



# Biomonitoring with Benthic Macroalgae and Direct Assay of Heavy Metals in Seawater of the Oporto Coast (Northwest Portugal)

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The concentrations of dissolved Cd, Cu, Hg and Pb in seawater and algae (*Enteromorpha* spp. and *Porphyra* spp.) were determined in parallel on three beaches located in the Oporto coast (Portugal), during 8 successive months, comprising periods of low and high biological productivity. The concentrations of dissolved metals in seawater varied markedly during the sampling period and exhibited seasonality. The concentrations of Cd were high in autumn and winter, but low in spring. The concentrations of Cu and Hg were high in autumn, low in winter and peaked in May. The concentrations of Pb were low in autumn and winter, and also peaked in May. It is likely that biological activity was, at least partially, responsible for the observed seasonal changes. The mean concentrations of dissolved metals in seawater were similar to those reported for polluted and industrialized european coastal areas. Metal concentration factors, calculated month-to-month for each alga, metal and site, varied markedly during the sampling period, indicating that concentration factors based on single or few determinations could be misleading. On the contrary, mean concentration factors (calculated by dividing the mean metal concentration in the algae by the mean metal concentration in seawater) were relatively constant at all three sites, indicating that in the Oporto coast, *Enteromorpha* spp. can be used to estimate the mean concentration of dissolved Cd, Cu and Hg in seawater, and *Porphyra* spp., the mean concentration of Cd, Cu and Pb. However, considering that future estimates of the concentrations of these metals in seawater should be based on determinations of the metal contents in *Enteromorpha* spp. and *Porphyra* spp. carried out on several occasions spanning a considerable period of time, the use of these algae as monitors of heavy metal pollution can have limited practical advantages over the direct assay of the metals in seawater. © 1997 Elsevier Science Ltd. All rights reserved

Oporto coast, comprising the administrative regions ('concelhos') of Matosinhos, Porto, Vila Nova de Gaia, Espinho and Ovar, is a heavily populated and industrialized area of Portugal (Fig. 1). The area (446 km<sup>2</sup>) corresponds to only 2% of the region north to Douro river, but the population (approx. 790 000) and industrial activity corresponds to 20–22% of the total of this region (INE, 1995a,b). Textile, tanning, metallurgical and chemical industries account for 70% of the Oporto total industrial activity (MESS, 1994). Industrial effluents and domestic wastewaters that are discharged without pre-treatment into the rivers or directly in the ocean, are considered to be the main sources of pollution in the Oporto coastal waters (CCRN, 1995).

Although there is a widespread concern with pollution levels in the waters of the Oporto coast, there have been few studies on this subject. Recent analysis of coastal superficial sediments and sand beach, at several sites near Matosinhos and Oporto cities, revealed the following total metal contents (mg kg<sup>-1</sup>): Cd: 0.4–1.9; Cu: 0.9–55.4; Pb: 6.1–34.7 (APDL, pers. comm.), which are within the range of medium contaminated areas (Bryan and Hummerstone, 1973; Sadiq, 1992; López-Sánchez *et al.*, 1996). Preliminary analysis of the water in Douro estuary showed however high metal pollution (Vasconcelos, unpub. data). Bacterial (faecal) pollution is also significant in the area, since in the last few years, the water in most of the beaches has exceeded the microbiological standards of water quality set by EEC (CCRN, 1995).

The aims of the present work were to assess contamination levels of dissolved Cd, Cu, Hg and Pb, in the seawater in the Oporto coast, and to evaluate the suitability of *Enteromorpha* spp. and *Porphyra* spp. as marine biomonitors of heavy metal pollution in these coastal waters.

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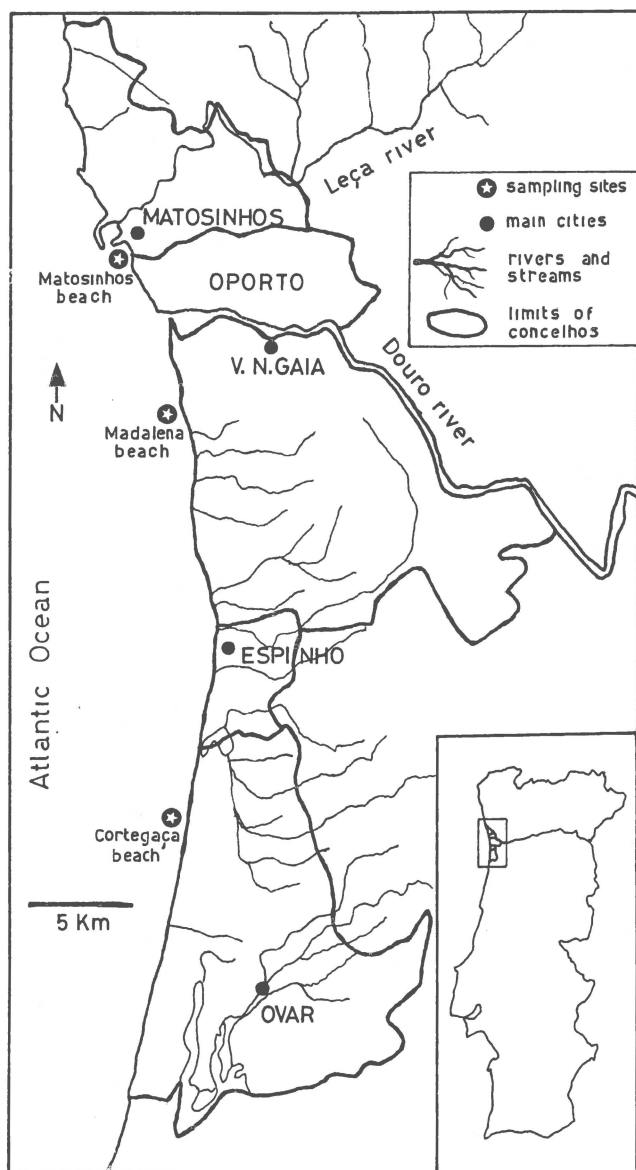


Fig. 1 Localization of sampling sites in Oporto coastal area (Portugal).

## Material and methods

### Sampling sites and dates

Seawater and algae were collected in parallel in three beaches, Matosinhos, Madalena and Cortegaça. The three chosen sampling sites are located in the Oporto coast (northwest Portugal) (Fig. 1), in an area of 30 km, but in different geographic contexts (MESS, 1994; CCRN, 1995; INE, 1995a,b). Matosinhos beach is located between the estuaries of Leça and Douro rivers, and close to Matosinhos, Oporto and Vila Nova de Gaia cities. Madalena beach is 5 km south of the estuary of Douro river, and Oporto and Vila Nova de Gaia cities. Cortegaça beach is located in a relatively low populated and industrialized area, 24 km south of Douro estuary. Sampling was carried out at low tide on the following dates: in 1994 on November 9 (I) and

December 20 (II); and in 1995 on January 19 (III), February 20 (IV), March 20 (V), April 6 (VI), May 2 (VII), June 1 (VIII) and June 27 (IX). The temperature of the water, measured during sampling, was at I–IX sampling dates, respectively, 14, 14, 13, 15, 16, 15, 15, 15 and 17°C.

### Algae

Preliminary observations of the sampling sites showed that only few species of intertidal benthic macroalgae were abundant at all three sites. Among these were *Enteromorpha intestinalis* (L.) Link, *Enteromorpha linza* (L.) J. Agardh (Bliding, 1963; Gallardo and Alvarez, 1985; Burrows, 1991; Villalard-Bohnsack, 1995), *Porphyra purpurea* (Roth) Agardh and *Porphyra umbilicaris* (L.) Agardh (Hiscock, 1986; Cabioc'h *et al.*, 1992). Since *E. intestinalis* and *E. linza*, *P. purpurea* and *P. umbilicaris*, formed mixed and dense populations on the surface of beach rocks, and displayed similar metal contents, we opted to sample both species together. The use of *Enteromorpha* spp. and *Porphyra* spp. as biological indicators or monitors of metal pollution is well documented in the literature (Hägerhäll, 1973; Seeliger and Cordazzo, 1982; Say *et al.*, 1986; Vasquez and Guerra, 1996).

### Preparation of the algae for metal analysis

The algae were collected by hand, randomly, at several places on each beach, to a total of approximately 20 g of each alga. The samples were bulked in a single plastic bag, but for the comparison of the variability in metal contents among sampling sites with the variability within each site, the samples were placed in separate plastic bags. Upon arrival at the laboratory, the algae were washed twice with NaCl (3.5%, w/v), blotted dry to remove excess water using laboratory paper towel, and stored in plastic containers at –20°C pending analysis (Say *et al.*, 1986). Microscopical examination of the algae revealed no significant presence of saprophytic organisms. For the determination of Hg, subsamples of approx. 0.3 g wet algae (used to avoid metal losses due to volatilization) were placed in closed quartz vessels with 2.00 ml of concentrated HNO<sub>3</sub> (Suprapure grade from BDH). Digestion was then carried out at high pressure in a microwave oven (Milestone, model MLS-1200 Mega). The liquid in the digestion vessels was transferred to 5-ml volumetric flasks, 100 µl of KMnO<sub>4</sub> solution (5%, w/v) and deionized water was added up to the mark. For the determination of Cd, Cu and Pb, subsamples of approx. 0.4 g dried algae were placed in closed vessels of Teflon with 4.00 ml of concentrated HNO<sub>3</sub>. Digestion was performed as described for Hg. The digests were transferred to 5 ml volumetric flasks, and deionized water was added up to the mark. The metal contents of the digested algae were then determined as described below. Subsamples were made in triplicate from each sampling site. To determine the water contents of the

algae, subsamples were dried at 100°C to constant weight. The mean values ( $\pm$ SD,  $n=10$ ) of the dry/fresh weight ratio resulted in  $0.217\pm0.019$  for *Enteromorpha* spp. and  $0.220\pm0.013$  for *Porphyra* spp.

#### Preparation of the seawater samples for metal analysis

The seawater was collected in plastic bags. Upon arrival at the laboratory, samples were filtered through 0.45- $\mu$ m pore size cellulose filters (Millipore), stored at room temperature, and analysed within 24 h. Chromatographic microcolumns, made of Perspex (5.0 cm length and 0.3 cm internal diameter), were filled with Chelamine (150–300  $\mu$ m; Fluka R04700), and then conditioned in ammonium acetate buffer (0.05 M  $\text{NH}_4\text{CH}_3\text{COOH}$ ; pH adjusted to 8.0 with NaOH; Olsen *et al.*, 1983). Three aliquots of the filtered seawater were applied to the chromatographic columns for on-line pre-concentration, and the metals removed from Chelamine by elution with 2 M  $\text{HNO}_3$ . Details about the analytical procedures used in the present work have been previously reported (Vasconcelos and Leal, 1997).

#### Determination of the concentration of Cd, Cu, Hg and Pb in the samples

The digested samples of the algae and the eluted solutions from the chromatographic columns were assayed for Cd, Cu, Hg and Pb. The determinations of Cd, Cu and Pb were performed by AAS with flame atomization using a Philips PU 9200X model spectrophotometer, linked to a Philips PM 8222 model chart recorder. The determination of Hg was carried out by AAS coupled to a Philips PU 9360X model hydride generation/mercury cold vapour system, using a  $\text{NaBH}_4$  (2%, w/v)–NaOH (0.1%, w/v) solution (previously filtrated by a 0.45- $\mu$ m pore size filter) as reducing agent. The calibration of the spectrophotometer was performed by using standard solutions of the different metals in ammonium acetate buffer, prepared daily from stock solutions (Spectrosol grade from BDH). Reference standard seaweed (CRM 279 *Ulva lactuca*; Griepink and Muntau, 1987) certified for Cd, Cu and Pb, and with indicative values for Hg, was used to check the accuracy of the digestion and analysis procedure (Table 1). The metal contents found were not significantly different from expected values by *t*-test ( $p < 0.05$ ). The detection limits were ( $\mu\text{g metal g}^{-1}$  dry wt): Cd, 0.04; Cu, 0.07; Hg, 0.012; and Pb, 0.13.

#### Reagents and solutions

All reagents were Analar grade, except otherwise stated. All solutions were prepared with deionized water, with resistivity of  $\geq 14 \text{ Mohm cm}^{-1}$ . All glassware and plastic-ware were leached for 24 h in 20% (w/v)  $\text{HNO}_3$ , rinsed several times with deionized water and dried.

TABLE 1

Analysis of *Ulva lactuca* reference material (CRM 279), certified for Cd, Cu and Pb, with indicative values for Hg ( $\mu\text{g metal g}^{-1}$  dry wt, algae).

Metal	Certified	Found***
Cd	$0.274\pm0.022^*$	$0.275\pm0.085^*$
Cu	$13.1\pm0.37^*$	$12.9\pm0.30^*$
Hg	$0.0662\pm0.0047^{**}$	$0.0701\pm0.0063^{**}$
Pb	$13.5\pm0.36^*$	$13.3\pm0.40^*$

\* Mean $\pm$ confidence interval ( $p=0.05$ ); \*\* Mean $\pm$ standard deviation; \*\*\* Six test portions were analysed; six analyses were made for each portion.

## Results

### Variability of the concentration of metals in seawater and algae

Firstly, we compared the variability among sampling sites with the variability within each sampling site, in order to test if the metal contents in seawater and algae differed significantly between Matosinhos, Madalena and Cortegaça (Phillips and Rainbow, 1994). For this purpose, five–six samples of seawater and algae were collected at random at each site, and the concentrations of Cd, Cu, Hg and Pb determined as described in Material and methods. The results (Tables 2 and 3) revealed low variability in the metal contents in seawater and algae within each sampling site. Analysis of variance (Zar, 1984) was applied to the results for

TABLE 2

Variability of the concentration of dissolved Cd, Cu, Hg and Pb in seawater at the studied sampling sites.

	Cd	Cu ( $\mu\text{g l}^{-1}$ )	Hg	Pb
Matosinhos				
	0.40	4.2	0.34	0.52
	0.47	4.0	0.28	0.52
	0.44	4.3	0.28	0.48
	0.40	4.1	0.32	0.56
	0.45	4.0	0.40	0.52
	0.36	4.2	0.34	0.50
CV(%)	9.5	3.2	13.8	5.3
Madalena				
	3.2	2.8	0.38	4.1
	3.5	2.9	0.30	3.9
	3.1	2.7	0.42	3.9
	2.9	2.9	0.44	4.1
	3.4	2.9	0.32	4.1
	3.2	2.8	0.36	4.1
CV(%)	6.0	3.0	14.8	2.3
Cortegaça				
	1.7	0.54	0.74	3.1
	1.6	0.57	0.76	3.2
	1.6	0.54	0.68	3.1
	1.7	0.52	0.64	3.0
	1.7	0.54	0.64	3.1
CV(%)	2.7	3.4	8.1	2.1
$F_{2,14}$	824	2000	78.2	4374

TABLE 3

Variability of the concentration of total Cd, Cu and Pb in *Enteromorpha* spp. and *Porphyra* spp. at the studied sampling sites.

	<i>Enteromorpha</i> spp.			<i>Porphyra</i> spp.		
	Cd	Cu	Pb ( $\mu\text{g g}^{-1}$ dry wt)	Cd	Cu	Pb
Matosinhos						
	0.45	11.9	2.3	0.14	9.5	0.50
	0.45	12.0	2.4	0.16	9.7	0.55
	0.50	12.2	2.8	0.17	10.3	0.59
	0.55	12.4	3.0	0.17	10.4	0.64
	0.55	12.7	3.1	0.18	10.7	0.68
	0.59	13.2	3.2	0.20	10.9	0.77
CV(%)	11.5	3.9	13.2	11.8	5.3	16.1
Madalena						
	0.41	14.5	4.2	0.38	10.4	1.1
	0.45	14.8	4.2	0.39	10.9	1.1
	0.50	15.3	4.4	0.42	11.2	1.2
	0.55	15.4	4.9	0.43	11.3	1.4
	0.55	16.5	5.1	0.43	11.4	1.6
	0.59	16.9	6.0	0.45	11.7	1.8
CV(%)	13.7	6.1	14.4	7.1	3.8	20.6
Cortegaça						
	0.09	6.4	3.8	0.26	5.8	3.1
	0.10	7.1	3.9	0.28	6.7	3.4
	0.10	7.5	3.9	0.28	6.8	3.4
	0.11	7.7	4.4	0.28	6.8	3.4
	0.12	8.1	5.3	0.28	7.1	3.5
	0.13	8.3	5.3	0.29	7.3	3.9
CV(%)	18.2	9.3	15.5	3.6	14.8	7.5
$F_{2,15}$	119	184	18.2	229	126	243

each metal and alga genus.  $F$ -values were highly significant ( $p < 0.00000005$  and  $0.0001$ , for seawater and algae, respectively) indicating that the variability in metal contents among stations was significantly greater than the variability within each station, and that there were significant differences in the metal contents in seawater and algae between the studied sampling sites. In light of these results, all subsequent analyses were carried out in bulked samples at each site.

#### Metal concentration levels in the seawater

Cd, Cu, Hg and Pb in seawater was assayed in the dissolved metal fraction, which was labile to Chelamine microcolumns. This fraction comprises the free metal plus the metal present in complexes that dissociate in the presence of Chelamine (the inorganic and a fraction of the organic complexes). Although lability is an operational parameter that is strictly dependent on the experimental conditions (namely on the stationary phase used in chromatographic columns), it may be a more suitable measure of the bioavailable metal in solution than the total metal concentration (Apte and Batley, 1995), and for this reason was used in the present study.

The concentrations of dissolved Cd, Cu, Hg and Pb in the seawater at Matosinhos, Madalena and Cortegaça, varied pronouncedly during the sampling period, and exhibited seasonality (Fig. 2). The concentrations of Cd were high in autumn and winter, but

low in spring. The concentrations of Cu and Hg were high in autumn, low in winter and peaked in May. The concentrations of Pb were low in autumn and winter, and also peaked in May. The highest mean concentration of Hg occurred at Matosinhos, Cu at Madalena, and Cd and Pb at Cortegaça (Table 4). Metal contamination levels in the seawater in the studied coastal area decreased in the following order: Pb > Cu > Cd >> Hg (Table 4).

#### Concentration of metals in marine algae

The concentrations of total Cd, Cu, Hg and Pb in *Enteromorpha* spp. and *Porphyra* spp., at Matosinhos, Madalena and Cortegaça, between November 94 and June 95 are shown in Fig. 2. Each metal displayed a characteristic pattern of variation during the sampling period. The concentrations of Cd were low in autumn but high in spring. The concentrations of Cu were relatively constant throughout the study. The concentrations of Hg increased gradually from autumn to spring. The concentrations of Pb were higher in spring than in autumn and winter. During the sampling period, the variation of the Cu contents in the algae was therefore relatively small, but the variation of the Cd, Hg and Pb contents was considerable (Table 4). However, the metal contents in the algae varied less than in seawater. The mean concentration of Cd in both algae was highest at Cortegaça, Cu at Madalena, and Hg at Matosinhos; the concentration of Pb in

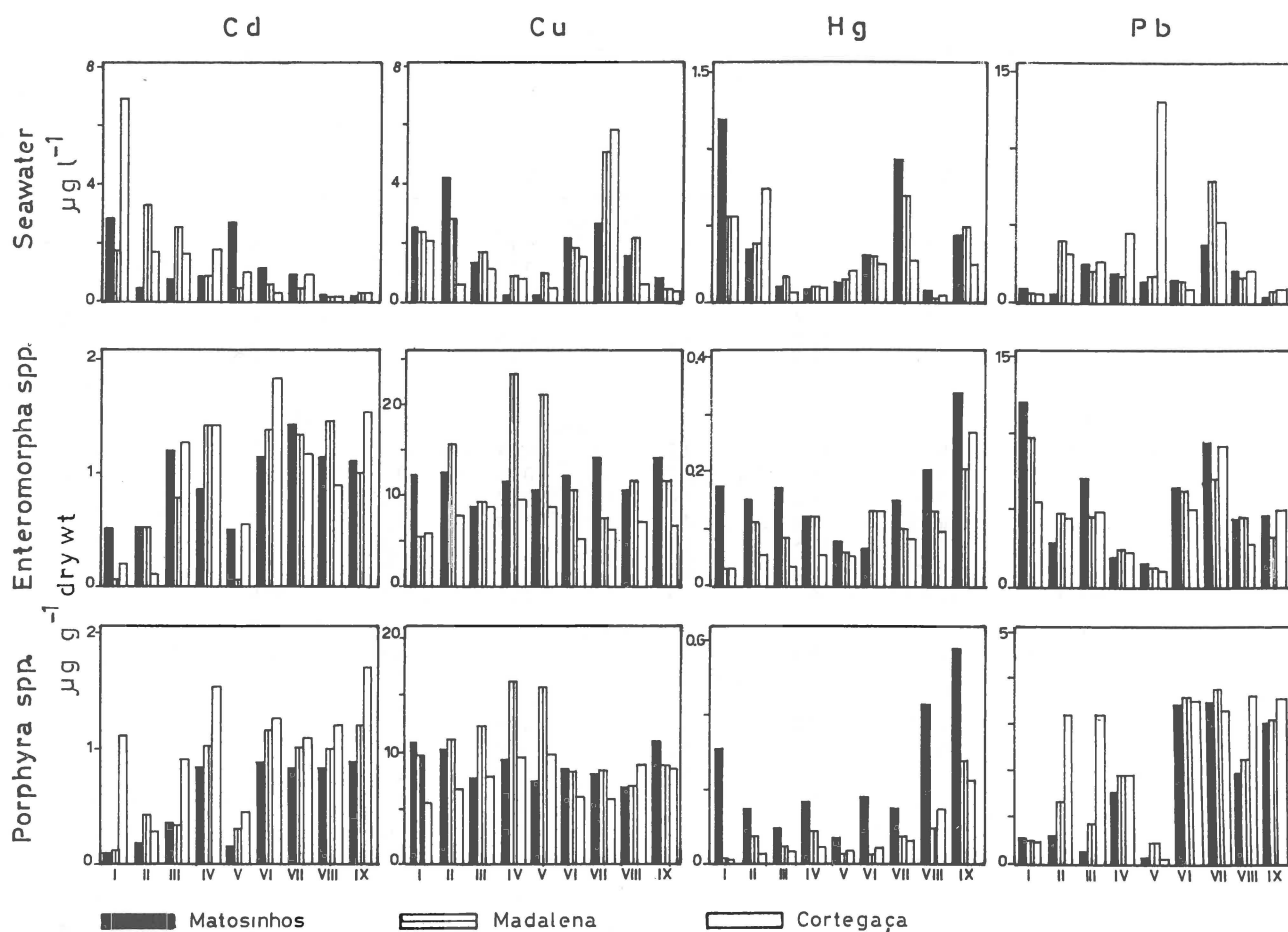


Fig. 2 Concentrations of Cd, Cu, Hg and Pb in seawater and marine algae at Matosinhos, Madalena and Cortegaça, between November 94 and June 95.

*Enteromorpha* spp. was highest at Matosinhos, but for *Porphyra* spp. this was at Cortegaça (Table 4). The mean concentration of Cd, Cu and Hg in *Enteromorpha* spp. and *Porphyra* spp. were similar, but *Enteromorpha* spp. had more Pb than *Porphyra* spp. (Table 4). The concentrations of the metals in these algae decreased in the following order: Cu > Pb > Cd > Hg (Table 4).

#### *Correlations between the concentration of the metals in marine algae and surrounding seawater*

During the sampling period, the pattern of variation of the concentrations of each metal in seawater and algae (*Enteromorpha* spp. and *Porphyra* spp.) were very different. Whereas the concentrations of Cd showed opposite trends in seawater and algae, no simple and

TABLE 4.

Mean concentration (and coefficient of variation, CV) of Cd, Cu, Hg and Pb in seawater and marine algae in the Oporto coast, between November 94 and June 95.

	Seawater ( $\mu\text{g l}^{-1}$ ) (CV, %)				<i>Enteromorpha</i> spp. ( $\mu\text{g g}^{-1}$ dry wt) (CV, %)				<i>Porphyra</i> spp.			
	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb
Matosinhos												
	1.1	1.8	0.40	1.7	0.90	12.0	0.16	4.7	0.56	9.0	0.24	2.0
	91	72	100	65	44	17	50	63	67	16	100	82
Madalena												
	1.1	2.0	0.32	2.4	0.88	13.0	0.11	4.1	0.73	11.0	0.085	2.0
	100	70	67	92	67	46	100	53	57	27	100	65
Cortegaça												
	1.6	1.5	0.27	3.8	0.99	7.2	0.094	4.1	1.1	7.7	0.075	2.8
	131	120	67	105	60	19	100	55	45	22	100	54

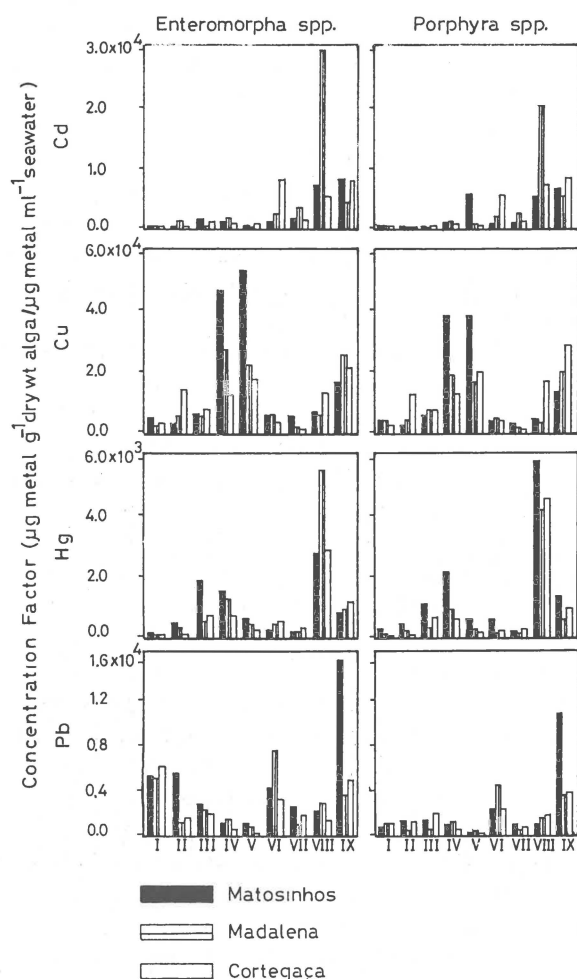


Fig. 3 Concentration factors for Cd, Cu, Hg and Pb in marine algae at Matosinhos, Madalena and Cortegaça, between November 94 and June 95.

constant relationship could be found between the concentrations of Cu, Hg and Pb in seawater and algae (Fig. 2). Concentration factors that were calculated month-to-month for each alga, metal and site, varied markedly during the sampling period (Fig. 3).

On the contrary, the mean metal contents in seawater and algae, for the period under study, was clearly

interrelated (except for Pb in *Enteromorpha* spp.) (Table 4). The mean Cd, Cu and Hg concentration in seawater and algae (*Enteromorpha* spp. and *Porphyra* spp.) decreased in the same way at the different locations:

Cd: Cortegaça > Madalena  $\cong$  Matosinhos

Cu: Madalena > Matosinhos > Cortegaça

Hg: Matosinhos > Madalena > Cortegaça

The mean Pb concentration in seawater and *Porphyra* spp. also decreased in the same order: Cortegaça > Madalena > Matosinhos, but the contents in *Enteromorpha* spp. showed an opposite trend.

Moreover, for each metal and algae, mean concentration factors (calculated by dividing the mean concentration of the metal in the algae by the mean concentration in seawater) were relatively constant at all three sites (except for Pb in *Enteromorpha* spp. and Hg in *Porphyra* spp.) (Table 5).

## Discussion

### Metal contamination levels in the seawater

The concentrations of dissolved heavy metals in seawater are affected by a number of processes, namely biological uptake, scavenging by particulate matter, release from bottom sediments, advection and mixing of water masses and aeolian transport of terrestrial materials (Moore, 1978; Yeats and Campbell, 1983; Jones and Murray, 1984; Wangersky, 1986). In some areas, the concentrations of heavy metals (namely Cd, and to a lesser extent Cu and Pb) are typically related to the concentration of nutrients and exhibit marked seasonality, with peak periods of primary productivity in spring-summer associated with a depletion of metals from surface waters due to sorption by phytoplankton cells (Knauer and Martin, 1973; Moore, 1978; Kremling, 1985; Noriki *et al.*, 1985; Wangersky, 1986; Valenta *et al.*, 1986; Kremling and Hydes, 1988; Burton *et al.*, 1993; Tappin *et al.*, 1993). In other areas, however, inputs from rivers and estuaries as well as from the atmosphere are very significant, and can be dominant, masking and counteracting biological

TABLE 5

Mean concentration factors\* for Cd, Cu, Hg and Pb in *Enteromorpha* spp. and *Porphyra* spp. in the Oporto coast.

Site	<i>Enteromorpha</i> spp.				<i>Porphyra</i> spp.			
	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb
Matosinhos	$8.3 \times 10^2$	$6.7 \times 10^3$	$4.0 \times 10^2$	$2.8 \times 10^3$	$5.1 \times 10^2$	$5.1 \times 10^3$	$6.0 \times 10^2$	$1.2 \times 10^3$
Madalena	$8.0 \times 10^2$	$6.4 \times 10^3$	$3.4 \times 10^2$	$1.7 \times 10^3$	$6.6 \times 10^2$	$5.4 \times 10^3$	$2.7 \times 10^2$	$8.3 \times 10^2$
Cortegaça	$6.2 \times 10^2$	$4.9 \times 10^3$	$3.5 \times 10^2$	$1.1 \times 10^3$	$6.7 \times 10^2$	$5.2 \times 10^3$	$2.8 \times 10^2$	$7.3 \times 10^2$
Mean	$7.5 \times 10^2$	$6.0 \times 10^3$	$3.6 \times 10^2$	$1.9 \times 10^3$	$6.2 \times 10^2$	$5.2 \times 10^3$	$3.8 \times 10^2$	$9.2 \times 10^2$
CV (%)	15	16	8.6	47	14	3.1	50	24

\*Calculated by dividing the mean metal contents in the algae by the mean metal concentration in seawater, and expressed in  $\mu\text{g metal g}^{-1}$  dry wt algae per  $\mu\text{g metal ml}^{-1}$  seawater.

removal associated with phytoplanktonic blooms (Jones and Murray, 1984; Kremling and Hydes, 1988; Kremling and Pohl, 1989; Burton *et al.*, 1993; Hydes and Kremling, 1993; Tappin *et al.*, 1993, 1995). In the Oporto coast, the concentrations of dissolved Cd, Cu, Hg and Pb varied markedly during the sampling period and exhibited seasonality. Although no assessment of phytoplanktonic populations was made in the present work, it is likely that biological activity was, at least partially, responsible for the observed seasonal changes, as reported for other areas throughout the world (Knauer and Martin, 1973; Morris, 1974; Valenta *et al.*, 1986; Kuwabara *et al.*, 1989; Gerringa *et al.*, 1996).

The mean concentrations of dissolved Cd, Cu, Hg and Pb in seawater in the studied sites were, respectively (ranges, in  $\mu\text{g l}^{-1}$ ) 1.1–1.6, 1.5–2.0, 0.27–0.40 and 1.7–3.8. For comparative purposes, selected values for the concentrations of Cd, Cu and Pb in European coastal waters were taken from the literature and are shown in Table 6. Values for Hg are scarce. For seas around the UK, Baker (1977) reported values within the range 0.0020–0.024  $\mu\text{g l}^{-1}$ . Gardner (1978) reported that in the Irish Sea (UK), although mean Hg concentrations varied between 0.017 and 0.058  $\mu\text{g l}^{-1}$ , in two areas (Solway Firth and Morecambe Bay) they ranged from 0.004 to 0.443  $\mu\text{g l}^{-1}$ . High values of dissolved Hg have also been found in the Loire estuary, with a range of 0.75–2.2  $\mu\text{g l}^{-1}$  (Frenet, 1981). It appears therefore that the concentrations of dissolved Cd, Cu, Hg and Pb in seawater in the Oporto coast were similar to those reported for polluted and industrialized European coastal areas.

#### Concentration of metals in marine algae

The concentrations of total Cd, Hg and Pb in both *Enteromorpha* spp. and *Porphyra* spp. varied appreciably during the sampling period, each metal displaying a characteristic pattern of variation. Similar high variability in the contents of heavy metals in marine macroalgae have been reported in the literature (Fuge and James, 1974; Eide *et al.*, 1980; Drude de Lacerda *et al.*, 1985).

It has been concluded that the metal contents in aquatic macrophytes are generally low in the warmer months of the year due to high growth rates that 'dilute' the accumulated metals (Phillips, 1994). However, Hägerhäll (1973) reported that the concentrations of Cu (as well as Ni and Zn) in *Ascophyllum nodosum* in Öresund (Sweden) were maximum in mid-July, and Drude de Lacerda *et al.* (1985) found that the concentrations of Cd, Cu and Pb (among other metals) in five marine algae at a sampling site in southern Brazil were generally maximum in January (summer) and minimum in August (winter). Similarly, in the present study we found that the concentrations of Cd, Hg and Pb in both *Enteromorpha* spp. and *Porphyra* spp. were higher in spring (April–June) than in winter (November–March).

#### The use of macroalgae as biomonitors of metal contamination levels in coastal waters

The contamination of seawater with heavy metals is one of the present problems in marine pollution. An objective assessment of contamination levels requires the determination of significant average concentrations, but also episodic peak values, since these can have drastic consequences for marine biota. As reported for several areas throughout the world, we found in the present study that the concentrations of Cd, Cu, Hg and Pb in the seawater at Matosinhos, Madalena and Cortegaça did vary appreciably from month-to-month and showed marked seasonality. It appears therefore that extensive sampling during a considerable period of time is indeed required for a significant assessment of the concentrations of Cd, Cu, Hg and Pb in the Oporto coastal waters.

As an alternative to the direct determination of heavy metals in seawater, these can be assayed in a suitable biomonitor, namely a marine macroalga, and knowing the corresponding concentration factors, the mean metal contents in seawater can be estimated. However, the advantages and accuracy of this procedure depends on meeting several criteria (Phillips, 1977; Rainbow and Phillips, 1993; Phillips, 1994; Phillips and Rainbow, 1994).

TABLE 6

Selected values (ranges) for the concentration of dissolved Cd, Cu and Pb in European coastal waters.

Locations	Cd	Cu ( $\mu\text{g l}^{-1}$ )	Pb
Sørfjorden, Norway <sup>1</sup>	0.81–2.2	3.7–6.3	4.3–8.9
Belgian and Dutch coast <sup>2</sup>	0.013–0.39	0.34–15	0.041–7.5
Ligurian and Northern Tyrrhenian shoreline, Italy <sup>2</sup>	0.005–0.45	0.13–3.4	0.019–2.4
Northern Adriatic <sup>3</sup>	0.03–0.11	0.9–15	0.70–1.9
Central and Southern North Sea, coast <sup>4</sup>	0.007–0.081	0.064–1.7	0.041–0.23
Oporto coast, Portugal, this work	1.1–1.6	1.5–2.0	1.7–3.8

<sup>1</sup>Melhuus *et al.* (1978); <sup>2</sup>Mart *et al.* (1982); <sup>3</sup>Munda and Hudnik (1991); <sup>4</sup>Tappin *et al.* (1995).

Among these, the following were tested in the present study for the Oporto coast, and are discussed below:

1. The concentrations of the metals under study should vary appreciably less in the alga than in the surrounding seawater. In the present study we found that the concentrations of Cd, Hg and Pb varied less in *Enteromorpha* spp. and *Porphyra* spp. than in seawater, but the variation in the algae was still considerable. The concentrations of Cu varied much less in both algae than in the seawater. Considering that similar variability in the contents of heavy metals in marine algae has been often reported in the literature (Fuge and James, 1974; Eide *et al.*, 1980; Drude de Lacerda *et al.*, 1985; Vasquez and Guerra, 1996), the assay of some heavy metals in the algae instead of directly in seawater may have only limited practical benefits.

2. The significance of concentration factors should be carefully evaluated. In the present study we found no apparent constant relationship between the variation of the concentrations of Cu, Hg and Pb in *Enteromorpha* spp. and *Porphyra* spp., and in seawater. Monthly concentration factors were very variable during the sampling period, indicating that concentration factors based on single or few determinations could be misleading, and instead only average values should be used. The results indicated that for the Oporto coast, mean concentration factors should be calculated by dividing the mean concentration of the metal in the alga by the mean concentration in seawater, both based on several determinations spanning over a considerable period of time.

3. Concentration factors should be relatively constant in the area under study, so that the chosen biomonitor can be used to estimate metal contamination levels in other sites within the area, or in the same sites in following years. In the present study, we did find that, for each studied metal, *Enteromorpha* spp. and *Porphyra* spp. had comparable mean concentration factors at Matosinhos, Madalena and Cortegaça (except for Pb in *Enteromorpha* spp. and Hg in *Porphyra* spp.). We therefore conclude that in the Oporto coast, under the present conditions, *Enteromorpha* spp. can be used to estimate the mean seawater contents in Cd, Cu and Hg, and *Porphyra* spp., the concentration Cd, Cu and Pb.

Constancy of mean concentration factors has been reported by several authors. Bryan and Hummerstone (1973) determined the concentration of metals in water and *Fucus vesiculosus* along two estuaries in the UK, and found similar mean concentration factors for Cu in these two areas. Foster (1976) determined the concentration of metals in seawater and two brown algae in the Menai Straits (UK), and found that the mean concentration factor for Cu in *Fucus vesiculosus* was similar to others reported in the literature. Hägerhäll (1973) determined the concentration of several metals in seawater and marine benthic algae in Öresund area (Sweden). Except for heavily contaminated areas, mean

concentration factors for Cu and Pb in *Enteromorpha intestinalis*, calculated from the data reported by this author, were relatively constant within the studied area. Seeliger and Cordazzo (1982) determined the concentration of Cu and Hg in *Enteromorpha* spp. and in water, at four localities in the Patos Lagoon estuary (Brazil). Mean concentration factors, calculated from the data reported by these authors, were relatively uniform within the area under study.

However, variability of concentration factors have also been reported in the literature. Melhuus *et al.* (1978) determined the concentration of Cd, Cu, Pb (and Zn) in five marine macroalgae and in seawater at four localities in the Sørkjorden fjord (Norway), and found that the concentration factors varied appreciably between the four sampling sites. However, although water samples were collected during various seasons in the period of 1972–1975, samples of algae were collected only in two periods in 1974. Woolston *et al.* (1982) determined the concentration of several metals in seawater and *Ascophyllum nodosum* at six different sites in the St Croix Estuary and Passamaquoddy Bay (Atlantic coast, boundary between Canada and USA) and found that concentration factors for Cd and Cu varied appreciably from site to site. However, samples of water and algae were taken in a single month. Considering that concentration factors for a given metal, alga and site, can vary pronouncedly throughout the year (see above), we would suggest that the variability of the concentration factors reported by these authors could have been due to the use of few determinations of the metal contents in the algae and/or in seawater.

All together, the results reported in the literature and those presented in this study indicate that some marine macroalgae can be used as bioindicators of heavy metal contamination levels in seawater, provided that representative concentration factors are used, and average values for the metal contents in the algae are determined. However, since these may require sampling of the algae at several occasions and spanning a considerable period of time, the assay of heavy metals in the algae instead of directly in seawater may have limited practical advantages.

## Conclusions

In the Oporto coast (northwest Portugal), the concentrations of dissolved Cd, Cu, Hg and Pb in seawater were similar to those reported for polluted and industrialized European coastal areas.

The marine benthic macroalgae *Enteromorpha* spp. and *Porphyra* spp. can be used as biomonitors of the seawater contents of Cd, Cu, Hg and Pb, in the Oporto coast. However, considering that future estimates of contamination levels in seawater must be based on extensive sampling of the algae during a considerable period of time, the advantages of using *Enteromorpha*

spp. and *Porphyra* spp. as monitors of metal contamination levels in the Oporto coast, instead of a direct assay in seawater, can be rather limited. As this limitation may be more general, it deserves to be investigated in other coastal areas.

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