Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2010, 22(8) 1151–1157
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Mercury in sediments and vegetation in a moderately contaminated salt marsh (Tagus Estuary, Portugal)

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Abstract

Depth variations of total mercury (Hg) and methylmercury (MeHg) concentrations were studied in cores from non-colonized sediments, sediments colonized by *Halimione portulacoides*, *Sarcocornia fruticosa* and *Spartina maritima* and belowground biomass, in a moderately contaminated salt marsh (Tagus Estuary, Portugal). Concentrations in belowground biomass exceeded up to 3 (Hg) and 15 (MeHg) times the levels in sediments, and up to 198 (Hg) and 308 (MeHg) times those found in aboveground parts. Methylmercury in colonized sediments reached 3% of the total Hg, 50 times above the maximum values found in non-colonized sediments. The absence of correlations between total Hg concentrations in sediments and the corresponding MeHg levels suggested that methylation was only dependent on the environmental and microbiological factors. The analysis of belowground biomass at high-depth resolution (2 cm) provided evidence that Hg and MeHg were actively absorbed from sediments, with higher enrichment factors at layers where higher microbial activity was probably occurring. The results obtained in this study indicated that the biotransformation of Hg to the toxic MeHg could increase the toxicity of plant-colonized sediments.

Key words: mercury; methylmercury; salt marshes; Tagus Estuary; Portugal
DOI: 10.1016/S1001-0742(09)60231-X

Introduction

Mercury is a globally dispersed pollutant which exists in several different forms in aquatic ecosystems (O'Driscoll et al., 2005). One of the most toxic forms is methylmercury (MeHg), a neurotoxin which may bioaccumulate in aquatic organisms and biomagnify in the food web to concentrations which result in adverse effects on reproduction and neural development in fish and mammals (e.g., Wang et al., 1998; Burgess and Meyer, 2008). Anoxic wetland sediments are known to be key areas of mercury methylation in freshwater ecosystems (O'Driscoll et al., 2005) due to the presence of sulphate reducing bacteria and/or other mercury methylating microorganisms (Compeau and Bartha, 1985; Fleming et al., 2006). Whereas mercury speciation has been extensively studied in freshwater wetlands, much less is known about the distribution and speciation of mercury in salt marshes. However, these ecosystems are of vital importance due to the high biological productivity, hydrological flux regulation, biogeochemical cycling of metals and nutrients, and habitat for fish and wildlife (e.g., Weis and Weis, 2004; Válega et al., 2008).

Studies in salt marshes showed high concentrations of both Hg and MeHg in the rhizosphere, weak accumulation of both into aerial parts of plants, release of Hg from leaves during plant transpiration, and a minor incorporation into leaves via atmospheric Hg deposition (e.g., De Sousa et al., 1999; Windham et al., 2001; Weis and Weis, 2004). In a previous work it was found that colonized sediments contain 70 times more MeHg (up to 18% of the total mercury concentration) than non-vegetation sediments (Canário et al., 2007a). These results were obtained in three salt marshes in Portugal with different degrees of Hg contamination and colonized by three plant species. Concentrations in belowground biomass were 9 (total Hg) and 44 (MeHg) times higher than in the corresponding colonized sediment. Moreover, belowground biomass was enriched 400 (total Hg) and 4700 (MeHg) in comparison to aboveground biomass, suggesting low translocation or weak retention of mercury species to/in aboveground parts.

This article expands the previous work (Canário et al., 2007a) by examining how the halophyte plants *Sarcocornia fruticosa*, *Halimione portulacoides* and *Spartina maritima* influence the partitioning of mercury species. The work was performed in a salt marsh of the Tagus Estuary that is moderately contaminated with mercury.

1 Experimental

1.1 Environmental setting

The study was carried out in the Alcochete marsh near the Tagus Estuary Natural Park, Portugal (Fig. 1). *S. fruticosa*, *H. portulacoides* and *S. maritima* cover 95% of

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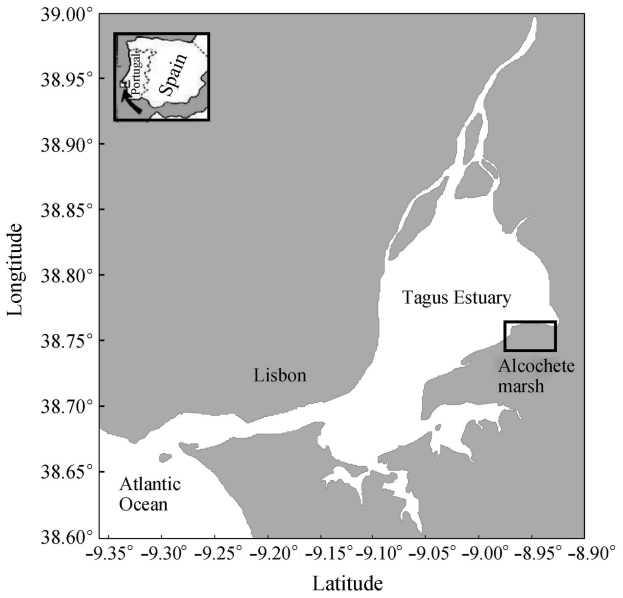

Fig. 1 Map of the Tagus Estuary with the study salt marsh.

the colonized area, which is inundated by the tide twice a day. Previous work indicated a moderate contamination by mercury (Canário et al., 2005, 2007b).

1.2 Field work and sample preparation

Salt-marsh plants and sediment cores were sampled in May 2007 from pure stands of *S. fruticosa*, *H. portulacoides* and *S. maritima*. Sediment cores were collected from colonized and non-vegetated (approximately 20 m distance from the pioneer plant, *S. maritima*) areas and sliced in layers of 2–5 cm sediment thickness. Before slicing, dissolved oxygen was measured using a Diamond Electro-Tech needle electrode (USA) following the method described by Caetano and Vale (2002). Measurements were done within 5 min after collection to avoid alteration from the air exposure. The non-colonized sediment core was sliced at the same depth intervals until 25 cm. Colonized sediments were sliced until O_2 concentration was detected.

Plant aboveground parts were removed at ground level and stored in plastic bags. At the laboratory they were washed with ultra pure Milli-Q water (18.2 MΩ) to remove dust and sediment, and then oven dried at 40°C. The belowground material of each layer was separated from the sediment carefully under a flux of Milli-Q water using a 212 μm mesh size sieve to remove any adhering particulate matter. Sediments and roots were oven dried at 40°C and weighed to determine belowground biomass (Gross et al., 1991; Caçador et al., 1999). Sediment and biological samples were homogenized with an agate mortar for chemical analysis.

1.3 Analysis of sediments

Sediment water content was estimated by weight loss at 105°C. Determinations of total Al, Fe and Mn were performed by mineralization of the sediment samples with a mixture of acids (HF, HNO_3 and HCl) according to the method described by Rantala and Loring (1975). Metal concentrations were obtained by flame-atomic absorption spectrometry (AAS) (AAnalyst 100, PerkinElmer, USA) using direct aspiration into N_2O-acetylene flame (Al) or air-acetylene flame (Fe and Mn). Mercury was determined by AAS using a silicon UV diode detector (AMA-254, LECO, Czech Republic) after pyrolysis of each sample in a combustion tube at 750°C under an oxygen atmosphere and collection on a gold amalgamator (Costley et al., 2000). Methylmercury was determined in dry sediments by alkaline digestion (KOH/MeOH), organic extraction with dichloromethane (DCM) pre-concentration in aqueous sulphide solution, back-extraction into DCM and quantification by GC-AFS in a Agilent Chomatograph (Mod. 6890, Agilent, USA) coupled with a pyroliser unit and a PSA fluorescence detector (PSA, UK) (Canário et al., 2004). Recoveries and the possible MeHg artifact formation were evaluated by spiking several samples with Hg(II) and MeHg standard solutions with different concentrations. Recoveries varied between 97% and 103% and no artifact MeHg formation was observed during our procedure. In all metal analysis, precision, expressed as relative standard deviation (RSD) of 4 replicate samples, was less then 4% ($p < 0.05$). International certified standards (MESS-2, PACS-1, IAEA-405 and BCR-580) were used to ensure the accuracy of procedures. For all metals investigated, obtained values were consistently within the ranges of certified values ($p < 0.05$). Total carbon sediment content was measured in homogenized and dried sediments, using a CHN Fissons NA 1500 analyser (NA 1500, Fissons, Italy). The calibration standard used was sulphanilamide. Procedural blanks were obtained by running several empty ashed tin capsules. Organic carbon was estimated by difference between total carbon and inorganic carbon after heating samples at 450°C for 2 hr to remove the organic carbon from the sediment. In all carbon analysis, precision expressed as RSD was less than 1% ($p < 0.05$) of 5 replicate samples.

1.4 Analysis of plant tissues

For MeHg determinations in vegetation a modified method based on the study of Canário et al. (2006) was used. Dried tissue samples were digested with a concentrated HBr (Merck suprapur) solution saturated with $CuSO_4$. Methylmercury in the digested solution was then extracted in a dichloromethane (DCM) solution pre-concentrated in a slight alkaline H_2S solution, back extracted into DCM and quantified by GC-AFS in an Agilent chromatograph coupled with a pyroliser unit and a PSA Hg fluorescence detector. Sediment analysis recoveries and the possible MeHg artifact formation were evaluated by spiking several samples with Hg(II) and MeHg standard solutions with different concentrations. Recoveries varied between 96% and 104% for all plant tissues investigated and no artifact MeHg formation was observed. For all the analysis, precision expressed as the relative standard deviation of three replicate samples, was less than 2.5% ($p < 0.05$). International certified standards CRM-60 (*Lagarosiphon major*, aquatic plant), CRM-61 (*Plantihypnidium riparioides*, aquatic moss) and IAEA-

140/TM (*Fucus* sea plant homogenate) were used to ensure the accuracy of the procedure. Mercury and MeHg concentrations were consistently within the ranges of certified values.

1.5 Statistical analysis

Statistical tests were performed using the computer software Statistica 9.0. The normality of all data was assessed by a Shapiro-Wilks test. Since some variables did not present a normal distribution, concentrations of the chemical parameters for each station were compared between the two months using the non-parametric Wilcoxon paired-sample (T) test. Correlations between chemical parameters were determined with Spearman correlation coefficient (r) (Zar, 1996).

2 Results and discussion

2.1 Sediment characteristics

Aluminium concentrations varied within a broad interval throughout the colonized and non-vegetated cores (5%–10%). Since Al is a proxy of clay fraction (Windom et al., 1989; Din, 1992), the variation content indicates that sediments from the Alcochete salt marsh are constituted by a mixture of fine and coarser materials. However, in all sampled cores the Al concentrations were similar in colonized and non-vegetated areas. Organic carbon content in sediments also varied within a broader range (0.8%–2.7%). The interval was narrower in colonized sediments (1.8% and 2.6%) and increased with the depth in non-vegetated sediments. Iron levels varied between 1.9% and 3.7% and Mn between 145 and 422 μg/g, with the higher concentrations found in colonized sediments. The enrichment factor, calculated as the ratio between levels in colonized and non-vegetated sediments, ranged between 1.3 and 1.8 for Fe and from 2.1 to 5.7 for Mn. These enrichment results from the intense mobilization of Fe and Mn in the vicinity of the roots as O_2 is released into anoxic sediments (Sundby et al., 2003). Previous studies reported the formation of iron plaques (e.g., St-Cyr et al., 1996) and Fe-oxide concretions (e.g., Vale et al., 2003) around the roots, as a consequence of the oxidation of iron sulphides and rapid precipitation of Fe(III) (Sundby et al., 1998).

2.2 Belowground biomass and dissolved oxygen

The belowground biomass was considerable higher in the areas colonized by *S. fruticosa* (3257 g/m^2) in comparison. with *H. portulacoides* (1166 g/m^2) and *S. maritima* (933 g/m^2). The amount of roots varied with the depth for the three plants. Whereas for *H. portulacoides* the maximum root quantities were found in 1-cm layer of the first 5-cm depth (max 446 g/m^2). For *S. fruticosa* and *S. maritima* the roots were more abundant between 5 and 14-cm depth. The differences on belowground biomass and root penetration depth usually reflect adaptive responses of plants to the environment (Sundby et al., 2003) like nutrients or the nature of sediment particles.

Dissolved oxygen in the colonized sediments was found until 56-cm depth in contrast to the non-vegetated sediments (< 1 cm). Similar profiles of dissolved O_2 concentrations have been measured in previous studies (Sundby et al., 2003) and interpreted as the delivery of oxygen by roots to the surrounding sediments (Caetano and Vale, 2002). It should be noticed that the presence of oxygen in the rooting sediments means that deliver of O_2 exceeds its consumption by the oxidation of organic matter and other associated redox reactions.

2.3 Total mercury and methylmercury in sediments

Figure 2 presents the vertical profile of Hg (μg/g) and MeHg (ng/g) concentrations in non-vegetated sediments.

In sediments where roots are present, concentrations of total Hg were in general higher (but not statistically different; $p > 0.05$) presenting a broader interval than in non-vegetated sediments for the three studied plants: 0.29–1.87 μg/g for *S. fruticosa*, 0.28–0.89 μg/g for *H. portulacoides*, 0.36–1.11 μg/g for 0.36–1.11 μg/g for *S. maritima* and 0.09–1.06 μg/g in non-vegetated sediments. Elevated levels in colonized sediments have been observed in previous works and interpreted as the result of Hg uptake by roots and subsequent retention in the buried litter, as well as incorporation in the abundant organic matter retained in sediments (Micaelo et al., 2003; Molina et al., 2006; Canário et al., 2007a).

The MeHg concentrations in colonized sediments differed two orders of magnitude of the non-vegetated sediments. The percentages of MeHg in non-vegetated sediments were lower than 0.08% of the total Hg, while in the sediments colonized by each of the three plants the values were statistically high ($p < 0.01$) and up to 3.8% (*H. portulacoides*; (0.59 ± 0.39)%; *S. fruticosa*: (3.78 ± 1.45)%; *S. maritima*: (1.61 ± 0.92)%). These differences pointed to a higher methylation in colonized sediments, which is consistent with previous studies in other marshes and in soils planted with rice and tobacco plants (e.g., Weber et al., 1998; Kongchum et al., 2006; Canário et

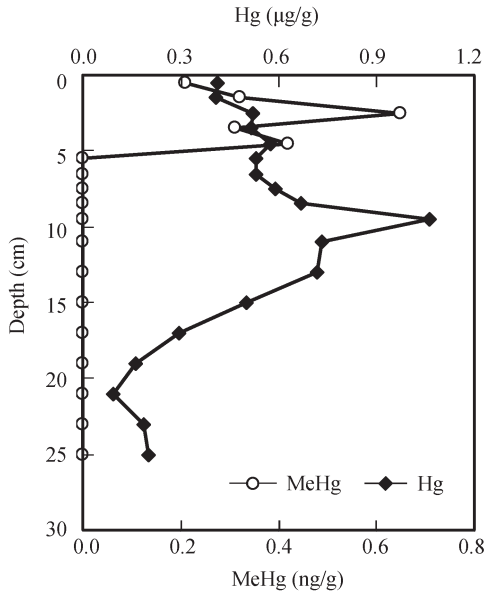

Fig. 2 Vertical profile of Hg and MeHg in non-colonized sediments.

al., 2007a). Moreover, the depth profiles of MeHg concentrations in colonized sediments showed that MeHg was quantifiable down to a depth of 58-cm in the presence of *H. portulacoides*, to 44-cm in the presence of *S. fruticosa*, and to 24-cm in the presence of *S. maritima*. The thickness of these layers contrasts with the thin MeHg layer (< 5 cm) observed in the non-vegetated sediments (Fig. 1). The network of roots, distributed heterogeneously throughout the sediment, creates a correspondingly heterogeneous pattern of sediment redox properties (Sundby et al., 2003) that appears to be ideal for a high microbial activity. The coupling with the reservoirs for bacterial respiration (e.g., organic carbon and sulphate) favors Hg methylation (e.g., Mason et al., 2003).

No correlation (at 95% confidence level) was found between total Hg concentrations and the corresponded MeHg levels in both non-vegetated and colonized sediments. This lack of relationship suggests that MeHg content in these salt-marsh sediments is mainly dependent on the environmental and microbiological factors affecting methylation process rather than the availability of Hg. The lowest concentrations of MeHg found in non-vegetated sediments do not appear to be related to the low available Hg but due to the absence of optimal biogeochemical conditions that promotes Hg methylation.

Figure 3 presents the relationships between total Hg and MeHg concentrations and the corresponded biomass content in the sediment colonized by the three plants. By considering the three plants all together, total mercury were not correlated with the amount of biomass, but for MeHg good relationships were found (r is better than 0.926). Additionally the slope for *S. maritima* were statistically higher (at 95% confidence level) than for the other two plants, suggesting that the transformation of Hg to MeHg is more efficient in sediments colonized by this species. These results are in line with the high proportion of MeHg found in colonized sediments.

In a previous work (Canário et al., 2007a), the lower proportion of MeHg was found in *S. maritima* from a salt marsh influenced by the Iberian pyrite belt (Caetano et al., 2007). Presumably Hg from geogenic origin is less available for methylation. In the present study the work was focused in a single salt marsh of the Tagus Estuary moderately contaminated by Hg due to human activities. *Spartina* appears to promote a rhizosphere environment more adequate for Hg methylation. These results indicate that the complexity of the salt-marsh environment and the inter-dependence among several variables influence methylation and contribute to this variability.

2.4 Total mercury and methylmercury in plants

Vertical profiles of Hg and MeHg concentrations in belowground biomass and the corresponding sediments for the three analyzed plants are presented in Fig. 4.

Mercury and MeHg levels are higher in belowground parts compared to the values obtained in sediments. Enrichment factors (EF) varied with the plant. For the three analyzed plants the EFs are up to 3 and 15 for Hg and MeHg, respectively. These results indicate that both Hg

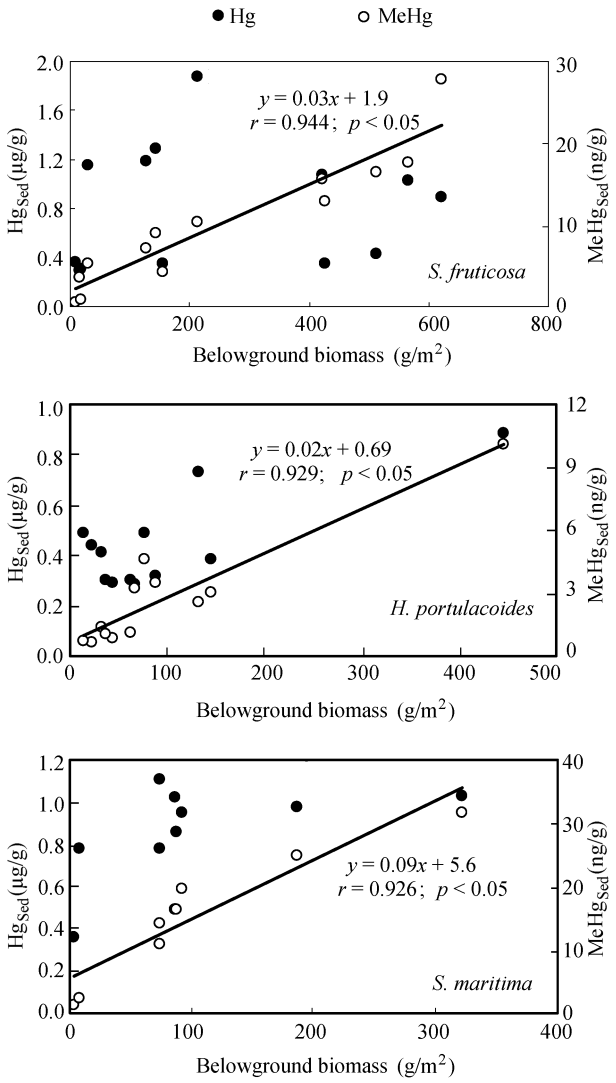

Fig. 3 Relationships between Hg and MeHg concentrations in layers of colonized sediments and the correspondent belowground biomass (regression lines refer to MeHg vs. belowground biomass).

and MeHg accumulate in root tissues of the salt-marsh plants. However, it is not clear whether Hg species are mainly bound to cell walls of root tissues or incorporated in the cell cytoplasm. Recent works (e.g., Wang et al., 2004; Skinner et al., 2007) showed that plant roots can effectively uptake Hg and MeHg by sequestration into two classes of cysteine-rich peptides, the metallothioneins and phytochelatins. The mechanism involves Hg binding with organic sulphur (R-SH) groups on the cysteine residues in these peptides being glutathionated and transported into vacuoles for long term sequestration (Brouwer et al., 1993). Mercury accumulation can also be explained by the existence of bacteria in the rhizosphere by processes involving the production of heat-labile compounds in roots exudates, which enhance Hg accumulation in plants (DeSousa et al., 1999), the transformation of Hg into MeHg more readily taken up into roots (Zayed et al., 1998), and the production of sulphate-transport protein in the root plasma membrane (Legget et al., 1956).

Another interesting aspect of the Hg accumulation in belowground biomass is that the levels of both Hg and

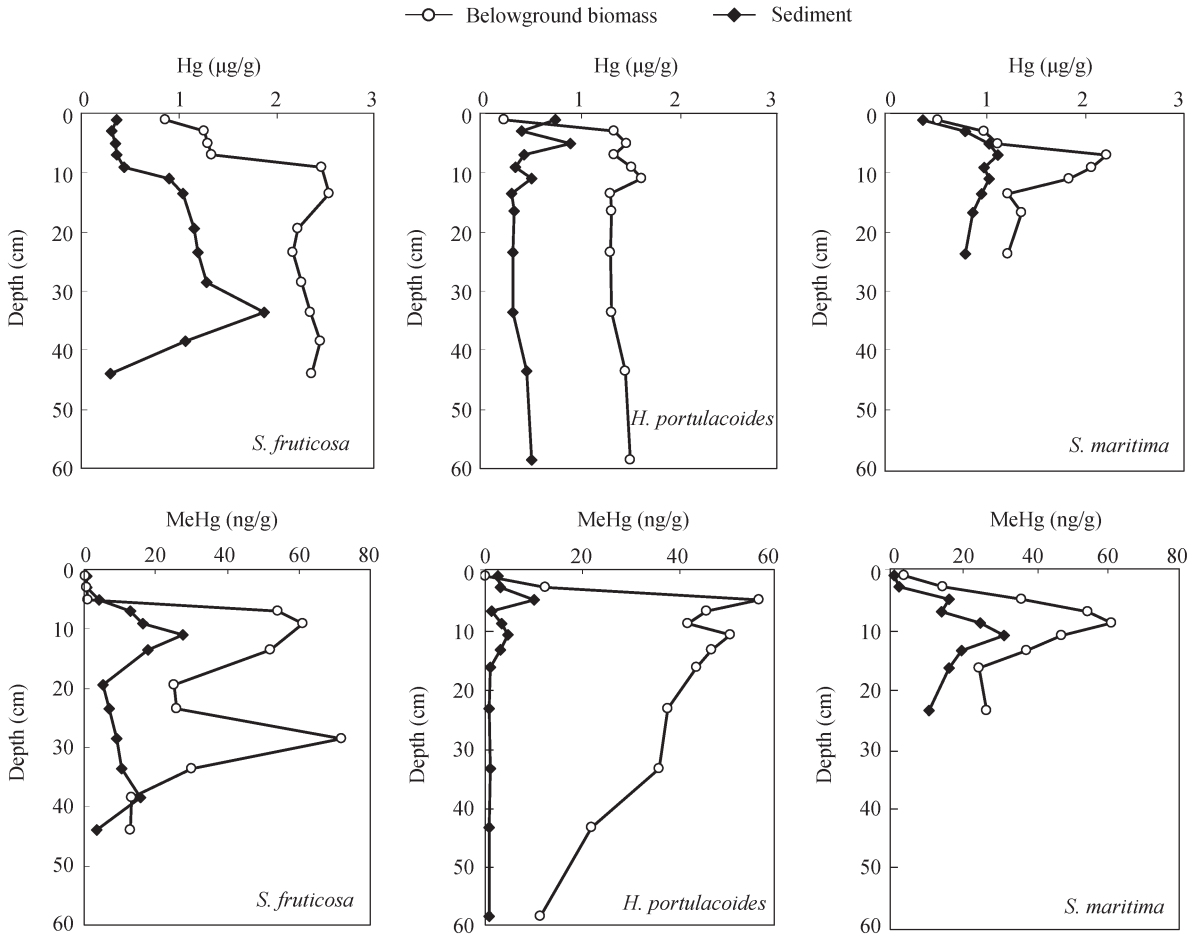

Fig. 4 Vertical profiles of Hg and MeHg concentrations in roots and corresponding sediment layers in the three studied plants.

MeHg varied with depth for all analyzed plants (Fig. 3). The depth variation indicates preferential layers of retention or transfer of Hg and MeHg from sediments to belowground tissues. This pattern was also observed in other marshes (e.g., Canário et al., 2007a) and may be related with the increase of Hg/MeHg phyto-availability or with root efficiency to remove mercury from sediments. The possibility of passive association of Hg with litter should also be taken into account since the analyzed belowground biomass includes live and dead material.

Table 1 presents the concentrations of Hg and MeHg in the above plant part, i.e., leaves and stems. For all analyzed plants both Hg and MeHg concentrations in aboveground parts are much lower than that determined in belowground plant parts. We estimated the partition factor (PF), calculated as the ratio between metal concentration on a weight basis in roots and in leaves or stems. In all analyzed plants the PF was always higher for the roots/stem ratio than for the roots/leaves one. For Hg the PF values varied between 23 for the leaves of *H. portulacoides* and 198 for the stems of *S. maritima*. For MeHg, the PF on *H. portulacoides* varied between 106 for leaves and 308 for stems. The observed partitioning of Hg and MeHg is in agreement with the findings of other researchers showing that roots are accumulators of mercury in various types of plants (Canário et al., 2007a; Válega et al., 2008). These results suggest that Hg mobility is mainly between sediments and roots and there is no evidence of substantial amounts translocated to the aboveground parts (Válega et al., 2008).

Table 1 Total Hg and MeHg concentrations in aboveground plant parts for the three studied plants

Plant	Tissue	Hg (μg/g)	MeHg (ng/g)	MeHg (%)
H. portulacoides	Stem	0.022 ± 0.005	0.11 ± 0.08	0.50 ± 0.06
	Leave	0.056 ± 0.011	0.32 ± 0.10	0.57 ± 0.10
S. fruticosa	Stem	0.018 ± 0.003	0.14 ± 0.06	0.77 ± 0.11
	Leave	0.021 ± 0.007	0.21 ± 0.04	0.99 ± 0.25
S. maritima	Stem	0.007 ± 0.004	< DL	–
	Leave	0.016 ± 0.007	0.12 ± 0.02	0.75 ± 0.18

Data are expressed as average ± SD (n = 4). DL: detection limit.

2.5 Stocks of mercury and methylmercury in the marsh

On the basis of the results obtained for belowground biomass, sediment density, water content, and mercury concentration in sediments and belowground tissues, we estimated the stocks of Hg and MeHg (g/m^2) in the 25-cm thick horizon that constitutes the average rooting zone for the three studied plants. Due to the much lower mercury concentrations and low biomass, the small contributions of aboveground stocks were not considered in this study. The stocks obtained for the three plants (without rooting sediment) were 8.7 g/m^2 of Hg and 0.55 g/m^2 of MeHg for *H. portulacoides*, 9.3 g/m^2 of Hg and 0.74 g/m^2 of MeHg for *S. fruticosa* and 2.2 g/ m^2 of Hg and 0.28 g/m^2 of MeHg for *S. maritima*. These elevated stocks were up to 10^2 (Hg)

and 10^4 (MeHg) times higher than the values reported by Canário et al. (2005) for the Tagus surface sediments (Hg 65×10^{-3} g/m^2; MeHg 59×10^{-6} – 81×10^{-6} g/m^2, considering a layer of 5-cm depth and the total area of the Tagus Estuary (320 km^2).

Moreover, considering the total area of this marsh (138,000 m^2) and that approximately 30% of this area are occupied by *H. portucaloides*, 45% by *S. fruticosa* and 20% by *S. maritma* (field observations), the total amount of Hg and MeHg stored can be estimated. The estimated values are 1358 kg of Hg and 75 kg of MeHg. These values indicate that 4.3% of the estimated amount of Hg is stored in only 0.05% of the subtidal area of the Tagus Estuary. This discrepancy is more pronounced for MeHg since the estimated values in this salt marsh were 3 times higher than the amount of MeHg in the subtidal area of the entire estuary (Canário et al., 2005).

Based on these results we may extrapolate the Hg and MeHg pool for the entire salt-marsh area of the Tagus Estuary (approximately 20 km^2). Assuming a similar plant distribution and similar Hg and MeHg concentrations in belowground biomass, the Hg and MeHg pool for the entire Tagus salt marshes can be as high as 197 tons of Hg and 10 tons of MeHg in a 25 cm depth horizon.

3 Conclusions

These results emphasize Hg methylation in salt marshes as a result of the complexity of their geochemistry and the inter-dependence of this process with several variables. In addition, our work emphasizes the importance considering stocks of Hg and MeHg in plant colonized areas for the budgets in aquatic ecosystems containing marshes.

Acknowledgments

The authors thank the financial supports providing by IPIMAR, Environment Canada and Reserva Natural do Estuário do Tejo. João Canário wish to thanks Fundação para a Ciência e Tecnologia for his PDF grant (SFRH/BPD/26324/2006).

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