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Macroalgae response to a mercury contamination gradient in a temperate coastal lagoon (Ria de Aveiro, Portugal)

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Abstract

Primary producers represent an important pathway for mercury incorporation in aquatic food webs. With eutrophication processes occurring worldwide, macroalgae may represent a substantial pool of mercury, as a result of its high growth rate and capacity to bind trace metals. The main aim of this work was to evaluate the response of the macroalgae to a human-induced environmental mercury gradient in a temperate coastal lagoon, by assessing the total and organic mercury contamination levels of the dominant species (*Enteromorpha*, *Fucus* and *Gracilaria*). Total mercury in the plant tissues ranged from 0.02 to 2.1 $\mu g\, g^{-1}$ dwt. *Fucus* was the most contaminated algae, followed by *Gracilaria* and *Enteromorpha*. As a whole, organic mercury never exceeded 15% of total mercury content, but tended to increase with distance to metal source on all macroalgae indicating complex physiological responses from these primary producers in areas of high and low mercury concentrations. Sessile macroalgae may be important mercury immobilisation agents, while free-floating algae (*Enteromorpha*) play an important role in mercury transport from contaminated areas (± 10 g ha^{-1}) to other areas of the lagoon and even to coastal waters. Based on the present results the use of macroalgal biomass from contaminated areas for direct or indirect human use (e.g. agricultural, industrial and food purposes) may result in health risks, due to the high bioaccumulation capacity (as high as 10^4 the dissolved mercury concentrations).

Keywords: total mercury; organic mercury; bioaccumulation; macroalgae; transport

1. Introduction

Mercury is a highly deleterious environmental pollutant with recognized mutagenic and teratogenic effects, although data on the mechanisms of such effects are very sparse and controversial in the available literature (Ariza et al., 1994; Calderón et al., 2003; Tchounwou et al., 2003). Problems derived from mercury contamination are enhanced when mercury enters the food chain, the predominant pathway of human exposure to methylmercury, which is the most toxic form of mercury (Tchounwou et al., 2003). Through the processes of biomethylation and bioaccumulation, methylmercury finds its way to species usually consumed by humans (Clarkson et al., 2003).

Primary producers represent an important pathway for mercury incorporation in estuarine food webs. Estuarine processes are therefore of great interest not only from a geochemical, economical and ecological point of view, been considered nurseries to many important faunal species and the location of the biggest cities in the World (Raffaelli et al., 1998; Flindt et al., 1999; Coelho et al., 2004; Lillebø et al., 2004), but also in a public health point of view, due to contamination of

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natural resources. With eutrophication processes occurring worldwide, estuaries shift from stable, macrophyte based systems to highly dynamic macroalgae based systems (Pardal et al., 2004). In such systems, macroalgae may represent a substantial pool of mercury, as a result of its reported capacity to bind trace metals (Radway et al., 2001; Vasconcelos and Leal, 2001) and high growth rate. By this mechanism mercury may be transferred up through the estuarine food webs and be incorporated in economically important species. Macroalgae may also play an important role in mercury transport and immobilisation. Several studies have been performed on the transport of mercury in contaminated sites, namely in the studied system (Pereira et al., 1998b; Boening, 2000; Ramalhosa et al., 2001; Monterroso et al., 2003), but the role of macroalgae has never been considered. Eutrophication is a worldwide problem, and macroalgal blooms (*Gracilaria* and *Enteromorpha* are dominant species in temperate latitudes) are common in such systems, with algal biomass reaching as high as 2 kg dwt m^{-2} (Raffaelli et al., 1998; Flindt et al., 1999; Pardal et al., 2004), which can be effectively transported with tides and represent a significant contaminant load to otherwise unaffected areas. Also, macroalgae are of economical interest, since at least 221 species are used as food or for medicinal, agricultural or industrial purposes worldwide (Zemke-White and Ohno, 1999). It is therefore important to have a correct assessment of heavy metal contamination in these natural resources.

Heavy metal biomonitoring with macroalgal species is frequent, despite some problems associated with seasonal variations, temperature and salinity conditions and intrinsic factors such as age and growth rate (Leal et al., 1997; Giusti, 2001; Villares et al., 2001; Barreiro et al., 2002; Caliceti et al., 2002; Tabudravu et al., 2002). However, studies specifically focused on mercury contamination are scarce, and have focused mainly on mercury uptake and kinetics by microalgae species (Miles et al., 2001; Moye et al., 2002). Species of the genera *Enteromorpha* are considered as good bioindicators, due to their laminar structure, structural and physiological uniform tissues and worldwide distribution. *Enteromorpha* was found also to reflect changes in environmental contamination faster than slower growing algae such as *Garcilaria* and *Fucus* (Villares et al., 2001). Algae from the genus *Fucus* have, in turn, been reported to produce methylated mercury and lead under polar conditions (Pongratz and Heumann, 1998), which raises the question about whether macroalgae may represent mercury methylation agents in temperate estuaries.

The main aim of the present work was to evaluate the response of the base of the trophic web to a human-induced environmental mercury gradient and the possible implications for the system, by assessing the total and organic mercury contamination levels of the dominant species of Chlorophyta (*Enteromorpha intestinalis*), Rodophyta (*Gracilaria verrucosa*) and Pheophyta (*Fucus vesiculosus*) in a temperate coastal lagoon (Ria de Aveiro, Portugal). A comprehensive interpretation of the results in relation to the ecology of the system, physiology of the plants and seaweed usage was attempted.

2. Materials and methods

2.1. Study site

The study was conducted in the Ria de Aveiro coastal lagoon, northwestern coast of Portugal (Fig. 1), that for the last 50 years has suffered from continuous mercury discharges originating from a chlor-alkali industry, inducing an environmental contamination gradient inside the lagoon (Pereira et al., 1998b). Mercury storage in the system is estimated to be 33×10^3 kg, of which 77% are stored in the Estarreja channel and Laranjo basin (Pereira et al., 1998a).

Fourteen sampling locations (A1–A14, Fig. 1) were selected along the four main arms of the lagoon with special focus on the most contaminated area, the Laranjo basin (stations A1–A5), near Estarreja where the industrial mercury discharges occurred. In addition, one sampling station was selected in the Mondego estuary (A15), 60 km south from the Ria. This station was considered to have pristine conditions referring to heavy metals (Vale et al., 2002) and served as a reference site, for comparison purposes.

2.2. Sampling

Algae samples were randomly collected by hand at each site and bulked in a single plastic bag, to a total of about 0.5 kg of alga (to account for within site variability). Sampling was conducted in late spring/early summer, when macroalgae blooms are common in the system due to its eutrophic status. Low tide situation was chosen and salinity measured whenever possible in the low water pools where most macroalgae were collected, as well as in the adjacent channel. At the laboratory samples were carefully washed with gentle rubbing to remove adherent sediment and epiphytes, dried to constant weight in a forced air oven at 60 °C, homogenized and stored in acid washed plastic flasks until analysis. Sediment samples were collected at the same sites, oven-dried to constant weight at 60 °C, homogenized and sieved through a 1 mm sieve before storage until analysis. Water sampling, sample treatment and analysis were performed using ultra-clean protocols (adapted from Bloom, 1995). Ultra-pure water was obtained from a Millipore Milli-Q model 185 system. All glassware was previously soaked for at

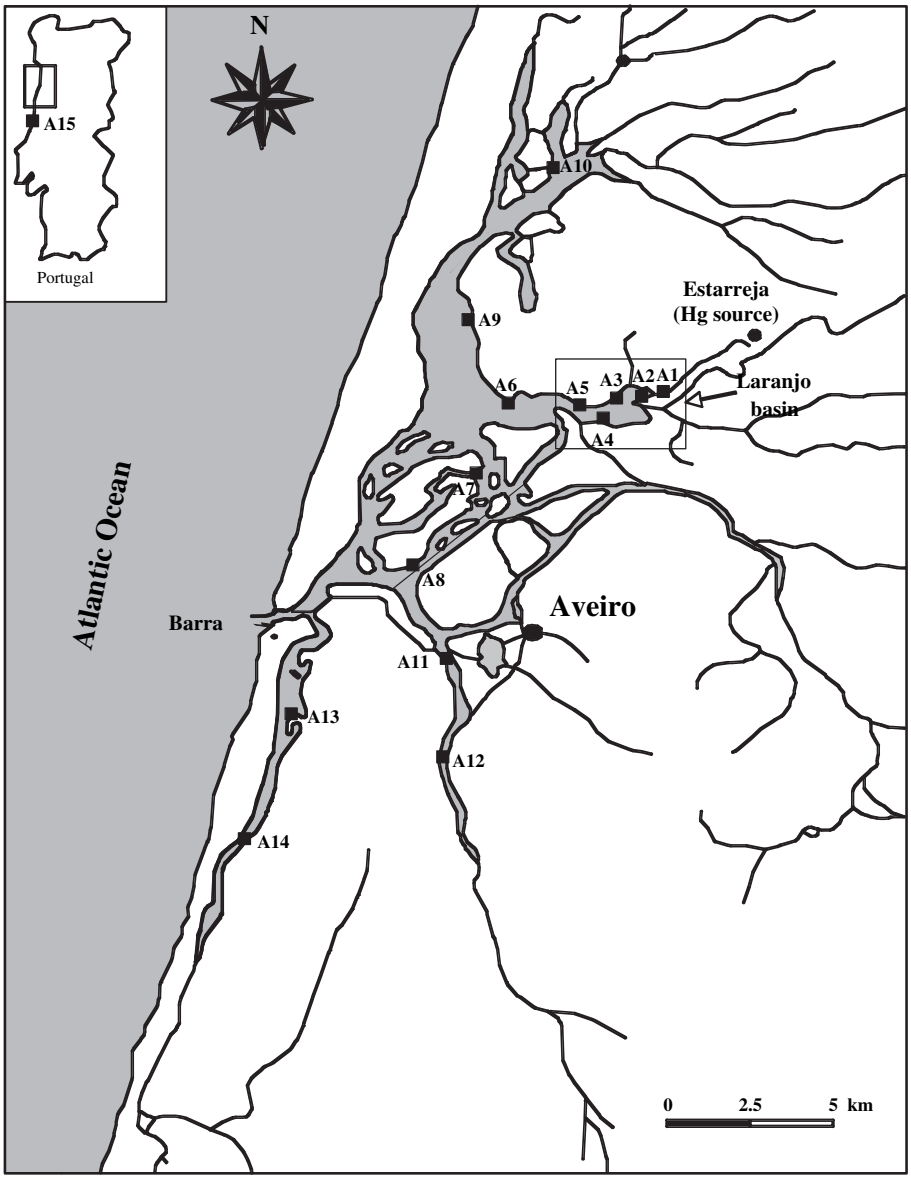

Fig. 1. Map of the Ria de Aveiro with the sampling sites indicated (A1–A15).

least 24 h in a bath containing 5% Decon, then in 25% HNO_3 and finally thoroughly rinsed with ultra-pure water. After sampling, water samples were transported to the laboratory and processed within a few hours. The water samples were filtered and the suspended particulate matter was collected on pre-weighed, 0.45 μm pore size Millipore filters for mercury determinations. In order to examine for any possible contamination during the filtration procedure, two blank solutions (100 mL of Milli-Q water) were acidified with 50 μL concentrated HNO_3 (Merck, "mercury-free") and were filtered in between the water samples through the same filtration unit used for those samples. The variability of replicates for filtration was assessed through analysis of two replicates of each sample, analysed three to four times each; the coefficient of variation was in the range from 2 to 6%. The method for mercury analysis in water has a mean analytical detection limit (defined as three times the standard deviation of the blank signal) of 0.42 ng L^{-1} ($n = 10$). Filters were dried at 60 °C and digested with HNO_3 4 mol L^{-1} for determination of the mercury concentration in suspended particulate matter (for detailed information on the method see Pereira et al., 1998b).

2.3. Analysis

Sediment and biological samples were analysed by pyrolysis atomic absorption spectrometry with gold amalgamation, using a LECO AMA-254 (Advanced Mercury Analyser), with no pre-treatment of samples. Dissolved reactive mercury and suspended particulate

matter mercury analyses were performed by cold-vapor atomic fluorescence spectrometry (CV-AFS) using a PSA model Merlin 10.023 equipped with a detector PSA model 10.003, with tin chloride as reducing agent (2% in 10% HCl). Total dissolved mercury concentrations were measured after addition of 500 μL of a saturated solution of potassium persulfate to 50 mL of filtered water and irradiation by a UV lamp (1000 W) for 30 min. Following irradiation, the excess of oxidant was reduced with 40 μL of hydroxylamine solution 12% (w/v) (Mucci et al., 1995).

Accuracy was assessed by taking measures of certified reference materials (CRM). The CRMs used were IAEA-356 (polluted marine sediment) and MESS-2 (marine sediment) for sediments and BCR-60 (*Lagarosiphon major*) for algae. The results were corrected according to the daily recovery percentage of the CRM analyses.

Organic mercury (quantified as total organic mercury) was quantified in the most contaminated area and in three sites progressively farther from the mercury source, through an extraction process involving acid leaching (KBr 18% in H_2SO_4 5%) and extraction of organic mercury in toluene (Cai et al., 1997). The aqueous fraction resulting from the addition of a $Na_2S_2O_3$ solution was then analysed by pyrolysis atomic absorption spectrometry with gold amalgamation. Efficiency of the extraction method for organic mercury was assessed through parallel analysis of CRM (IAEA-140 TM, *Fucus* sp. homogenate) (Coquery et al., 2000) together with the real samples.

All statistical analysis was performed with SPSS 11 software package.

3. Results

CRM analyses showed high efficiency (close to or above 90%) in mercury recovery for all the matrices, and excellent reproducibility (Table 1).

Salinity values ranged from 13 to 35 in the water column, and from 18.5 to 38 in the intertidal water pools formed in low tide, at the time of sampling (Fig. 2). A salinity gradient was present from the most contaminated area seawards (Fig. 2), but intertidal pools where most algae were collected from consistently had higher salinity values than the adjacent channel, probably due to evaporation processes, and did not show a clear pattern.

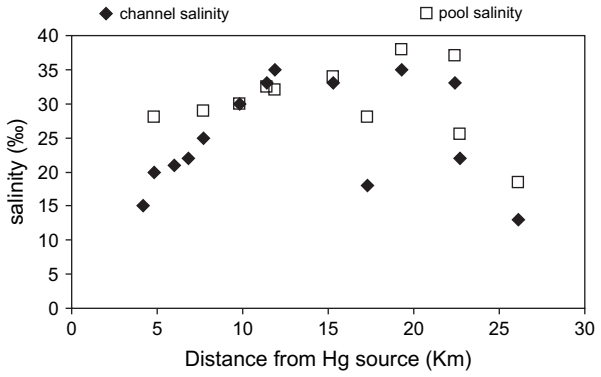

Fig. 2. Salinity in the water column and in low water pools in the sampling sites, in relation to distance from mercury source.

Mercury in the water (dissolved and suspended particulate matter) and total mercury in sediments were low in most sampling stations, except in the most contaminated area (Laranjo basin, stations A1–A5). So, two distinct scenarios are present in the Aveiro lagoon where mercury contamination problems seem therefore to be confined to the Laranjo basin. For the remnant of the sampled system, mercury contamination was comparable to that of the reference site (A15) (Table 2). The partition coefficient for dissolved mercury ($K_d = C_s/C_w$, where C_s (mg Hg kg^{-1}) is the measured non-reactive Hg (total minus reactive concentration) (mg Hg L^{-1}) divided by the SPM concentration (kg SPM L^{-1}), and C_w

Table 1
Obtained and certified mercury concentrations and extraction efficiency for analyses of CRM. n = number of analyses; C. Var. (%) = coefficient of variation

Reference material	[Hg] (μg g^{-1})	C. Var. (%)	n	[Hg] cert. (μg g^{-1})	Efficiency (%)
BCR-60	0.30	6.7	43	0.34	88.2
MESS-2	0.086	4.8	23	0.092	93.4
IAEA-356	7.45	12.8	8	7.62	97.8

Table 2
Total mercury in sediments (μg g^{-1}), reactive and total dissolved mercury (ng L^{-1}) and suspended particulate matter (μg g^{-1}) (mean values ± std. dev.)

Station	Sediment Hg (μg g^{-1})	Reactive dissolved Hg (ng L^{-1})	Total dissolved Hg (ng L^{-1})	Suspended particulate matter Hg (μg g^{-1})
A1	51.7 ± 4.8	60.5 ± 9.6	275.4 ± 12.6	25.8 ± 0.4
A2	6.8 ± 0.3	15.8 ± 1.0	73.2 ± 4.0	20.1 ± 2.6
A3	5.2 ± 0.1	24.0 ± 2.3	97.8 ± 0	9.0 ± 0.5
A4	2.5 ± 0.1	28.3 ± 1.8	107.7 ± 12.5	6.5 ± 0.2
A5	6.2 ± 0.1	9.0 ± 1.7	34.4 ± 3.4	8.9 ± 0.5
A6	0.4 ± 0	2.9 ± 0.4	10.0 ± 1.5	1.1 ± 0
A7	0.1 ± 0	4.0 ± 0.6	6.8 ± 1.7	0.7 ± 0.1
A8	0.2 ± 0	2.6 ± 0.3	4.4 ± 0.7	0.8 ± 0.1
A9	0.1 ± 0			0.8 ± 0.2
A10	0.2 ± 0.1	0.9 ± 0.2	2.8 ± 0.6	0.5 ± 0.2
A11	0.3 ± 0.1	1.1 ± 0.5	1.9 ± 1.1	1.0 ± 0.1
A12	0.2 ± 0	1.4 ± 0.4	2.2 ± 1.1	0.3 ± 0
A13	0.2 ± 0	0.6 ± 0.3	1.0 ± 0.6	0.6 ± 0.2
A14	0.1 ± 0.1	1.1 ± 0.3	3.7 ± 1.0	0.4 ± 0
A15	0.1 ± 0	1.5 ± 0.4	4.6 ± 1.3	1.2 ± 0.5

is the reactive Hg concentration (mg Hg L^{-1}), which expresses the ratio between colloidal bound and dissolved mercury (Lindström, 2001). K_d tended to decrease with increasing saline influence (Fig. 3), opposite to the percentage of mercury associated with suspended particulate matter, which would indicate lower bioavailability of mercury seawards from the contamination source and hence influence macroalgae Hg uptake in the lower reaches of the system.

Globally, mercury levels for all the three algae were higher in the most contaminated area (Fig. 4A), reaching as high as 2.1 μg g^{-1}. *Fucus* accumulated more mercury than *Gracilaria* except in sites A2 and A3, where there were no significant differences between the two algae (Tukey HSD, $p = 0.124$), whereas *Enteromorpha* had the lowest mercury concentrations in the most contaminated area (Laranjo basin). For the rest of the system, *Enteromorpha* had the highest mercury values, and significant differences for the three algae were found (ANOVA with Tukey HSD Post Hoc test, $p < 0.05$). *Enteromorpha* showed no significant correlations to dissolved mercury ($r = 0.37$ for reactive and $r = 0.40$ for total dissolved mercury), sediment ($r = 0.51$), but a significant correlation was found to suspended particulate matter ($r = 0.71$, $p < 0.01$) mercury levels. On the contrary, both *Gracilaria* ($r = 0.66$, $p < 0.05$; $r = 0.72$, $p < 0.01$; $r = 0.85$, $p < 0.01$; $r = 0.95$, $p < 0.01$, respectively) and especially *Fucus* ($r = 0.82$, $p < 0.01$; $r = 0.86$, $p < 0.01$; $r = 0.92$, $p < 0.01$; $r = 0.93$, $p < 0.01$ respectively) were in good agreement with environmental mercury contamination in a given site, which would suggest them to be good bioindicators of Hg contamination.

Mean concentration factors (CF) (calculated by dividing the mercury concentration in the algae by the mean total mercury concentration in water) were quite variable between sites and no clear pattern could be found, except for *Enteromorpha* (Fig. 5A–F), where significant correlations were found between *Enteromorpha* CF and dissolved reactive mercury ($r = 0.73$, $p < 0.05$). Significant differences were found between algal species in all sampling stations (ANOVA with Tukey HSD Post Hoc test, $p < 0.05$), except between *Gracilaria* and *Fucus* in sites A3 ($p = 0.12$) and A7 ($p = 0.68$) and between *Enteromorpha* and *Fucus* in site A10 ($p = 0.88$). These results suggest that only *Enteromorpha* Hg concentrations seem to be dependent of water mercury levels, suggesting differential responses to mercury contamination by the algae. Both the low dissolved mercury concentrations (Table 2) and the variability in water mercury levels might account for the oscillation in concentration factors from site to site.

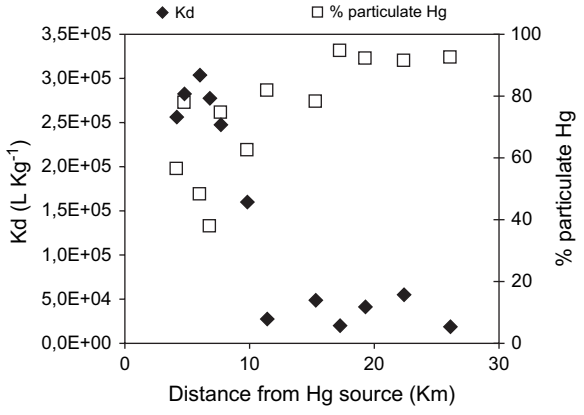

Fig. 3. Partition coefficients (K_d – dm^3 kg^{-1}) and % of particulate mercury to total mercury in the water column in the sampling sites, in relation to distance from mercury source.

As expected the absolute values of organic mercury in all macroalgae decrease with distance to source, and represent a minor fraction of the total mercury load in macroalgae (Fig. 4A and B). Significant differences were found between algae in sites A2 (Kruskal–Wallis, $p = 0.018$), A5 (Kruskal–Wallis, $p = 0.030$) and A6 (Kruskal–Wallis, $p = 0.048$), whereas no differences where found in the other sites. Nevertheless a different scenario was observed concerning the percentage of organic mercury in relation to the total mercury load. Two different scenarios in terms of organic mercury contamination emerge, for high (Fig. 6A) and low (Fig. 6B) contamination situations. In low contamination sites, the organic mercury fraction tended to increase in all algae with distance to source, while in the high contaminated area algal response was species-specific. *Fucus* organic mercury levels were lowest in the most contaminated area, and increased with distance from sampling station A5, whereas *Gracilaria* levels decreased till station A5 (Fig. 6A) and increased seawards from that point (Fig. 6B). *Enteromorpha* revealed the opposite behaviour, increasing with distance to source up to A5, where an abrupt drop occurs, and then increasing again. Station A5 seems therefore to be an important turning point in terms of organic mercury content for macroalgal samples.

4. Discussion

The results confirm that macroalgae may represent an important role in the global mercury cycle in contaminated aquatic systems, since all the algae responded to the environmental mercury gradient and showed very high concentration factors in relation to the water column, suggesting high bioaccumulation ability for mercury. This feature has been reported previously (Leal et al., 1997; Giusti, 2001; Villares et al., 2001; Tabudravu et al., 2002), and has important ecological implications. Being at the base of the trophic web, macroalgae may be responsible for the transfer of mercury from the environment to the biota, and

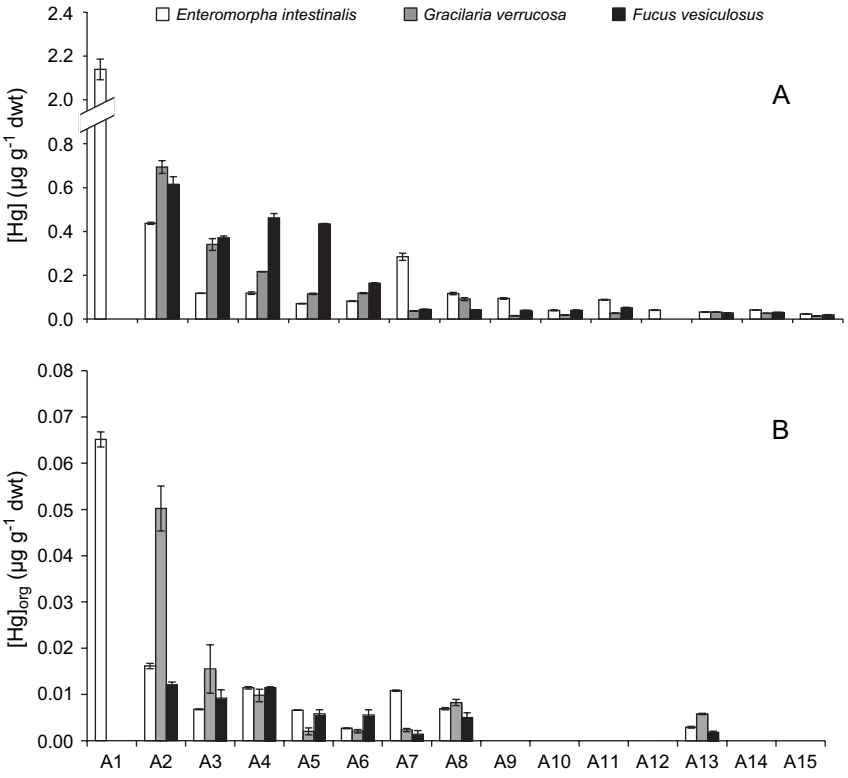

Fig. 4. Total mercury (A) and organic mercury (B) concentrations (µg g^{-1} dwt) in *Enteromorpha intestinalis*, *Gracilaria verrucosa* and *Fucus vesiculosus* in the Ria de Aveiro (error bars represent standard deviation).

biomagnification processes through the food web can result in severe health risks.

Mercury accumulation in macroalgae will primarily be related to water mercury concentrations, and also the salinity gradient. The higher water mercury concentrations were found in the Laranjo basin, and K_d values found in the system suggest higher bioavailability of mercury in this area, consistent with the higher mercury contamination values in all the algae. Downstream both mercury concentrations and K_d values are minor, which would explain the inferior algal contamination.

Differences in mercury contamination between species will probably result from various factors. The differential mobility (mainly of *Enteromorpha*) may explain the poorer correlations found to environmental mercury levels, since tidal transport to and from the contaminated area will influence the algae distribution. The higher correlations found for both *Gracilaria* and *Fucus* with environmental levels are consistent with the more sessile characteristics of these algal species. Physiological differences may also contribute to differentiate the algae in terms of mercury content, since the amount of metal accumulated is usually proportional to the growth rate of the algae (Round, 1981). *Fucus* and *Gracilaria* are perennial, slow growing algae (Round, 1981; Pedersen and Borum, 1996; Hughes and Otto, 1999) with typical growth rates of 2–3 cm $month^{-1}$ (Knight and Parke, 1950 in Giusti, 2001) whereas *Enteromorpha* has very high growth rates (Martins et al., 1999), which would suggest *Fucus* and *Gracilaria* to be the algae least responsive to contamination. The results evidence the opposite situation, probably as a result of the greater age of the portions analysed, therefore subject to mercury for a longer period. Differences between the two perennial algae may result from the different surface/volume ratios, which will influence the mercury uptake rate and thus explain the higher mercury concentrations found in *Fucus* when compared to *Gracilaria*.

Some macroalgae are capable of producing exudates (low molecular weight proteins, glutathione, phytochelatins and phyto-metallothionins) that will compete with the algae sites for metals, reducing metal incorporation into the cells (Lobban and Harrison, 1997; Vasconcelos and Leal, 2001). Distinct metabolism may hence justify the differential accumulation of mercury by the three algae, both in the inorganic and organic form.

Despite the good correlations found between *Fucus* and *Gracilaria* and environmental contamination, the oscillation found in the concentration factors from site to site and the lack of significant correlations to water mercury levels do not allow considering them suitable bioindicators of mercury contamination. Moreover, concentration factors were found to have seasonal fluctuations (Leal et al., 1997), which would furthermore influence the results.

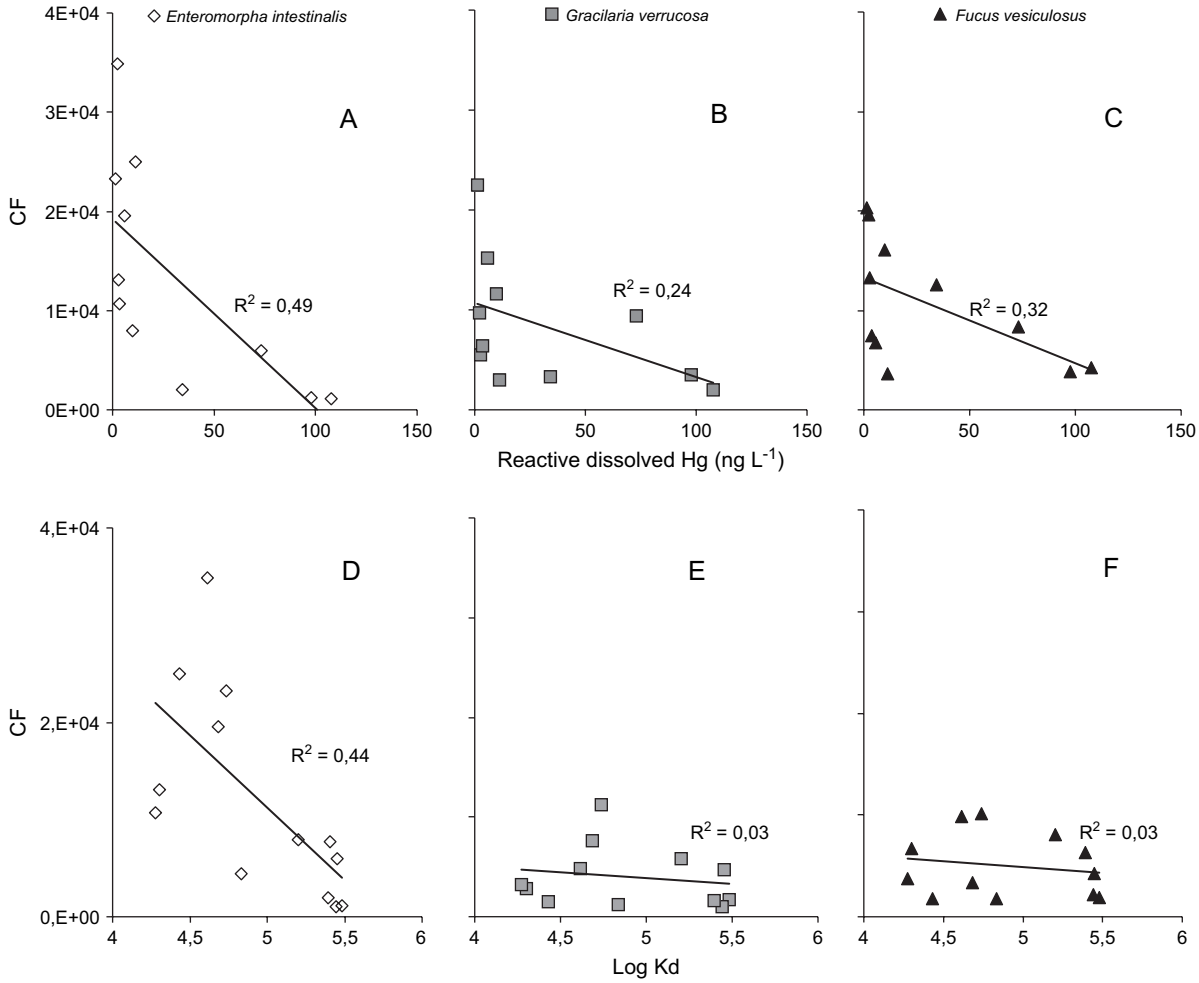

Fig. 5. Concentration factors (CF) ([Hg] algae/[Hg] water) to dissolved reactive [Hg] for *Enteromorpha* (A), *Gracilaria* (B) and *Fucus* (C) in all sampling sites; CF to log K_d for *Enteromorpha* (D), *Gracilaria* (E) and *Fucus* (F) in all sampling sites.

The percentage of organic mercury in macroalgae was low, generally below 15% (Fig. 6A and B), and tended to increase with distance to metal source. Dissolved mercury in estuarine waters occurs mainly in the inorganic form (Coquery et al., 1997; Horvat et al., 1999), but our results ranged from 22% (in the upper, low salinity area) to 60% (downstream areas) of total dissolved mercury (total minus reactive mercury). Organic mercury content may result both from direct uptake of organic mercury forms or from methylation by the algae (Pongratz and Heumann (1998) have reported Hg methylation by *Fucus distichus* in polar conditions), and thus be dependent on the metabolism of each algae. This could explain the different organic mercury fraction in the three algae, especially in the most contaminated area where species-specific factors must be the base for the differential organic mercury accumulation. The production of phytochelatins by the algae (discussed earlier) may also influence the uptake of available dissolved organic mercury. The distinct scenarios found for high and low contamination situations probably reflect differential physiological responses to environmental stress. In low contamination sites the three macroalgae present similar responses, suggesting that no specific decontamination strategy is occurring. Organic mercury results show a similar pattern (increased percentage of organic mercury in low contamination situation) to that of planktonic communities from contaminated and pristine environments (Schaefer et al., 2004). Adaptation to contamination and enhanced reductive demethylation rates may explain this feature (Schaefer et al., 2004).

Through their high growth rate and metal binding capacity, macroalgae can incorporate large amounts of mercury, which may then be transported by tidal currents (mainly *Enteromorpha*, the most important free-floating algae) away from the contamination source. A rough calculation assuming an average mercury concentration of 0.5 $\mu g\, g^{-1}$ and a mean *Enteromorpha* biomass of 0.5 kg dwt m^{-2} (during macroalgal blooms these values are common, personal observation), in the most contaminated area of the Aveiro lagoon (with an macroalgae covered intertidal area of around 1 ha) 10 g

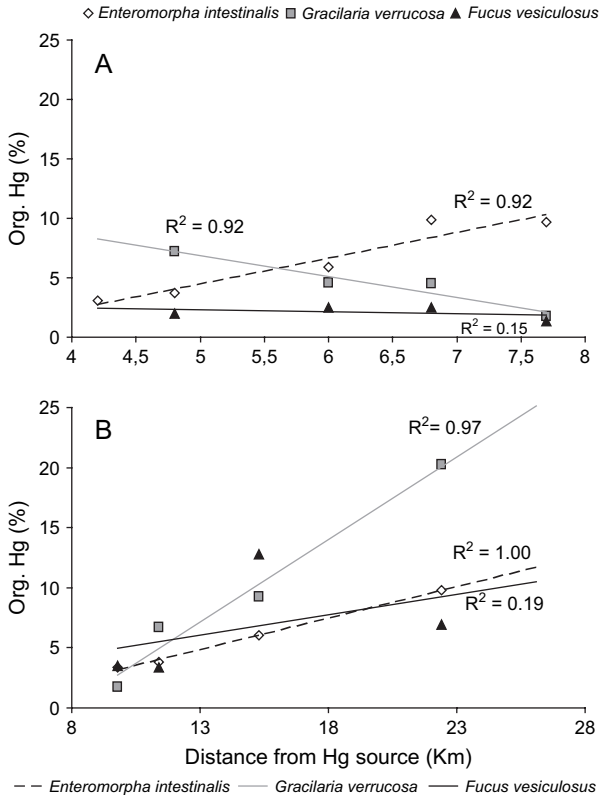

Fig. 6. Percentage of organic mercury to total mercury in the algal samples in high contamination (A) and low contamination (B) situations.

of mercury are integrated in seaweed tissue, available for tidal export. Reports on dissolved, particulate and phytoplankton (52 g, 82 g and 11 g per tidal cycle, respectively) transport (Ramalhosa et al., 2001; Monterroso et al., 2003) suggest the percentage exported by macroalgae may not be very significant, however further investigation is needed.

When considering sessile algal forms such as *Fucus*, the mercury removed from the water is incorporated and rendered immobile, remobilisation occurring through algal decay or consumption.

Considering that macroalgae are commonly used for food and agricultural and industrial purposes (e.g. *Gracilaria* is widely used for agar production, *Enteromorpha* is consumed directly by human populations and *Fucus* is used in the cosmetic industry), mercury levels found in this study can be considered preoccupant and a periodic monitoring should be considered. Additionally, an effort should be made to clarify the role of macroalgae in the mercury cycle in estuarine ecosystems, both in terms of transport and methylation processes. In addition, better insight of the mechanisms of organic mercury contamination in algae is necessary, as the percentage of organic mercury was found to increase in low contamination situations and may therefore represent a risk to human populations.

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