastoris* can potentially provide useful information on the contamination of oligohaline environments; these environments are often impacted by amounts of anthropogenic pollutants, which, in combination with low salinities, may further stress the organisms.

The present study aims to provide information on the capacity of *G. bursa-pastoris* to act as an efficient bioindicator of metals in oligohaline coastal environments. We described the spatial and seasonal variation in iron, zinc, copper, lead and cadmium concentrations in *G. bursa-pastoris* in the coastal section of the Evros Delta, Aegean Sea and investigated whether the variation in metal contents in this macroalga reflects the variation in their ambient abundances and if it is significantly affected by certain variables. Provided that metal contents in seaweeds often indicate their ambient bioavailability [1], we predicted that *G. bursa-pastoris* would reflect the ambient abundances of the metals analyzed, i.e. that it satisfies the basic pre-requisite for its use as bioindicator of these elements in oligohaline coastal environments.

MATERIALS AND METHODS

Study area

Gracilaria bursa-pastoris along with *Ulva rigida* C. Agardh are the most common and abundant macroalgae in the coastal section of the Evros River Delta, Northern Aegean Sea, Mediterranean Sea, where extremely low salinities frequently occur almost throughout the annual cycle [9]. The Evros River Delta is one of the most important estuarine areas in Southern Europe and is protected as a wetland of international value according to the RAMSAR convention. Nevertheless, considerable amounts of metals, originating from human polluting activities, possibly reach this estuarine area through Evros River (e.g. [10]).

Evros is the greatest river in the Balkan Peninsula, rising from the mountain Rila in Bulgaria and following south direction. Its total length is 540 km, while the river basin is approximately 53 000 km^2. Evros River receives sewage from large urban centres and agricultural runoff from intensively cultivated plains in Bulgaria, Turkey and Greece as well as wastes from a heavy industrial activity mainly in Bulgaria, including metal works, wood processing, textiles, tanneries, production of chemicals, paints, batteries, etc. (e.g. [10]). Evros River is divided in two branches, before falling into the Aegean Sea, the Eastern Branch and the Western Branch, and forms a delta; the part of the delta that belongs to Greece is located between 40°44′ N and 40°51′N and between 25°53′E and 26°08′E (Fig. 1). Fresh water reaches the delta area mainly through the Eastern Branch of Evros River.

Sampling

Samples were collected at stations B (40° 45′ N and 26° 01′ E), D (40° 47′ N and 26° 01′ E) and E (40° 48′ N and 26° 00′ E) located in the coastal section of the Evros Delta at increasing distance from the estuary of the Eastern Branch to a northwest direction (Fig. 1); the Eastern Branch is the main branch of Evros River and, thereby, the potential major input pathway of metals to the delta area. All three stations were located in sheltered areas with immediate communication with the sea. The distance of each station from the fluvial end-member of the Eastern Branch of Evros River (40° 46′ N and 26° 07′ E) was estimated with Google Earth tools as the minimal path along the northern part of the Eastern Branch and the coasts of the Evros Delta (Fig. 1). Samples were also collected from a sheltered site which communicated immediately with the sea in the estuarine area of Nestos River (station N_2, 40° 51′ N and 24° 37′ E, Fig 1), for comparison (control site); Nestos River estuary is considered as a relatively uncontaminated area [11].

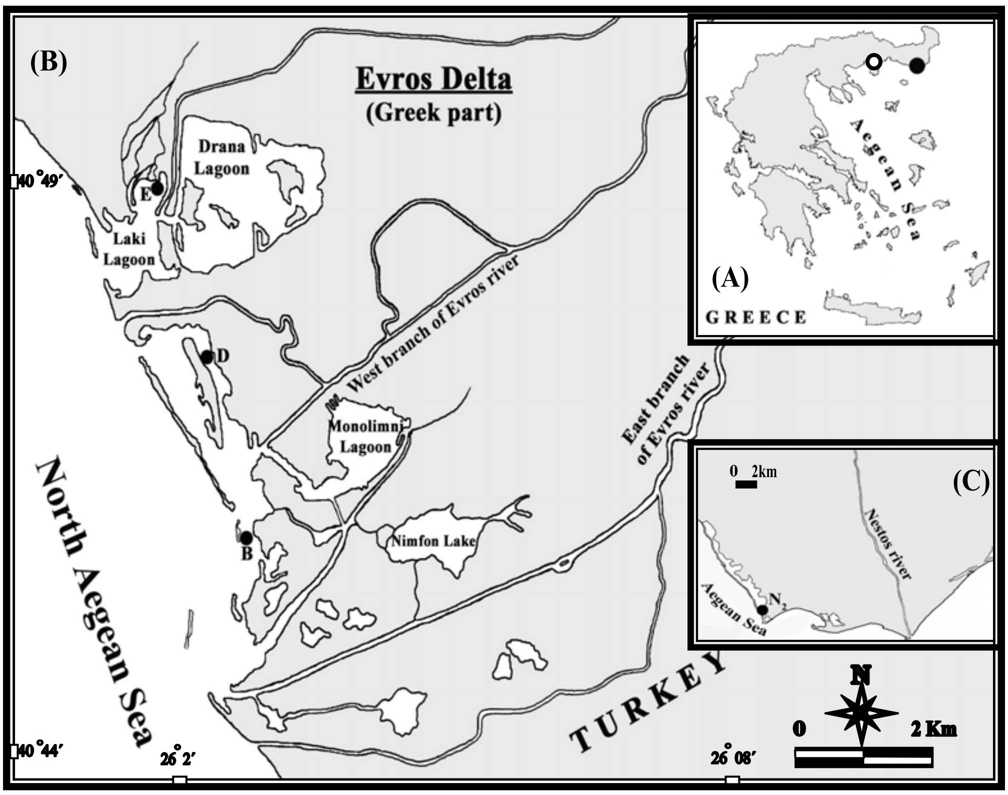

FIGURE 1 - (A) Geographical location of the study area (Evros River Delta, black circle) and of the area of the control site (estuarine area of Nestos River, open circle). (B) Map of the Evros River Delta showing the sampling stations. (C) Map of the estuarine area of Nestos River showing the control site.

A total of five sampling cruises took place; in particular, samples were collected in May, August and October 1998 and January 1999 at stations B, D and E and, additionally, in May 1999 at stations B and D. Each sampling date at stations B, D and E, depth and salinity, temperature and pH of water near the bottom were measured; in addition, at least three random samples of water using 1 l plastic tanks, of surface sediments using an 8 cm diameter plastic corer penetrating to a depth of 10 cm and of *G. bursa-pastoris*, hand-picked wearing plastic gloves and using a 25 · 25 cm plastic frame were collected. Algal samples were washed in estuarine water at the sampling site and placed in plastic bags. Additional water samples for the estimation of macronutrient concentrations were also taken. Samples were transferred to the laboratory under refrigerated conditions.

Salinity was also measured and three random samples of *G. bursa-pastoris* were also collected in May 1999 at station N_2 (control site).

Sample pretreatment and analytical procedure

In the laboratory, water, sediment and algal samples collected on a sampling date at the same station were pooled, offering a normalization option towards spatial variability within the sampling station. Estuarine water samples were acidified (1.5 ml l^{-1} conc. HNO_3) and filtered through an acid-washed grass-fibre (0.45 μm). Algal samples were washed in bidistilled water and any epiphyte and sediments were carefully removed with nylon brushes. All samples were then frozen (-20 °C) until analysis. After being dried to a constant weight (80 °C), sediment samples were sieved through a nylon net with a mesh size of 63 μm; the < 63μm sediment fraction (silt and clay) was used in the procedures followed (e.g. [12]). Plant samples were dried to a constant weight (80 °C) and ground in an agate mill. Three sub-samples of each powdered algal and sediment sample were digested in Teflon vessels with HNO_3/ $HClO_4$ (4/1) for 12h. The previous procedures have been frequently applied in previous studies (see [3, 13, 14]).

The total iron and zinc concentrations in the sediments and in the seaweed and the dissolved iron and zinc concentrations in the estuarine water were measured with flame Atomic Absorption Spectrophotometry (AAS Perkin-Elmer 2100); the limits of detection for the analysis of iron and zinc in sediments and plant tissues were 500 ng g^{-1} dry wt and 50 ng g^{-1} dry wt respectively and in water 5 ng ml^{-1} and 0.5 ng ml^{-1} respectively. The concentrations of dissolved copper, lead and cadmium in the estuarine water and the total copper, lead and cadmium concentrations in the sediments and in the alga were measured using graphite furnace AAS (Perkin-Elmer 2100) with Deuterium background correction; the limits of detection for the analysis of copper, lead and cadmium in sediments and plant tissues were 2 ng g^{-1} dry wt, 5 ng g^{-1} dry wt and 0.3 ng g^{-1} dry wt, respectively and in water 0.02 ng ml^{-1}, 0.05 ng ml^{-1} and 0.003 ng ml^{-1}, respectively. Pro-analysis grade reagents (Merck) were used and reagent blank was run concurrently. The accuracy of the technique was checked with the analysis of the standard reference material of Sea Lettuce (*Ulva lactuca* no 279, Community Bureau of Reference); results were in agreement with certified values (Table 1). The precision of the analysis of iron, zinc, copper, lead and cadmium was calculated by the mean of coefficients of variation (CV %), which was calculated by measuring three independent sub-samples of each sample; the results for *G. bursa-pastoris* were 4.1, 5.0, 4.2, 5.2 and 3.6 % respectively.

TABLE 1 - Analysis of certified reference material BCR 279 (sea lettuce): certified values, found values (mean ± standard deviation, n = 6) and recovery (%).

	certified (μg g^{-1} dry wt)	found (μg g^{-1} dry wt)	recovery %
Fe	$2.3 \cdot 10^3 \pm 0.1 \cdot 10^{3*}$	$2.27 \cdot 10^3 \pm 0.07 \cdot 10^3$	99
Zn	51.3 ± 1.2	50.4 ± 1.7	98
Cu	13.14 ± 0.37	13.45 ± 0.46	102
Pb	13.48 ± 0.36	13.85 ± 0.58	103
Cd	0.274 ± 0.022	0.280 ± 0.020	102

*: not certified values

Nutrients (NO^-_3, NO^-_2 and NH^+_4) were measured using HACH DR/2000 direct reading spectrophotometer with a cadmium reduction method.

Data analysis

The significance of the spatial and seasonal variation in metal concentrations was tested with Kruskal-Wallis one-way analysis of variance; as for the spatial variation the values recorded at each station during all seasonal samplings and as for the seasonal variation the values recorded in each season at all sampling stations were used. Spearman's rank correlation coefficient (r) was applied to identify correlations. Only one of those environmental variables found to be highly inter-correlated was used in the in the correlations with metal concentrations.

RESULTS AND DISCUSSION

Water physico-chemical variables

The distance of stations B, D and E from the fluvial end-member of the Eastern Branch of Evros River as well as the values of physical and chemical parameters of water at each station during the sampling period are shown in Table 2. Oligohaline waters mostly occurred in the coastal section of the Evros Delta throughout the sampling period (Table 2). Nitrite concentrations were highly correlated with water salinity ($r = -0.950$, $p<0.0001$, $n = 14$) and, thereby, were not used in the correlations with metal concentrations.

Ambient metal concentrations

Mean (± standard deviation) and range of iron, zinc, copper, lead and cadmium concentrations in the water and sediments in the study area are shown in Table 3. Mean metal concentrations in the water decreased in the order Fe > Zn > Pb > Cu > Cd and in the sediments in the order

TABLE 2 - Distance of stations B, D and E from the fluvial end-member of the Eastern Branch of Evros River and values of water physico-chemical variables at these stations in May, August, October 1998, January and May 1999.

	station B					station D					station E			
	M98	A	O	J	M99	M98	A	O	J	M99	M98	A	O	J
Distance (km)			10.70					14.82				19.32		
Depth (cm)	25	25	30	50	20		15	25	30	30	25	15	20	25
Salinity (psu)	0.3	6.4	4.6	2.8	1	4.9	6.9	5.8	5.1	3.4	4	6.8	6	0.8
Temperature (°C)	17	26.5	18	7.3	21.6	19.2	27.7	14.8	9.2	21.8	17.3	21.8	16.9	9.5
pH	7.95	8.19	7.34	8.45	8.39	7.46	8.35	8.23	8.02	8.17	7.1	8.1	8.51	8.06
NO^-_3 (mg l^{-1})	3.08	0.44	6.16			15.4	0.88	4.4			10.12	13.64	4.84	
NO^-_2 (mg l^{-1})	0.149	0.007	0.046	0.092	0.231	0.046	0	0.033	0.04	0.063	0.066	0.01	0.02	0.063
NH^+_4 (mg l^{-1})	0.813	8.26	1.2			2.903	8.51	1.3			2.451	10.77	0.9	

Fe > Cu > Pb > Zn > Cd (Table 3). Mean dissolved copper and zinc concentrations and to a lesser extent lead ones exceeded the European Environmental Quality Standards (EQSs) of waterborne contamination in estuaries, whereas those of iron and cadmium did not (<5.0 µg l^{-1}, <40 µg l^{-1}, <25 µg l^{-1}, <1.0 mg l^{-1} and <5.0 µg l^{-1} respectively, according to McLusky and Elliott [15]). In addition, mean copper and lead levels in surface sediments and to a lesser extent zinc and cadmium ones exceeded the baseline levels in estuarine sediments (approx.10 µg g^{-1} dry wt, 25 µg g^{-1} dry wt, <100 µg g^{-1} dry wt and 0.2 µg g^{-1} dry wt respectively, according to Kennish [16]).

No metal concentrations in the water displayed a significant seasonal or local variation (Table 4, Fig. 2). Iron sediment contents showed no significant local variation and all five metal ones no significant seasonal variation (Table 4).

On the other hand, zinc, copper, lead and cadmium sediment contents showed a significant spatial variation (Table 4); the highest mean concentrations of all four metals (180.6, 383.2, 155.9 and 1.0 µg g^{-1} dry wt respectively) occurred at station B (Fig. 2), located nearer than the other stations to the estuary of the Eastern Branch (Fig. 1, Table 2). In addition, zinc, copper, lead and cadmium sediment concentrations showed a significant negative correlation with the distance from the fluvial end-member of the Eastern Branch ($r = -0.860$, $p < 0.001$; $r = - 0.687$, $p < 0.01$; $r = -0.757$, $p < 0.01$; $r = -0.802$, $p < 0.001$, respectively; $n = 14$).

TABLE 3 - Mean value (± standard deviation) and range of metal concentrations in the water, sediments and *Gracilaria bursa-pastoris* in the coastal section of the Evros River Delta, regardless of season and station.

		mean (±SD)	range
Water (µg l^{-1})	**Fe**	524 (± 292)	202-1227
	Zn	61.0 (± 30.2)	20.7-115.7
	Cu	8.7 (± 7.0)	1.4-24.0
	Pb	31.6 (± 11.4)	17.5-52.9
	Cd	1.0 (± 0.8)	BDL-3.4
Sediments (µg g^{-1} dry wt)	**Fe**	25035 (± 3145)	19270-31310
	Zn	119.4 (± 54.7)	68.7-246.5
	Cu	188.3 (± 185.1)	58.7-710.0
	Pb	123.3 (± 40.5)	66.0-219.4
	Cd	0.5 (± 0.4)	0.2-1.6
***G. bursa-pastoris* (µg g^{-1} dry wt)**	**Fe**	817 (± 366)	370-1610
	Zn	43.6 (± 7.0)	31.8-54.6
	Cu	12.3 (± 3.1)	8.9-19.1
	Pb	8.6 (± 3.5)	2.7-14.1
	Cd	0.5 (± 0.2)	0.2-0.9

BDL: Below detectable limit

TABLE 4 - Values of statistic H (Kruskal-Wallis one-way analysis of variance) of the spatial and seasonal variation in metal concentrations in the water, sediments and *Gracilaria bursa-pastoris*; n_j: number of observations within each group.

		Spatial Variation		Seasonal Variation	
		H	nj	H	nj
Water	**Fe**	1.53	4,5,4	4.23	3,3,3,2,2
	Zn	0.91	"	1.29	"
	Cu	0.83	"	5.74	"
	Pb	0.30	"	3.10	"
	Cd	1.83	"	4.76	"
Sediments	**Fe**	1.18	4,5,5	1.05	3,3,3,3,2
	Zn	9.93**	"	0.13	"
	Cu	7.49*	"	1.05	"
	Pb	8.46*	"	0.23	"
	Cd	9.35**	"	2.06	"
G. bursa-pastoris	**Fe**	4.89	4,5,5	3.1	3,3,3,3,2
	Zn	8.39*	"	2.83	"
	Cu	2.83	"	2.69	"
	Pb	3.65	"	1.97	"
	Cd	8.45*	"	2.87	"

*: significant at p<0.05; **: significant at p<0.01

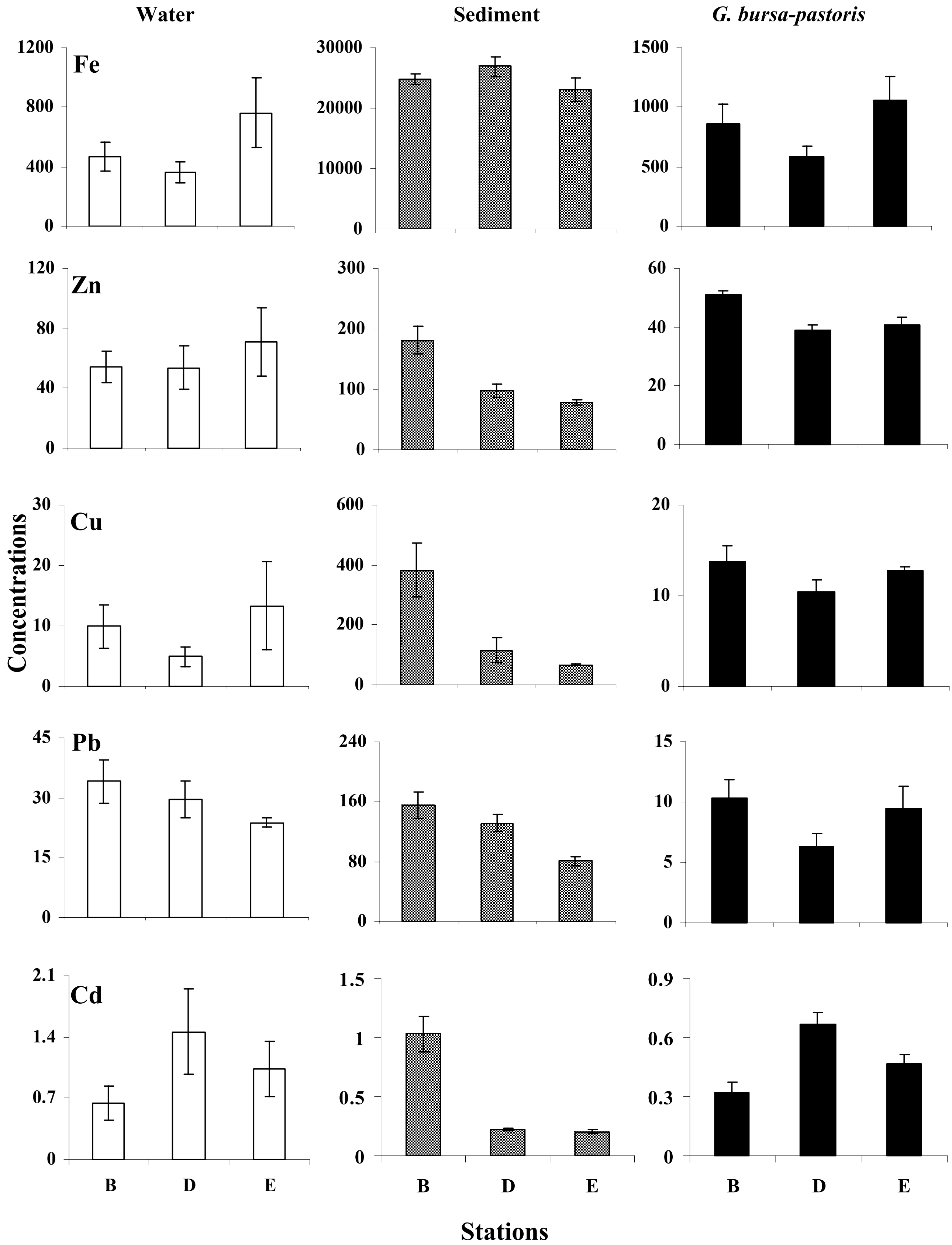

FIGURE 2 - Local variation in iron, zinc, copper, lead and cadmium concentrations in water (μg l^{-1}), surface sediments (μg g^{-1} dry wt) and *Gracilaria bursa-pastoris* tissues (μg g^{-1} dry wt) in the coastal section of the Evros River Delta. Each column represents the mean of the values recorded at a station during all sampling dates; bars represent the standard error.

TABLE 5 - Bibliographical data on metal concentrations in *Gracilaria* species (μg g^{-1} dry wt) from various geographical areas; [a]: mean; [b]: range; [c]: mean and range; [d]: range of mean [e]: individual concentration; BDL: Below detectable limit.

Species	Geographical area	Fe	Zn	Cu	Pb	Cd	References
G. bursa-pastoris	Evros Delta, Aegean Sea	817(370-1610)	44.1(31.8-54.6)	12.3(8.0-19.1)	8.6(2.7-14.1)	0.49(0.2-0.9)	present study[c]
	Nestos Delta, Aegean Sea	250	65.15	4.5	2.3	0.5	present study[e]
G.cornea	Yucatan, Caribbean Sea	493.4	1.19	19.11	25.13		[33][a]
G.corticata	Saurashtra coast, Arabian Sea		24.71-27.96	6.74-10.05			[34][b]
	Tamil Nadu, coast of India	257-630	40-65	34-35			[35][b]
G.gracilis	Venice Lagoon, Adriatic Sea	471(224-1080)	163(36-240)	8(4-12)	6.9(2.8-20.6)	0.4(0.1-0.6)	[36][c]
	" "	23 (BDL-405)		4.35(1.44-10.89)	2.54(0.76-5.8)	0.1(0.04-0.17)	[37][c]
	" "	353		9.77		0.29	[38] (in Favero and Frigo [37])[a]
	" "	379		4.47		0.10	Favero, unpubl. data (in Favero and Frigo[37])[a]
	" "	530-1790	52-823	5.4-16.3	1.1-6.6	0.24-0.25	[39][d]
	Thermaikos Gulf, Aegean Sea	1.5-22.0		0.25-3.30			[40][d]
	" "	95.1(63.0-121.0)	53.4(35.0-67.7)	2.0(1.1-3.2)	14.4(9.5-19.0)	1.8(0.8-3.1)	[41][c]
	Sinop, Black Sea	781-2777	31.4-73.4	4.75-9.11	6.76-7.95	1.03-2.12	[42][b]
G.pachydermatica	Loreto Bay, Baja California Sur	4500	21				[43][e]

The above suggests that amounts of heavy metals, mainly of copper, zinc, lead and to a lesser extent of cadmium, mostly transported by the Evros River, reached the coastal section of the delta area. The flocculation and sedimentation of riverborne metals [16-18] most probably resulted in the decrease in zinc, copper, lead and cadmium sediment contents in the coastal section of the Evros Delta with increasing distance from the Eastern Branch's estuary.

Metal concentrations in Gracilaria bursa-pastoris

The descriptive statistics of metal concentrations in *G. bursa-pastoris* in the study area is shown in Table 3; mean metal concentrations decreased in the order Fe > Zn > Cu > Pb > Cd.

Comparison with *Gracilaria* species from other areas

The concentrations of the metals analyzed in *G. bursa-pastoris* were generally higher than those in the same species from the control site (station N_2, Nestos River Delta) at 6.2 psu salinity in May 1999 and in the range of those previously reported for *Gracilaria* species from other brackish and marine coastal areas (Table 5), some of which are recipients of domestic and industrial wastes (e.g. central part of Venice Lagoon, Thermaikos Gulf); in particular, copper contents in *G. bursa-pastoris* are among the highest ones and iron and lead contents relatively high (Table 5). The above indicate that the study area was mainly contaminated by copper.

Spatial Variation

Iron contents in the macroalga showed no significant spatial variation (Table 4), just as its ambient concentrations; the maximum mean iron concentration (1063 μg g^{-1} dry wt) occurred at station E (Fig. 2).

Zinc contents displayed a significant local variation, just as its sediment contents, whereas copper and lead ones did not (Table 4); the highest mean concentrations of these three metals (50.90, 13.79 and 10.33 μg g^{-1} dry wt respectively) were recorded at station B (Fig. 2), located nearer than the other stations to the fluvial end-member of the Eastern Branch of Evros River (Fig. 1, Table 2), just as their sediment contents (Fig. 2). The above suggests that *G. bursa-pastoris* reflects, to a certain extent, the sediment abundances of zinc and, to a lesser extent, those of copper and lead. The concentrations of metals in surface sediments reflect, to a certain extent, their levels in the overlying water and give an integrated and stable picture of contamination over time (e.g. [19]). In addition, *G. bursa-pastoris* may accumulate metals not only from solution, but also directly from sediments or suspended inorganic particulates; seaweed tissues, contacting surface sediments and suspended particulates in shallow, turbid estuarine environments, may scavenge metals whose strength of binding to seaweed tissues exceeds their strength of binding to particles [20].

Cadmium contents in the macroalga displayed a significant local variation (Table 4). However, the pattern of this metal was almost reverse to that of its sediment contents and to those of zinc, copper and lead ones both in sediments and in the macroalga; the highest mean cadmium concentration (0.67 μg g^{-1} dry wt) occurred at station D (Fig. 2).

Seasonal Variation

No metal concentrations in *G. bursa-pastoris* showed a significant seasonal variation (Table 4), just as their ambient concentrations. This suggests that seasonal changes in environmental factors and in metabolic factors did not markedly affect the accumulation of these metals in the red macroalga tissues. However, zinc and cadmium displayed clear patterns of seasonal periodicity which were similar in all three stations (Fig. 3). The seasonal variation of zinc concentrations in *G. bursa-pastoris* tissues was probably associated with the growth dynamics of the macroalga, since the levels of this metal were lowest in summer (Fig. 3), when growth rates may have been high; annual growth maximum of *G. bursa-pastoris* in a euhaline Mediterranean lagoon (Thau, France) occurred in spring, while biomass peaked in early summer when water temperature attained 20 ^{o}C [6, 7]. High growth rates may result in dilution of the accumulated metals and, thereby, in reduction of their concentrations (e.g. [1]). The seasonal variation of cadmium concentrations in *G. bursa-pastoris* tissues (Fig. 3) was probably associated with tissue age; this metal content mainly decreased in winter, namely when young individuals may have predominated over the population and progressively increased from spring to autumn, probably with increasing tissue age. The main period of reproduction of *G. bursa-pastoris* in the euhaline Thau Lagoon, France was in late spring-early summer; mortality of female thalli bearing carposporophytes extended until autumn, most of the tetrasporophangial thalli disappeared during summer, while thalli without reproductive structures predominated over the population mainly in early winter [7]. This potential increase with tissue age could be attributed to the fact that binding sites of the seaweed increase with age and cadmium is taken up irreversibly by it [21].

Intermetal correlations

The correlation matrix of metal concentrations in seaweed tissues showed a significant negative correlation between zinc and cadmium (Table 6). This suggests an antagonistic interaction between these metals. In addition, provided that the spatial pattern of cadmium contents in the macroalga was almost reverse to that of its sediment contents as well as to those of zinc ones both in the macroalga and in the sediments, this finding particularly suggests that zinc negatively affected the accumulation of cadmium in *G. bursa-pastoris* tissues. Antagonism between zinc and cadmium is probably expected, since the uptake of both metals by the macroalga may be achieved through the same processes. In a kinetic study, it was indicated that zinc and cadmium uptake by *Gracilaria blodgettii* and *Ulva lactuca* was processed through passive diffusion or a facilitated transport [22]; it was suggested that facilitated transport by binding with protein ligands is important in the transport of both metals [22]. Zinc may behave similarly to cadmium because of their

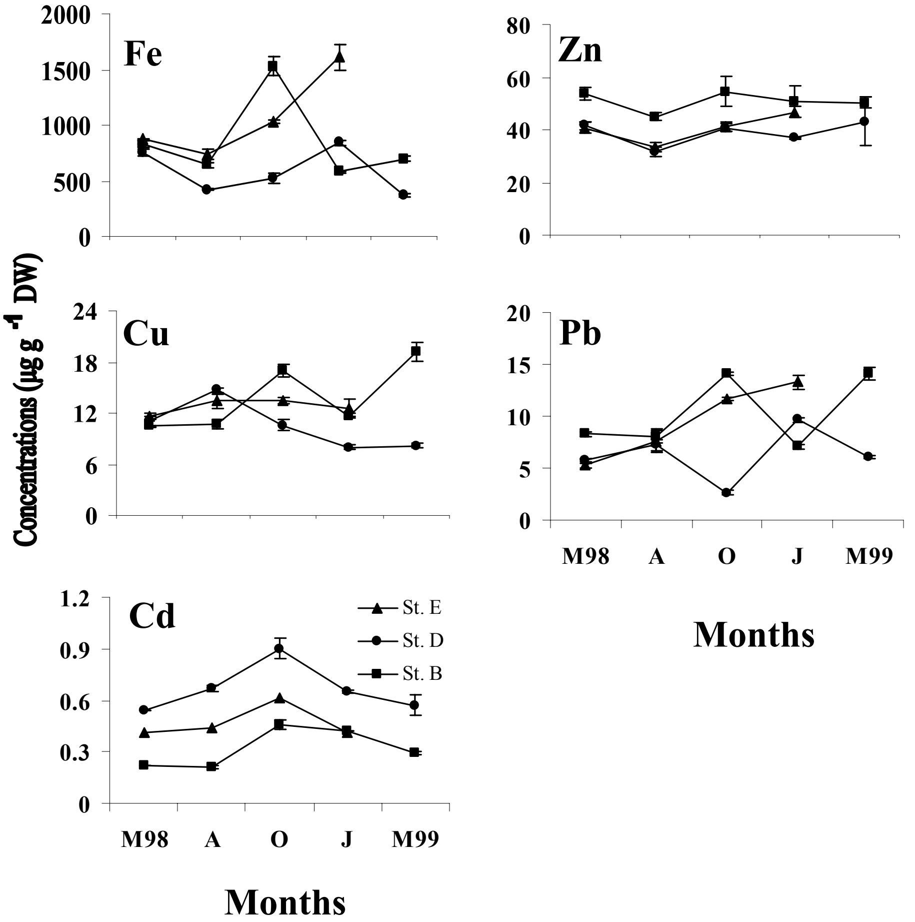

FIGURE 3 - Seasonal variation in iron, zinc, copper, lead and cadmium concentrations in *Gracilaria bursa-pastoris* tissues (μg g^{-1} dry wt) per station in the coastal section of the Evros River Delta. Each dot represents the mean of at least three subsamples of each sample; bars represent the standard error. M98: May 1998; A: August 1998; O: October 1998; J: January 1999; M99: May 1999.

close chemical relationships and possible competition for the same binding sites; zinc has a stronger affinity to common binding sites than cadmium, while other metal-specific bindings may also be available for each one of these elements [23]. Evidence for zinc-cadmium antagonism in macroalgae, which were incubated mainly at salinities higher than those occurring in the study area, has been given in some laboratory studies (e.g. [24-26]). Zinc-cadmium interactions are also commonly observed in terrestrial plants; in most cases, zinc reduces the uptake of cadmium by both root and foliar systems [27].

TABLE 6 - Spearman's rank correlation coefficient between concentrations of different metal pairs in *Gracilaria bursa-pastoris*; n=14.

	Zn	Cu	Pb	Cd
Fe	0.262	0.222	0.530	-0.242
Zn		0.125	0.442	-0.616*
Cu			0.481	-0.095
Pb				-0.323

*: significant at $p<0.05$

Associated environmental variables

Zinc concentrations in *G. bursa-pastoris* showed a significant positive correlation with its sediment contents (Table 7); thereby, as for zinc, this red macroalga fulfils the most likely important criterion for an ideal bioindicator, that is the pollutant contents of the organism and the environment should be correlated (e.g. [1, 28]). No other metal contents in *G. bursa-pastoris* showed a significant positive correlation with its ambient concentrations (Table 7). On the other hand, cadmium concentrations in *G. bursa-pastoris* tissues were negatively correlated with its sediment contents (Table 7); this negative correlation could be considered as a result of the potential zinc-cadmium antagonistic interaction in *G. bursa-pastoris* tissues.

TABLE 7 - Spearman's rank correlation coefficient between metal concentrations in *Gracilaria bursa-pastoris* and in the water and sediments; n=14.

	Fe	Zn	Cu	Pb	Cd
G. bursa-pastoris-water	-0.236	-0.099	0.098	0.033	0.231
G. bursa-pastoris -sediments	-0.134	0.604*	-0.174	0.011	-0.632*

*: significant at $p<0.05$

Zinc contents in *G. bursa-pastoris* displayed a significant negative correlation with the distance from the fluvial end-member of the Eastern Branch (Table 8), just as its sediment contents, as well as with water salinity (Table 8). No other metal concentrations in *G. bursa-pastoris* showed a significant correlation with an examined environmental variable (Table 8). The negative correlation between zinc contents in the seaweed and water salinity may be due to a higher metal burden in freshwater flowing into the study area. However, it may also suggest a decrease in zinc accumulation by this red seaweed with increasing salinity; a decrease in free ion concentration of zinc with increasing salinity, because of the complexes formed between this metal and the chloride ions, and an increased competition of zinc with sodium ions at uptake sites on the plasma membrane and the cell wall may have contributed to a decreased zinc accumulation at higher salinities (e.g. [22, 29, 30]). A decrease in zinc accumulation in two submerged plants (*Elodea canadensis* and *Potamogeton natans*) with increasing salinity from 0 to 5 psu was observed under laboratory conditions [30]. Evidence for a decrease in zinc accumulation by seaweeds, among which *Gracilaria blodgettii*, with increasing salinity of the media, but within a range of higher salinity values (e.g. from 10 to 28 psu, or from approx. 8 to 32 or 38.6 psu), has been also given in some laboratory studies (e.g. [22, 31, 32]). The above suggest that salinity variation in coastal oligohaline environments may partly interfere with the use of *G. bursa-pastoris* as a bioindicator of zinc.

TABLE 8 - Spearman's rank correlation coefficient between metal concentrations in *Gracilaria bursa-pastoris* and the distance from the fluvial end-member of the Eastern Branch of Evros River as well as the values of water physico-chemical variables.

	Fe	Zn	Cu	Pb	Cd	n
Distance	0.305	-0.648*	0.019	-0.226	0.345	14
Salinity	-0.262	-0.741**	0.116	-0.235	0.508	14
Temperature	-0.429	-0.218	0.249	-0.062	-0.106	14
pH	-0.486	-0.108	0.279	0.103	0.194	14
NO^-_3	0.433	-0.05	0.217	-0.183	0.1	9
NH^+_4	-0.633	-0.617	0.217	-0.4	0	9

*:significant at $p<0.05$; **: significant at $p<0.01$

CONCLUSIONS

As hypothesized, our results suggest that *G. bursa-pastoris* efficiently reflects the ambient abundances of zinc in oligohaline coastal environments, i.e. satisfies the basic pre-requisite for its use as a bioindicator of this trace element in these environments; several examined variables, possibly except for water salinity, do not significantly affect the accumulation of zinc by this red macroalga, namely do not markedly interfere with its use as a bioindicator of zinc.

However, our data also suggest that *G. bursa-pastoris* does not reflect the ambient abundances of cadmium, partially refuting our initial hypothesis; zinc may significantly reduce the accumulation of cadmium in this red seaweed tissues, interfering with its use as a bioindicator of cadmium. Our results also suggest that *G. bursa-pastoris* does not efficiently reflect the sediment abundances of copper and lead, whereas, whether this red macroalga reflects the ambient abundances of iron could not be appreciated, as this metal concentrations in the water and sediments did not significantly vary in the study area.

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