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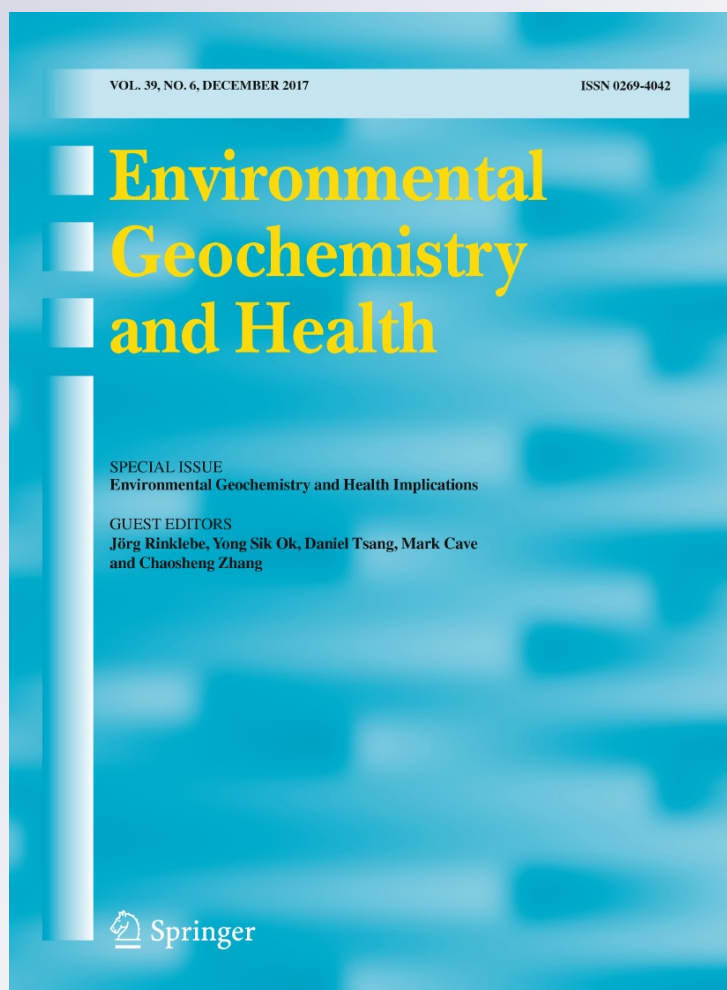
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Mercury uptake by halophytes in response to a long-term contamination in coastal wetland salt marshes (northern Adriatic Sea)

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Abstract Mercury (Hg) distribution in saltmarsh sediments and in three selected halophytes (*Limonium narbonense*, *Sarcocornia fruticosa* and *Atriplex portulacoides*) of a wetland system (Marano and Grado Lagoon, Italy) following a contamination gradient in sediments was investigated. The Hg uptake was evaluated at the root system level by calculating the enrichment factor (EF) and in the aboveground tissues by means of the translocation factor (TF). The related methylmercury (MeHg) concentrations in the halophytes were also investigated with regard to the location of the sites and their degree of contamination. Hg concentration in halophytes seemed poorly correlated both with the total Hg in rhizo-sediments and with the specific plant considered, supporting the

evidence that the chemico-physical parameters of sediments could significantly affect metal availability for plants. Hg concentrations in roots increased with depth and were 20-fold higher than content measured in related rhizo-sediments (high EF). A low content of Hg is translocated in aboveground tissues (very low TF values), thus highlighting a kind of avoidance strategy of these halophytes against Hg toxicity. MeHg values were comparable between the two sites and among species, but the translocation from below- to aboveground plant tissues was more active.

Keywords Mercury · Halophytes · Sediments · Bioaccumulation · Enrichment factor · Translocation factor

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Introduction

Salt marshes are among the most important ecosystems in wetland environments. Several are formally protected by the Ramsar Convention on Wetlands of International Importance (1971), the subsequent Convention on Biological Diversity and Intergovernmental Agreement in 1992, and all salt marshes are protected by the Water Framework Directive (WFD/2000/60 EU) under the transitional waters annex. One of the main aspects is their function as ecosystem services. As extensively reported in the literature, they ensure system functionality by exchanging marine and fresh waters, act as protection for internal coasts from erosion and storm events (maintenance of the sedimentary budget), are active in carbon sequestration and preserve halophile habitats which are of vital importance for the conservation of biodiversity (Bonometto 2005; Foster et al. 2013).

Along with their role as a “sedimentary trap”, salt marshes act as a sink for contaminants (Válega et al. 2008a) and as a secondary source considering both vegetation–atmosphere Hg^0 fluxes (Canário et al. 2017) and erosive processes (Bloom et al. 2004), resulting in high levels of exposure for both aquatic and terrestrial biota (Pereira et al. 2009).

Among trace metal(oid)s, mercury (Hg) is a global environmental concern, and its organic form methylmercury (MeHg) can negatively affect both human and wildlife health (Mergler et al. 2007). The Marano and Grado Lagoon belongs to the extended wetland system of the northern Adriatic Sea (Italy). In this area, Hg was supplied by two different sources. The first source was the historical mining activity (beginning in the sixteenth century), which took place in Idrija (Slovenia) until its definitive closure in 1996 (Horvat et al. 1999; Covelli et al. 2001). The mine residues, mainly as the insoluble form cinnabar (HgS), associated with sediment particles (Biester et al. 2000), are still transported through the Isonzo River into the Gulf of Trieste, and within the lagoon system during periods of high floods (Covelli et al. 2007). Here, a secondary source is due to the uncontrolled Hg discharge originated from a chlor-alkali plant (CAP), which operated from 1949 to 1984, and reached the lagoon through the Aussa-Corno river system. Until this time, the level and extension of Hg contamination in the lagoon have been extensively investigated. In the “MIRACLE” (Mercury Interdisciplinary

Research for Appropriate Clam farming in a Lagoon Environment) research project (Covelli 2012), the results reported the decreasing concentration gradient of Hg contamination in bottom sediments moving from east to west ($>11\text{--}0.7\text{ mg kg}^{-1}$), the significant enrichment in the central sector (up to 5 mg kg^{-1}), the presence of Hg exceeding the background value up to 1 m in deep core sediments. Moreover, a preliminary rounded-down gross estimate of total Hg “trapped” in the lagoon sediments amounted to 251 tons (Acquavita et al. 2012; Covelli et al. 2012).

Piani et al. (2005) demonstrated using a specific thermo-desorption technique, that Hg speciation in bottom sediments of the central lagoon sector is quite complex. The most insoluble cinnabar (HgS) form is not homogeneously distributed, and the non-cinnabar form, potentially involved in biogeochemical transformations, showed the highest level near the Aussa-Corno river mouth. Moreover, the distinction between HgS and non-HgS compounds by means of a one-step chemical extraction method confirmed the previous findings (Acquavita et al. 2012). The same authors stated that MeHg content is very low, comparable to other less impacted systems, and it is not strictly related to total Hg concentration in sediments.

Salt marshes can be colonised by several plant species, namely halophytes which are adapted to grow in these hypersaline environments, as reported in Flowers and Colmer (2008). At the same time, halophytes can uptake trace metal(oid)s in their root systems and translocate small amounts of these compounds to the aerial parts such as stems and leaves (Reboreda and Caçador 2007). Mercury contents in both sediment and plants of saltmarsh systems are well documented (e.g. Válega et al. 2008b; Canário et al. 2010; Anjum et al. 2012), such as changes in metal availability determined by physico-chemical properties of sediments (Castro et al. 2009) or seasonal fluctuations (Anjum et al. 2011), as well as behaviour along soil profiles (Canário et al. 2007).

In the Marano and Grado Lagoon, the degree of bioaccumulation was investigated at different levels of the food chain such as molluscs and fish (Brambati 1997, 2001; Giani et al. 2012). Recently, Petranich et al. (2016) explored the presence of anomalous contents of several trace metal(oid)s in saltmarsh sediments and halophytes (*Sarcocornia fruticosa* and *Limonium narbonense*) at two selected sites. The authors stated that root tissues accumulate significant

amounts of metal(oid)s, but the translocation to the aboveground tissues seems to be very limited. These data also suggested the importance of the vegetation coverage in phytoremediation of contaminated sites, bearing in mind that bioaccumulation is specific for each element and plant species, and it is site-specific (Windham et al. 2003; Weis and Weis 2004; Canário et al. 2007).

Regarding Hg bioaccumulation and phytoremediation, halophytes can tolerate high Hg levels and partition both Hg and MeHg in different organs to avoid toxic effects (e.g. Castro et al. 2009; Canário et al. 2010). Generally, the root system shows the highest degree of Hg uptake both on cell walls and into cell cytoplasm by means of cysteine-rich peptides (metallothioneins and phytochelatins) (Skinner et al. 2007) and by the activity of specific bacteria which are capable of enhancing Hg accumulation (De Sousa et al. 1999).

The aim of this work was to expand our knowledge on Hg and MeHg in salt marshes of the Marano and Grado Lagoon and to investigate the potential of bioaccumulation (translocation to aboveground tissues) in some selected halophytes (*L. narbonense*, *S. fruticosa* and *Atriplex portulacoides*) commonly present in this wetland system. The study was designed in order to detect any site-specific differences between the salt marshes, and to investigate differences in halophytes behaviour due to their possible importance in phytoremediation.

Materials and methods

Environmental setting

The Marano and Grado Lagoon, which is one of the best conserved wetlands of the Adriatic Sea (Italy), is located between the Tagliamento and Isonzo river deltas and belongs to the extended transitional system network along with the Pialassa Baiona, the Venice and Caorle lagoons. It covers about 160 km² with an average width of 5 km and a length of 32 km. The lagoon is an optimal nursery and recruitment zone for some fish species and provides shelter to many bird colonies. This system has been partially under the protection of the Ramsar Convention since 1971, and following the implementation of the Habitats Directive (92/43/EC) it was also designated for the whole

area as a Site of Community Importance (SCI IT3320037). Despite being under protection, the lagoon system hosts several economic, tourism (marine areas) and industrial services (mostly inland in the drainage basin), fishing, clam harvesting (mainly *Ruditapes philippinarum*) and fish-farming comprising the most important resources for local inhabitants (Sladonja et al. 2011).

On the basis of morphological and hydro-dynamic characteristics, the Marano and Grado basins are quite distinct from each other. The first has few areas above sea-level and is subjected to main freshwater inputs, especially from the Stella (36.1 m³ s⁻¹) and Cormor (10.7 m³ s⁻¹) rivers. Here, salinity values are very low (0.5–7) close to the river mouths and increasing towards the tidal inlets where it reaches up to 30, and the input of nutrients is significant (Acquavita et al. 2015). On the contrary, the Grado basin shows less hydrodynamism, a remarkable marine feature, and more complex morphologies such as salt marshes and tidal flats.

To date, no accurate estimate is available regarding sediment loads (Fontolan et al. 2012): it is commonly recognised that particulate matter inputs from small rivers are of secondary importance, being the Tagliamento and Isonzo river deltas (silty and clayey particles, respectively) the primary source of sediments delivered to the lagoon. In addition, the erosion of the barrier islands also contributes to sand materials. Historical transformations of the salt marshes were analysed using aerial photographs (dates considered 1954, 1990, 2006), and with the support of historical maps and topographic surveys. The present extent and distribution of the salt marshes inside the lagoon (760 ha) show a total reduction of 16% (about 144 ha) if compared to 1954. Direct human actions played a significant role in the budget, since total loss due to land reclamation and dredging during this period amounted to 126 ha (Fontolan et al. 2012).

The selection of the sampling sites was done by integrating the information regarding the distribution and characteristics of the salt marshes with the presence of the three target species (Poldini et al. 2006; Fontolan et al. 2012). According to the Hg concentration gradient, five salt marshes were selected (Fig. 1). G1B is located in the Primero basin (easternmost part of the lagoon) and has an area of about 0.95 ha. Among the selected sites, it is the most impacted by Hg contamination with more than

13 mg kg⁻¹ in bottom sediments. Further west, G2 salt marsh (5.8 ha) is close to a lagoon channel called *Belvedere*: its origin is both natural and anthropogenic, since part of the sedimentary material was disposed following dredge operations conducted in the channel. G3 is situated in a complex morphological area with the presence of decommissioned fish farms of the Buso basin (*Isole della Gran Chiusa*). Here, the saltmarsh complex has an area of 8.7 ha and is periodically subjected to high stress due to the adjacent navigation channel. Finally, two salt marshes were chosen in the Marano sector. The first one, M1 (0.30 ha), close to the village of Marano Lagunare, is characterised by low Hg levels in bottom sediments and displays a very homogeneous morphology. On the contrary, M2 is a back barrier with an irregular morphology and is crossed by an intricate network of secondary tidal channels.

Selected plant species

Three halophytes widely spread along the whole lagoon system were selected for this study.

Limonium narbonense Mill. (syn. *L. vulgare* Mill., *L. serotinum* (Rchb.) Pignatti, *Statice brunii* Migl.-Gar., *S. limonium* Auct.) belongs to a taxonomically

complex group of plants (*Limonium* Mill. genus, *Plumbaginaceae* Juss. family, *Caryophyllales* Juss. ex Bercht. & J.Presl order) usually found in Atlantic Europe and in the Mediterranean region (Cortinhas et al. 2015). The plant is perennial herbaceous and reaches a height of about 30–70 cm and exhibits lanceolate–spatulate leaves located in a basal rosette. The flowers are white to pale violet and its flowering period extends from June to September. This species inhabits the lowest part of the salt marshes, especially in clayey soils flooded daily with seawater, and shows a high secretion rate of both Na⁺ and Cl⁻ (Rozema et al. 1981).

Sarcocornia fruticosa (L.) A.J. Scott (syn. *Arthrocnemum fruticosum* (L.) Moq., *Salicornia europaea* var. *fruticosa* L., *S. fruticosa* (L.) L.) is a succulent and perennial chamaephyte, belonging to the genus *Sarcocornia* A.J. Scott (*Chenopodiaceae* Vent. family, *Caryophyllales* Juss. ex Bercht. & J.Presl order) which includes species with similar morphological traits. *S. fruticosa* occupies coastal salty soils, salt marshes and regularly flooded brackish marshes. It tolerates high levels of salinity and blooms from July until early September.

Atriplex portulacoides L. (syn. *Halimione portulacoides* (L.) Aellen, *Obione cupani* (Tineo) Nicotra, O.

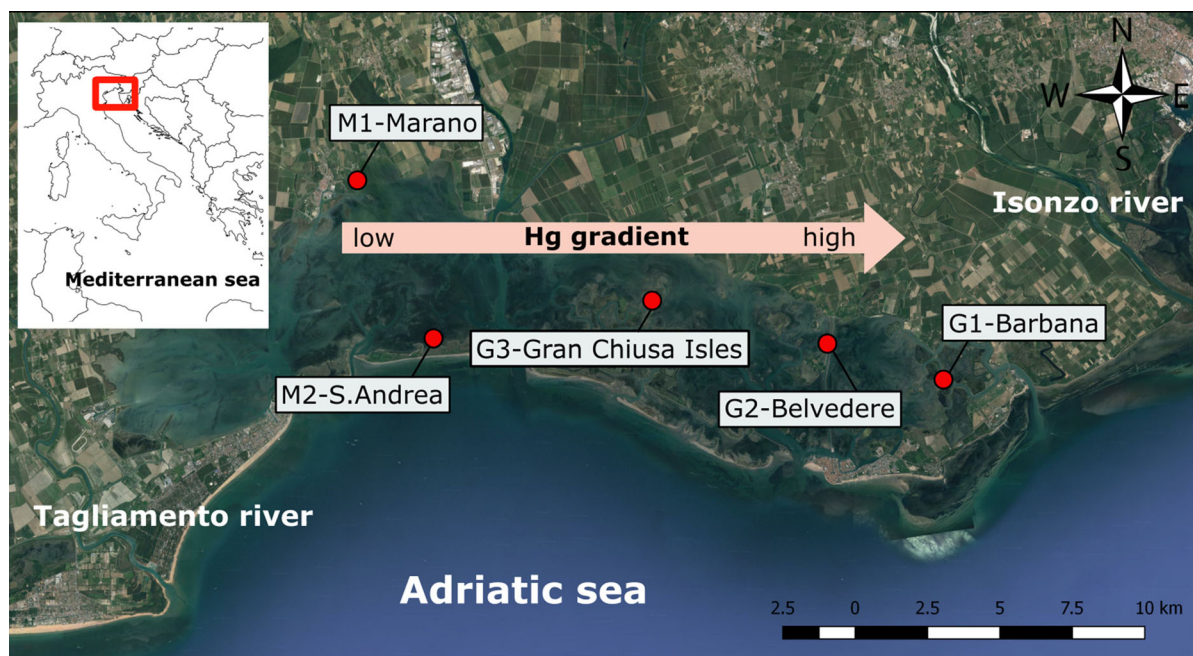


Fig. 1 Index map of the study area showing the locations of the salt marshes investigated in the Marano and Grado Lagoon

portulacoides (L.) Moq.) belongs to the genus *Atriplex* L. (*Chenopodiaceae* Vent. family, *Caryophyllales* Juss. ex Bercht. & J.Presl order) and it is a widespread perennial halophyte common in the salt marshes of Europe, North Africa and Southwest Asia. This species is susceptible to water stagnation, thus preferring elevated saltmarsh areas, and must tolerate higher salinity values during periods of soil desiccation (Cazzin et al. 2009). It flowers in June–July. Recent data suggest that this plant is a source of valuable phytochemicals, including phenolic compounds (Rodrigues et al. 2014; Vilela et al. 2014). High productivity and capacity to grow in soils contaminated with heavy metals (Sousa et al. 2008) also make this species potentially useful for saline soil reclamation and phytoremediation purposes and even as fodder plants.

Sarcocornia fruticosa and *Atriplex portulacoides* are often employed in bioaccumulation and phytoremediation as reported in the scientific literature (Válega et al. 2008a; Castro et al. 2009; Canário et al. 2010). Photographs of these three species are available in Figure S1 (Supplementary material).

Sampling and laboratory analysis

At each site, three individuals of the selected plants were collected together with the related rhizo-sediment. The first step was the collection of the aboveground biomass (stems and leaves), whereas the belowground biomass (roots) and the attached rhizo-sediment were subsequently sampled using a single gouge auger sampler (i.d. = 5.5 cm, l = 50 cm, cod. 01.11.SE, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). The recovered sediment cores (including roots) were sectioned in the field to obtain three subsamples corresponding to 0–5, 5–15 and 15–25 cm depth levels, respectively. At the same time, Eh, pH, dissolved oxygen and conductivity were measured in the field (pH25, Crison Instrument, Barcelona, Spain), in both rhizo-sediment and the saltwater marsh. Finally, the samples were sealed in plastic bags, transported to the laboratory and stored at -4 °C until subsequent operations.

In the laboratory, the aerial parts of plants were divided into leaves and stems, while the roots were carefully separated from the rhizo-sediment. All samples were rinsed with abundant distilled water and cleaned in an ultrasonic bath (Sigma-Aldrich®),

and rinsed again with Milli-Q, prior to freeze-drying (CoolSafe 55–4, ScanVac, Labogene, Lyngø, Denmark), finally ground and homogenised.

For grain-size analysis, an aliquot (15–20 g) of fresh rhizo-sediment was preliminarily treated with hydrogen peroxide (H_2O_2 10 v/v) to remove organic matter (t = 48 h) and subsequently wet-sieved through a 2-mm sieve. Grain size was determined on 2-ml aliquots using a Mastersizer 2000 laser granulometer (Malvern Instruments Ltd., Worcestershire, UK).

Total carbon (C_{tot}) and nitrogen (N_{tot}) contents were determined in freeze-dried (CoolSafe 55–4, ScanVac, Labogene, Lyngø, Denmark) and homogenised samples at a combustion temperature of 1020 °C (ECS 4010 Elemental Combustion System, Costech Analytical Technologies Inc., Valencia, CA, USA). Organic carbon (C_{org}) was determined through the same equipment at a combustion temperature of 920 °C (Hedges and Stern 1984), prior to progressive acidification with 0.1–1.0 M HCl. Acetanilide was used as a standard compound for calibration. The detection limit for C_{org} and C_{tot} was 0.1%. The precision for C_{tot} , C_{org} and N_{tot} was 3%.

Mercury contents in both rhizo-sediment and plant tissues were determined using a Direct Mercury Analyser (DMA-80, Milestone, Bergamo, Italy) on approximately 30 mg of material according to US-EPA Method 7473. Quality control was tested using certified reference materials (PACS-2 harbour sediment, NCR-CNRC, Canada, certified values $3.040 \pm 0.2 \mu\text{g g}^{-1}$). Results obtained were in good agreement with the certified value, with recovery being between 98.2 and 100.3% (n = 14).

For MeHg determination in vegetation, only two sampling stations were considered (G1, the easternmost one and M1, the westernmost one). A modified method was used based on Canário et al. (2006). Dried tissues were digested with a concentrated HBr (Merck suprapur) solution saturated with CuSO_4 . Methyl mercury 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 (7890 Model—USA) coupled with a pyrolysis unit and a PSA Hg fluorescence detector (PSA Analytical—UK). Analysis recoveries and the possible MeHg artefact 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 artefact MeHg formation was observed. For all the analysis, precision expressed as the relative standard deviation of 3 replicate samples was $<2.5\%$ ($p < 0.05$). 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. Obtained CRM concentrations were consistent with the certified values and precision of three (for each CRM) replicates were $<3\%$.

Data analysis

The ANOVA test was applied after verifying the assumption of the normality of data distribution (Shapiro–Wilks test > 0.05) and equality of variances (Bartlett's test > 0.05), by normalising data when necessary, for the characterisation of rhizo-sediment parameters (Eh, grain size, N_{tot} , C_{tot} and C_{org}). The nonparametric Kruskal–Wallis' test was applied after verifying the non-normal distribution (Shapiro–Wilks test < 0.05) and the unequal variances (Bartlett's test < 0.05) of Hg datasets in order to compare sites, species and depth levels. Pearson's correlation index (r) was applied to highlight possible interactions between Hg uptake and soil features (e.g. Eh, C_{org} , N_{tot}) as well as between Hg and MeHg content in plant tissues. All the analyses were computed using R software (R Core Team 2013).

Bioaccumulation factors

To evaluate the bioaccumulation/phytoremediation potential in the plant species examined, two factors were calculated: (1) enrichment factor (EF = metal concentration in roots/metal concentration in rhizo-sediments) and (2) translocation factor (TF = metal concentration in leaves or stems/metal concentration in roots) (Yoon et al. 2006; Anjum et al. 2011). In case of $EF > 1$, there is an active bioaccumulation; otherwise, for $EF < 1$, no bioaccumulation can be stated. Similarly, $TF > 1$ indicates that the element of interest has been translocated in the aerial part (stems and leaves) of the plant species.

Results

Physical and chemical parameters and estimation of the plant biomass

Several authors emphasise the importance of Eh and pH in Hg bioaccumulation by plants, suggesting that in rhizo-sediments, an optimal range ($-150 \text{ mV} < \text{Eh} < 200 \text{ mV}$) could enhance metal(oid) mobility (Jackson 1998; Caetano et al. 2008; Caçador et al. 2009; Marques et al. 2011).

In this work, the Eh values measured *in situ* in saltwater marshes ranged approximately -100 and $+100 \text{ mV}$, with the only exception of G3, where values up to -370 mV (strongly reducing condition) were found in correspondence with *S. fruticososa* and *L. narbonense* vegetated areas, and to a lesser extent at M2. On the contrary, when the sampling of *A. portulacoides* occurred the Eh values were always positive. Similar behaviour was recorded in rhizo-sediments, where values in G3 and M2 were negative and conversely, positive in the other salt marshes ($+25$ to $+133 \text{ mV}$). Plant roots are able to acidify the surrounding media, through pumping oxygen/exudation of organic compound (Válega et al. 2008b and references therein), and the Eh values tend to be more positive than in non-vegetated sediment. This evidence suggests that the presence of plants influences the rhizo-sediment properties; as a result of the input of root exudates, the rhizo-sediment had different chemical, physical and microbial properties from the non-rhizo-sediment. The predominant properties of the rhizo-sediment were high in organic content matter, stable physical structure and high microbial activity. Generally, the Eh values clearly decreased moving downcore.

The pH value in water was comparable (from neutral to slightly acid classification) among all salt marshes considered (from 6.23 to 7.53) and did not exhibit significant differences among plants.

Dissolved oxygen (O_2) ranged from 1.0 to 5.4 mg l^{-1} . Generally, the system was well oxygenated probably as a result of the release from root activity which, in turn, favours the Hg microbial transformation as suggested by Válega et al. (2008b). The G2 site, however, showed almost hypoxic/anoxic conditions ($O_2 < 2.0 \text{ mg l}^{-1}$). As already stated for pH, the conductivity was comparable among all sites and in the specifically vegetated area.

Among the species considered, *L. narbonense* exhibited the lowest epigeal biomass (stems and leaves), whereas, as reported in Canário et al. (2010), *S. fruticosa* can be distinguished by its huge biomass (on average 25.6 and 16.1 g in leaves and stems, respectively). In all the species, the root biomass was prevalent in the first level considered (0–5 cm) and to a lesser extent at 5–15 cm, whereas moving downcore (15–25 cm) the values obtained were onefold lower. In rhizo-sediments, *L. narbonense* showed the highest biomass (on average 15.5 g).

Sediment characteristics

According to Shepard's classification (Shepard 1954), in almost all rhizo-sediments (86% of the samples) the sandy-silt component represented the predominant fraction. Silt was the predominant fraction in all salt marshes ranging from 59 ± 23.6 and $73.4 \pm 3.5\%$ at G3 (0–5 cm) and M1 (5–15 cm), respectively (Table 1). The clay fraction, which was subordinate, accounted for about 10% in all samples, whereas the sand component showed the highest variability (20.8 ± 5.6 to 42.2 ± 26.1). These data are comparable with those reported in Petranich et al. (2016). No clear pattern was observed moving downcore, such as no significant differences among the plant species considered.

The N_{tot} contents in rhizo-sediment ranged from 0.01 to 0.98% and showed on average the highest values at 0–5 cm level. The same trends were also found for C_{tot} (3.28–12.45%) and C_{org} (0.12–11.2%). Moreover, M1 and M2 salt marshes (Marano basin) were found to be the most enriched in these elements. These data are quite high if compared to results found in bottom sediments of the intertidal and subtidal areas in the same lagoon (Acquavita et al. 2012; Covelli et al. 2012), which reported a value of $1.23 \pm 0.52\%$ for C_{org} , and also showed the opposite trend moving downcore. One explanation is that exudates from roots and their decomposition enhance the presence of this abundant organic matter (OM) in a saltmarsh environment and in our case, at the core top (Drake et al. 2015 and references therein).

The application of $C_{\text{org}}/N_{\text{tot}}$ molar ratio is widely employed to discriminate the OM source especially in coastal environments where terrestrial and marine contributions are mixed. Goñi et al. (2003), stated that the OM of marine origin displays a ratio between 4 and

10, whereas vascular terrestrial plants produce ratios >20 . In bottom sediments of the Marano and Grado Lagoon, an average value of 8.6 ± 1.5 was found, which is typical of the presence of algae residues, zooplankton, phytoplankton and bacteria (Acquavita et al. 2012). Recently, Petranich et al. (2016) reported values slightly higher in saltmarsh environments (about 12). In this work, the $C_{\text{org}}/N_{\text{tot}}$ ratio calculated was 10.1 ± 0.70 , consistent with the coexistence of both autochthonous and allochthonous materials. However, the intense microbial activity on OM occurring in the rhizo-sphere can affect the original content (especially considering nitrogen), thus explaining the evidence that the parameter decreased with depth.

Total mercury in rhizo-sediments

As already stated, bottom sediments of Marano and Grado Lagoon (northern Adriatic Sea) are strongly enriched in Hg if compared to the natural background value of the area ($0.13 \pm 0.04 \mu\text{g g}^{-1}$) (Covelli et al. 2012). Total Hg in rhizo-sediment showed a high degree of variability ranging from 0.01 to $27 \mu\text{g g}^{-1}$. According to the gradient previously reported for bottom sediments (Piani and Covelli 2001; Piani et al. 2005; Acquavita et al. 2012), the G1 salt marsh was the most contaminated site with an average Hg concentration of $10.6 \pm 5.5 \mu\text{g g}^{-1}$ (Table 1), whereas the least impacted site was M1 ($1.19 \pm 0.52 \mu\text{g g}^{-1}$). The other salt marshes exhibited intermediate values with 3.22 ± 2.73 , 4.76 ± 2.84 and $5.21 \pm 2.78 \mu\text{g g}^{-1}$ in G3, M2 and G2, respectively. These values are higher than those reported for the non-vegetated sediment (referred to Acquavita et al. 2012; Covelli et al. 2012), probably as a consequence of roots which are capable of Hg uptake and the subsequent release in the buried litter and in addition to the presence of abundant OM complexing Hg. Moreover, the redox state (i.e. Eh positive and good oxygenation, see above) of rhizo-sediments favours the scavenging of Hg by precipitation with iron oxides (Gagnon et al. 1997) as already reported by Válega et al. (2008b). Differences in Hg contents are statistically significant ($p < 0.0001$), with the exception of G2 and M2 ($p = 0.3$).

A clear increase in Hg contamination was observed moving downcore, especially at G1, whereas at M1 no significant trend was observed. Kruskal–Wallis' test returned significant differences between all the levels

Table 1 Sediment characteristics at each site and depth level

Depth level (cm)	Site	Sand	Silt	Clay	C _{tot}	N	C _{org}
0–5	G1	20.8 ± 5.6	67.5 ± 2.1	8.8 ± 3.5	7.5 ± 0.4	0.29 ± 0.06	3.1 ± 0.7
	G2	26.5 ± 2.7	73.4 ± 3.5	11.1 ± 1.2	7.3 ± 0.7	0.36 ± 0.08	3.8 ± 0.9
	G3	23.3 ± 5.2	59.5 ± 23.6	8.1 ± 3.6	7.3 ± 1.4	0.53 ± 0.14	4.9 ± 1.5
	M1	30.7 ± 3.6	71.3 ± 4.8	7.1 ± 1.6	10.2 ± 1.4	0.75 ± 0.11	7.7 ± 3.3
	M2	33.3 ± 1.2	66.3 ± 2.8	5.8 ± 2	9 ± 1.1	0.74 ± 0.11	7.4 ± 0.9
5–15	G1	21.1 ± 2.9	65.1 ± 2.9	8.9 ± 2.8	6.9 ± 0.4	0.19 ± 0.03	2 ± 0.4
	G2	24.1 ± 4.6	70.5 ± 2.7	9.5 ± 1.7	6.3 ± 0.5	0.17 ± 0.04	1.7 ± 0.4
	G3	26.6 ± 8.6	67.6 ± 9.2	8.7 ± 2.4	6.5 ± 1.2	0.42 ± 0.11	4.1 ± 1.3
	M1	29.9 ± 2.2	74.5 ± 3.1	8.2 ± 1.2	6.8 ± 0.4	0.45 ± 0.04	4.1 ± 1.9
	M2	32.5 ± 3.9	63 ± 12	5.7 ± 2	7 ± 1.3	0.28 ± 0.1	3.2 ± 0.9
15–25	G1	21.5 ± 3.0	68 ± 3.1	8.4 ± 3.3	6.6 ± 0.3	0.13 ± 0.02	1.2 ± 0.3
	G2	42.2 ± 26.1	73.9 ± 2.8	9.5 ± 1.7	7.6 ± 1.7	0.09 ± 0.07	0.9 ± 0.6
	G3	24.7 ± 4.9	65 ± 11.7	8.9 ± 1.6	5.4 ± 0.7	0.32 ± 0.08	3.1 ± 0.9
	M1	22.3 ± 3.1	68.2 ± 4.4	6.3 ± 1.8	3.9 ± 0.4	0.22 ± 0.02	2.2 ± 0.5
	M2	39.4 ± 12.3	62.2 ± 8	4.4 ± 1.4	7.1 ± 1.6	0.15 ± 0.11	1.7 ± 1.2

All variables are expressed in %

considered only in G1 ($p < 0.02$) and in G3 ($p < 0.01$), and between the first and the third levels in G2 ($p = 0.05$). Considering the whole dataset, no clear relationship was found between total Hg content and grain size such as with C_{org}.

Total mercury and methylmercury in halophytes

Total Hg in stems and leaves were two orders of magnitude lower than in rhizo-sediments, ranging from $< \text{LOD}$ ($0.01 \mu\text{g g}^{-1}$) up to $0.14 \mu\text{g g}^{-1}$. On the contrary, roots showed high Hg values (maximum $121 \mu\text{g g}^{-1}$) and a clear exponential increase moving downcore (Fig. 2), being that the values obtained at the 0–5 cm level were almost negligible. In more detail, Hg at 0–5 cm level slightly varied considering both species and sites; in fact, it ranged from a minimum of $0.20 \pm 0.03 \mu\text{g g}^{-1}$ in *L. narbonense* to a maximum of $0.70 \pm 0.39 \mu\text{g g}^{-1}$ in *S. fruticosus* at site M2, whereas differences between salt marshes at the 5–15 level became more evident.

At the deepest level (15–25 cm), higher concentrations in the roots exceeded up to 20 times the Hg content recorded in the related rhizo-sediments, and the variability of Hg content also increased. It ranged from values $< \text{LOD}$ in *S. fruticosus* (M2) to

$54.6 \pm 49.0 \mu\text{g g}^{-1}$ in *A. portulacoides* (G2), also showing a higher variability among plant replicates.

Statistical analysis confirmed the significant difference among the three levels at G1 ($p < 0.02$) and G2 ($p < 0.03$) and between the first and the third level at M2 ($p = 0.03$), thus highlighting the possible existence of “preferential” layers of retention or transfer of Hg as already reported in previous studies (Canário et al. 2007). Moreover, a high correlation was found between Hg concentrations in rhizo-sediments and belowground tissues ($r = 0.68$), thus suggesting the influence of the total Hg content in sediments and its phytoavailability on Hg uptake by plants, despite the difference noticed among salt marsh sites. Among the three species, *A. portulacoides* seemed to accumulate less in the hypogean part (66% of total Hg) (Table 2), but in general the uptake seems to be linked to site-specific conditions rather than to a particular plant species (among species, $p = 0.4$).

MeHg content in roots varied from 2 up to 192 ng g^{-1} at M1 and from 0 to 499 ng g^{-1} at G1. The maximum concentrations are in the same order of magnitude of the highest belowground biomass levels measured by Canário et al. (2007) in industrially contaminated Portuguese estuaries (up to 941 ng g^{-1} , Table 4). The percentage of MeHg in the roots with

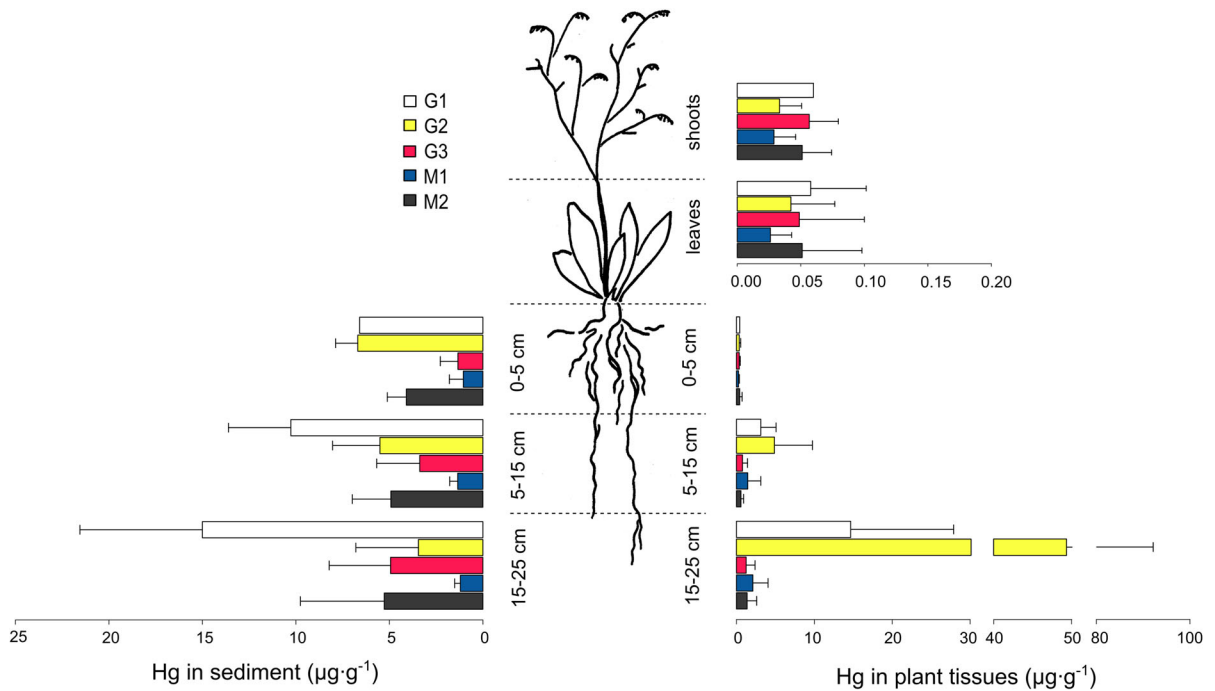


Fig. 2 Mercury concentration ($\mu\text{g g}^{-1}$) in sediments and roots in the three depth levels analysed (0–5, 5–15 and 15–25 cm depth) and in the aerial parts (leaves and stems) of the plant at

the five salt marshes. The average values \pm SD (standard deviation) for the three species of halophytes are reported as *bar charts*

respect to the total Hg content was similar among species considered and in the two investigated salt marshes accounted on average for $5.15 \pm 2.12\%$ (sites M1 and G1 were not discerned). Nevertheless, considering the total amount of MeHg at different depths, a significant increase was noticed downcore (in particular at site G1; Fig. 3), similar to what was observed for Hg in roots. A positive correlation was found between MeHg and Hg, both in rhizo-sediments and roots ($r = 0.70$ and 0.98 , respectively). The highly significant correlation found between Hg and MeHg in plant tissues, especially in roots ($r = 0.98$), confirmed that the MeHg content is a function of the Hg total content in plants, as already observed by Canário et al. (2007). Furthermore, unlike Hg, MeHg

(%) in roots seemed more equally distributed among the three depth levels and among species (Table 3), ranging from 1.70 up to 10.8% in *S. fruticososa* (one sample at site G1).

As expected, the aboveground biomass showed values (0.09 – 0.16 and 0.08 – 0.60 ng g^{-1} at M1 and G1, respectively), about two orders of magnitude lower than the corresponding content in the roots. These concentrations are in accordance with the results reported in other contaminated salt marshes such as in California (Best et al. 2008) and in Portugal (Canário et al. 2007, 2010). On average, MeHg accounts for $0.34 \pm 0.15\%$ and $0.53 \pm 0.29\%$ of the total Hg measured in plant samples at M1 and G1, respectively. These values are comparable to those

Table 2 Percentage of Hg in leaves, stems and roots in the three species analysed

Species	Hg in leaves (%)	Hg in stems (%)	Hg in roots (%)
<i>Atriplex portulacoides</i>	15.3	18.4	66.3
<i>Limonium narbonense</i>	4.0	0.5	95.5
<i>Sarcocornia fruticososa</i>	8.4	10.7	80.9

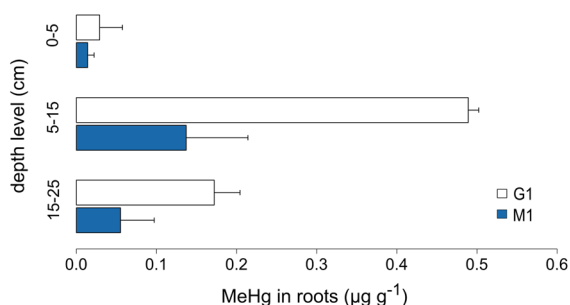


Fig. 3 Bar charts of methylmercury (MeHg) concentrations ($\mu\text{g g}^{-1}$) in the roots expressed as the average values \pm SD (standard deviation) for the three species of halophytes in the two salt marshes analysed, the least contaminated (M1) and the most contaminated (G1) sites

Table 3 Percentage of MeHg in roots in the three species analysed among the different depth levels

Species	0–5 cm (%)	5–15 cm (%)	15–25 cm (%)
<i>Atriplex portulacoides</i>	4.79 ± 1.82	4.49 ± 1.57	n.a.
<i>Limonium narbonense</i>	4.80 ± 1.75	4.81 ± 2.17	6.24 ± 1.49
<i>Sarcocornia fruticosa</i>	4.33 ± 3.99	3.33 ± 1.94	4.83^a

^a Means one sample available

reported by Válega et al. (2008d) for *A. portulacoides* and *S. fruticosa* in the inner part of the Aveiro Lagoon, in Portugal, highly contaminated from a chlor-alkali plant, whereas MeHg reached up to 17 and 14% of the total Hg in *A. portulacoides* stems and *S. fruticosa* shoots, respectively.

Mercury uptake and translocation in plant tissues

Enrichment factor (EF) showed a great variability ranging from <1 up to 81. As depicted in Fig. 4, the enrichment occurred prevalently in the deepest layer (15–25 cm, average EF = 5.2), whereas no significant difference was observed by the application of statistical tests for both plants and salt marshes ($p < 0.0001$). The only exception was represented by site G2.

A prevalent EF > 1 was recorded also for site M1, whereas only a few samples showed metal enrichments in the deepest level such as at both sites M2 (*L. narbonense*, two samples) and G1 (*L. narbonense* and

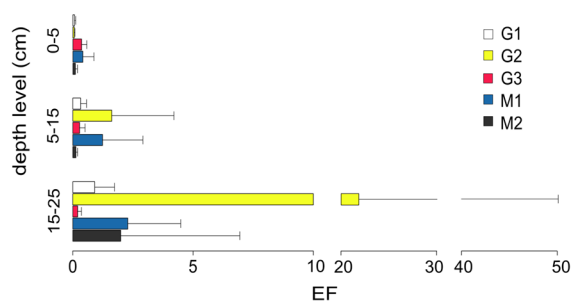


Fig. 4 Mercury enrichment factor (EF), calculated as the ratio between the concentration of the metal in the roots and in the corresponding rhizo-sediment, for each of the three subsampled layers (0–5, 5–15 and 15–25 cm depth) in the three species of halophytes. Values are reported as average \pm SD (standard deviation)

S. fruticosa, two samples). On the contrary, no bioaccumulation (EF < 1) was recorded for site G3 (Table S1).

Comparing species, despite the absence of statistical significance ($p > 0.05$), *L. narbonense* showed the highest EF values (Table S1), probably due to its deeper and wider development of the root system than *A. portulacoides* and *S. fruticosa* (Vittori Antisari et al. 2016).

The TF varied from very low values of 0.0005 in *L. narbonense* stems (in M2) up to 0.18 in leaves (in G2) and 0.37 in stems (in G1) of *A. portulacoides*, thus highlighting that there was no evidence of Hg translocation. Any statistical difference was recorded among species or salt marshes ($p > 0.05$). *A. portulacoides* showed in general the highest variability, also reaching the highest TF values (Fig. 5).

Discussion

In this work, we attempted to extend our knowledge about Hg contamination in salt marshes sites capable of retaining a high degree of contaminants and to understand whether bioaccumulation/phytoremediation processes are active through selected plant species. These plants are indeed able to tolerate high Hg levels and partition both Hg and MeHg in different organs to avoid toxic effects (e.g. Castro et al. 2009; Canário et al. 2010).

The extent and dynamics of Hg contamination, previously described as a net positive gradient moving eastwards (Brambati 1997; Acquavita et al. 2012),

were also confirmed in rhizo-sediments of the five selected salt marshes, as evidenced by the lowest level of Hg at the M1 site and the highest one at the G1 site. This site is in a confined area of the *Primero* lagoon sub-basin directly affected by Hg inputs through the Isonzo River (Covelli et al. 2007). Moreover, Hg levels were comparable to those reported in the literature for the contaminated Portuguese estuarine sediments (Table 4) which incorporate large quantities of Hg discharged mainly by industries and urban areas but also deriving from mining and the extraction of metals since the Roman Age (Canário et al. 2007).

In salt marshes, it has been shown that the concentration of metals in rhizo-sediment is greatly influenced by many site-specific factors, i.e. pH, redox status of sediments, organic matter (Burke et al. 2000; Windham et al. 2003), and by the presence of vegetation (Weis et al. 2002; Reboreda and Caçador 2007). In the Marano and Grado Lagoon salt marshes, these effects seem to enhance the level of both Hg and MeHg in rhizo-sediments, compared to non-vegetated sediments, by the formation of an ideal environment in terms of physico-chemical parameters such Eh, pH and OM contents overall, which can form complexes with Hg and MeHg (Frohne et al. 2012; Frohne and Rinklebe 2013). It is well known that specific redox conditions enhance the bacterial activity that favours Hg methylation (Ulrich et al. 2001) despite some

authors recording a lower influence of the redox changes on Hg mobility in anoxic sediments (Frohne et al. 2012). Similarly, the pH effect on metals availability seems controversial: some authors supported the direct effect on metal solubility and toxicity for biota (Devai et al. 2005), whereas others suggested dealing with the many other variables interacting with each other (Frohne et al. 2012). In this work, MeHg in rhizo-sediment was not determined, but its accumulation in the roots (Table 4) suggests that the salt marshes of Marano and Grado act as active sites of methylation activity, although to a lesser extent than other systems. The preferential accumulation of Hg in rhizo-sphere could also be due to the root system network, which in some species creates a radial oxygen barrier that inhibits the entry of potentially toxic substances (St-Cyr and Campbell 1996). Although the specific plant species could be more or less prone to passive Hg uptake and differently affect the metal concentration in soils (Reboreda and Caçador 2007; Válega et al. 2008b; Anjum et al. 2011, Frohne and Rinklebe 2013), no statistical difference was noted among the three halophytes considered in this study. Similar results were also reported by Canário et al. (2007) for the salt marshes of the Tagus, Sado and Guadiana estuaries in Portugal. The main factors affecting Hg uptake in plant tissues are not easily determined, being Hg bioavailability the

Fig. 5 Average \pm SD (standard deviation) values of the translocation factor (TF = Hg concentration in leaves or stems/Hg concentration in roots) for each of the three species of halophytes investigated in each sampling site

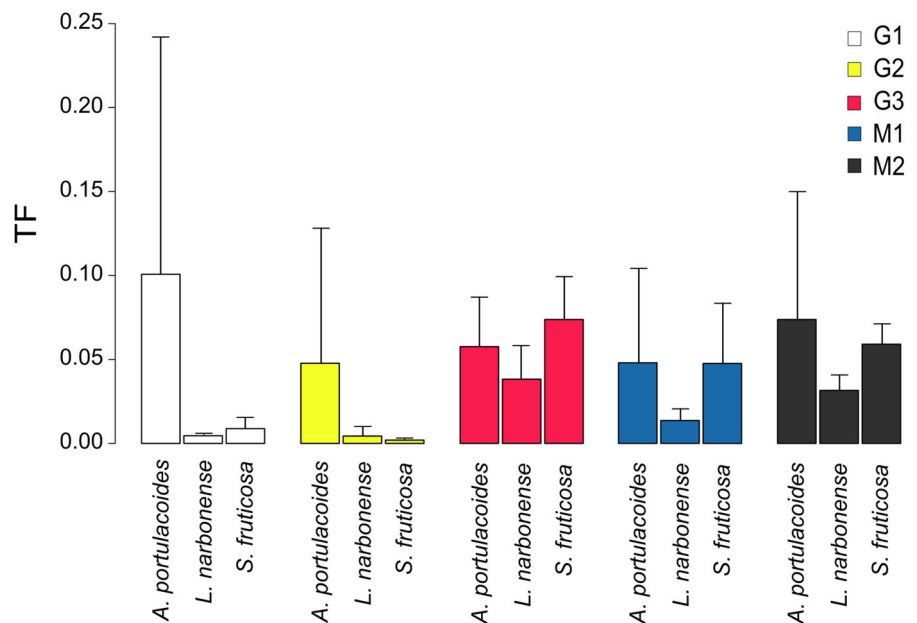


Table 4 Comparison of Hg and MeHg data available in the literature, regarding sediment concentration, metal partitioning in plant tissues, enrichment factor (ratio roots/sediment Hg content) and translocation factor (ratio shoots/roots Hg content) in the worldwide salt marshes

References	Halophytes investigated	Hg sediment ($\mu\text{g g}^{-1}$)	Hg roots ($\mu\text{g g}^{-1}$)	Hg shoots ($\mu\text{g g}^{-1}$)	EF	TF	MeHg roots (ng g^{-1})	MeHg shoots (ng g^{-1})
Present study	<i>A. portulacoides</i> <i>L. narbonense</i> <i>S. fruticosa</i>	0.01–27	0.2–9.8 ^a	<0.01–0.14	up to 1.9 ^b	up to 0.37	0–499	0.08–0.60
Canário et al. (2017) Portuguese estuaries	<i>A. portulacoides</i> <i>S. fruticosa</i> <i>Spartina maritima</i>	0.41–0.48	0.8–1.2	0.005–0.07	–	–	–	–
Marques et al. (2011) Rio de Aveira, Portugal	<i>Juncus maritimus</i> <i>Scirpus maritimus</i>	14–43 ^c	0.9–1.33 ^c	–	–	–	–	–
Anjum et al. (2011) Rio de Aveira, Portugal	<i>A. portulacoides</i> <i>J. maritimus</i>	0.6–104	0.03–23	0.016–0.97	0.02–0.5	0–120	–	–
O'Driscoll et al. (2011) Nova Scotia, Canada	<i>Spartina alterniflora</i>	0.001–0.050	0.007–0.223	0.014–0.045	1.5 ^c	0.2 ^c	0–0.9	LOD-0.003
Canário et al. (2010) Tagus estuary	<i>A. portulacoides</i> <i>S. fruticosa</i> <i>S. maritima</i>	0.3–1.9	0.01–2.5	0.01–0.06	up to 3	–	0–75	0.11–0.32
Castro et al. (2009) Rio de Aveira, Portugal	<i>A. portulacoides</i> <i>J. maritimus</i> <i>S. fruticosa</i> <i>Triglochin maritima</i>	1.6–20.8	0.06–2.9	0.02–0.35	0.05–0.34	0.02–0.47	–	–
Válega et al. (2008c) Portuguese estuaries	<i>A. portulacoides</i>	0.03–17	0.01–12.9	0.002–0.12	0.3–2.2	0.7–152.4	–	–
Best et al. (2008) San Pablo Bay, California	<i>Salicornia virginica</i> <i>Spartina foliosa</i>	–	0.09–0.11	0.01–0.05	–	–	1.2–2.4	0.2–1.8
Canário et al. (2007) Portuguese estuaries	<i>A. portulacoides</i> <i>S. fruticosa</i> <i>S. maritima</i>	0.27–2.79	0.17–13	0.018–0.22	up to 9	–	up to 941	0.11–0.72
Windham et al. (2003) New Jersey, USA	<i>Phragmites australis</i> <i>Spartina alterniflora</i>	2.91 ^c	0.006–1.81	0.001–0.11	–	0.01–0.2	–	–
Kraus et al. (1986) New Jersey, USA	<i>Spartina alterniflora</i>	0.22–18.17 ^c	–	0.02–0.16 ^c	–	–	–	–
Breteler et al. (1981) Northeast USA	<i>Spartina alterniflora</i>	0.10–0.33 ^c	–	0.011–0.19 ^c	–	–	–	–
Gardner et al. (1978) Georgia, USA	<i>Spartina alterniflora</i>	0.63 ^c	–	0.05 ^c	–	–	–	–

Table 4 continued

References	Halophytes investigated	Hg sediment ($\mu\text{g g}^{-1}$)	Hg roots ($\mu\text{g g}^{-1}$)	Hg shoots ($\mu\text{g g}^{-1}$)	EF	TF	MeHg roots (ng g^{-1})	MeHg shoots (ng g^{-1})
Windom et al. (1976) Georgia, USA	<i>Spartina alterniflora</i>	0.95 ^c	–	0.07 ^c	–	–	–	–

^a Hg concentration in the total belowground biomass, calculated as weighted average of Hg concentrations in roots in the three depth levels based on correspondent tissue biomass

^b Calculated for the total belowground biomass using weighted average of Hg concentrations of roots and the average of Hg concentration in sediments recorded for the three levels of depth

^c Average value

results of several factors that can also vary seasonally and temporally (Anjum et al. 2011). Compared to leaves or stems, the root system seems to be the preferential part for Hg uptake by plants as already emphasised in the literature (e.g. Canário et al. 2010; Anjum et al. 2011), protecting the aerial parts of the plant against metal toxicity. The highest values recorded in root tissues in the G2 salt marsh, which did not show the highest Hg content in rhizosediments, suggest the presence of site-specific factors which promote Hg uptake prevailing on the selection of a specific plant species. These factors are probably linked to the partial anthropic origin of this salt marsh, enlarged with the addition of dredging sediments from the adjacent ship channel. This statement, however, should be confirmed by accurate Hg speciation analyses. Previous research focused on Hg speciation in bottom sediments of the central sector of the lagoon distinguished between cinnabar (HgS) and non-cinnabar compounds through a thermo-desorption technique (Piani et al. 2005). The last component was found to occur in high percentages in fine-grained and organic-rich material, which is similar to the saltmarsh sediment. In accordance with the results, a part of Hg seems to be passively adsorbed to the finest particles in the form of Hg(II), Hg(I) and Hg(0), due the higher specific surface area of each particle although the presence of micro-crystalline cinnabar cannot be excluded. Moreover, the predominance of Hg as “sulphide” (53.5%) and “elemental/strongly complexed” Hg (43.6%) in the surface sediments of the eastern sector (Grado) of the lagoon was then confirmed by a more detailed speciation analysis through a selective sequential extraction (Covelli et al. 2011). The authors, however, do not exclude that following diagenetic processes and the change in

redox conditions and pH lowering in deeper sediments a slow dissolution of Hg-binding solid phases into pore waters occurs. This amount of Hg would be able to re-precipitate in other phases (such as complexes with OM and Fe/Mn oxyhydroxides) and thus would contribute to increase those fractions of metal which are easily or moderately exchangeable. These fractions would be eventually bioavailable for plant root uptake although we can only speculate on this point since direct speciation evidence is not currently available for saltmarsh sediments.

Mercury levels in the three considered species are comparable to those reported for other halophytes being in the same order of magnitude, for both roots and stems, and similarly also occur for the EF values calculated as the ratio between Hg levels in roots and sediments. Despite these similarities, the choice to consider different depth levels allowed for the detection of very high values of Hg in the roots (up to $95 \mu\text{g g}^{-1}$) and consequently of EF (up to 81) in that specific level, which is the deepest level taken into consideration (15–25 cm) in our study. The existence of these preferential layers for Hg uptake, for all sampling sites and species investigated, is also supported by other case studies (Canário et al. 2007) and could be explained in two main ways: (1) the higher availability of the metal with depth, or (2) the absence of physical barriers to metal passive uptake in the youngest and less developed roots. In addition, the shape of the roots could be very important because this may increase the specific area available for methylation or uptake, both between different layers and plant species.

Low TF values highlighted some avoidance strategies in these plants regarding metal translocation, similar to what was reported by Castro et al. (2009).

Therefore, the three analysed species are not to be considered proper “bioaccumulator” plants, but rather Hg-“tolerant” plants due to the Hg enrichment in the roots which showed only in the deepest levels.

MeHg is the most toxic form of Hg, due to its easy uptake, bioaccumulation and biomagnification within the trophic chain. Anjum et al. (2014) discussed some effects of MeHg presence in plants considering physiological, genetic and biochemical consequences. Nevertheless, data in the literature are scarce (Table 4) and often opposite conclusions were highlighted regarding the influences of plants or soil features on metal concentration in sediments as well as on Hg or MeHg partitioning in plant organs.

In this work, MeHg appeared highly correlated with the total Hg content in plant tissues but more equally distributed inside the plant, also showing less differences between the two sites considered. The absence of significant differences between high and low contaminated sites and the lower concentration of both Hg and MeHg in aboveground plant tissues has led us to hypothesise that these species are equipped with “control mechanisms” which enable them to keep Hg and MeHg levels under the damage thresholds, preventing direct toxic effects. In general, MeHg levels, especially for aboveground tissues, are comparable to those reported in the literature for contaminated sites and MeHg concentration in roots are higher than in stems (see, for instance, Canário et al. 2007, 2010; Best et al. 2008 in Table 4).

Conclusions

The effects of Hg uptake on plants are not to be considered less important, being directly linked to animal and human health. In fact, the uptake and the accumulation of the metal in plant tissues could be considered a source of toxic compounds for herbivores as well as carnivores along the food chain (Leady and Gottgens 2001) or birds, considering possible translocation through the phloem up to the seeds (Lucena et al. 1993). In this context, many halophytes exhibit avoidance strategies against toxic metals (e.g. Válega et al. 2009) and Hg seems to be easily compartmented in the belowground biomass, apparently not affecting the plant health status. These features are confirmed in this study, conducted in one of the most Hg-impacted lagoon systems worldwide. Our results indicated that

investigated salt marshes act as a sink for Hg species, but despite the accumulation in halophytes’ roots the translocation to the aboveground biomass is almost negligible. The degree of bioaccumulation suggests that phytoremediation could be important in the management of this contaminated wetland system. Due to its complexity, this process needs deeper analysis and physiological investigations in field and in laboratory in order to clarify plant responses regarding metal uptake, especially evaluating how environmental and pedological factors as well as human activities could influence Hg availability.

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