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Saltmarsh plants role in metals retention and the potential of vegetation for metal removal in the long term

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ABSTRACT

Estuaries along with their saltmarshes are extremely relevant areas to what ecosystems conservation is concerned. Not only do they provide unique conditions to house numerous species but can also play an important role in pollution mitigation. This study aimed to evaluate the role of saltmarsh plants in metals retention in the long term, using a previously monitored estuary as a case study (Lima river estuary). Seasonal sampling campaigns were carried out in 2022 to determine the metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) concentrations in vegetated and non-vegetated sediments, as well as in saltmarsh plants at three sites within the estuarine area, Canogem, Salinas and Srª das Areias. Results showed saltmarsh plants, despite some seasonal variability, are concentrating metals in their rhizosediments (ratio metal in vegetated sediment / metal in non-vegetated sediment >1) and in their roots, namely Cd, Cu and Zn (ratio metal in plant roots / metal in non-vegetated sediment >1). This role seems to be maintained in the long term, with plant metal retention levels similar in 2009 and 2022, indicating plants are probably contributing to remove metals from the surface water. However, this feature seems to be decreasing in one of the sites, Srª das Areias, that shows signs of degradation. Thus, saltmarsh plants have the potential to retain metals in estuarine areas, contributing to reduce metals present in the aquatic environment and preventing them from spreading through the estuarine area, from reaching coastal areas and eventually from reaching underlying aquifers. Protection of this environment is mandatory and the promotion of re-vegetation of non-vegetated estuarine areas is needed so that the saltmarsh works as a nature-based solution that prevents and/or recovers impacted environments, in order that saltmarshes can continue to deliver their multiple co-benefits.

Introduction

Estuaries are open bodies of water that receive both saline water from the sea and freshwater from the rivers, generating different levels of salinity. This feature gives estuaries unique properties which result in distinct habitats for several organisms [1]. Despite being known as places for recreational activities and general enjoyment, estuaries are also relevant for many other reasons. For example, these locations act as cleaning agents because the water that flows through them is filtered by their sediments and saltmarshes, allowing the removal of nutrients and other pollutants, generating cleaner water for all living organisms [2] and preventing them from reaching coastal areas. Since estuaries are naturally rich areas that favor the development of numerous economic activities, they also suffer from the urban pressure caused by the growing population living around these areas. Agriculture, animal farms, industries, aquaculture, ports and urban areas are the main factors that contribute to anthropogenic pressure in estuaries, leading to changes in the ecosystems' dynamic [3]. Furthermore, estuaries can be receivers of industrial and municipal wastes, as well as treated wastewater (that might still contain some pollutants) or direct sewage

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disposal. Frequently, these sites are contaminated with several compounds and the continuous exposure of the organisms to these pollutants can have a long-term impact on ecosystems' health [4].

Metals are one of the contaminants that have been identified as potential source of stress for these ecosystems [5]. Their hazards towards life are extensive because these compounds have proven to be persistent and bioaccumulative and their toxicity towards aquatic life may result in poisoning of species, leading to ecosystems imbalance [6].

Conventional physical and chemical methods to clean contaminated sites are widely used and present high efficiencies. However, they can be of high cost, some of them are limited to small areas, may require additional land for waste storage, and are dependent on the type of contaminant present [7]. Moreover, they can cause ecological disturbances and might not be applicable in contaminated areas such as estuarine ones. Phytoremediation refers to the use of plants and associated microorganisms in the removal of different contaminants, such as organic pollutants and metals, from diverse matrices like sludge, soil, sediments, surface water and wastewater. This method has the sun as energy source and is applied in situ, reducing the overall costs, and is an eco-friendly alternative to physicochemical technologies [8]. Native flora has proven to be phytoremediation agents in the retention and/or removal of contaminants such as metals [9]. For instance, saltmarsh plants are usually present in areas contaminated with different pollutants and, therefore, they have been studied to understand their potential use in phytoremediation processes [10], namely in estuarine areas [11].

The potential of saltmarsh plants, such as *Juncus maritimus, Phragmites australis, Spartina patens, Triglochin striata* and *S. alterniflora*, for metal phytoremediation has been observed in different estuaries [11]. A previous work conducted in the estuary of Lima river in Portugal (NW, Portugal) concluded that the presence of saltmarsh plants allowed the retention of metals, either in the plants underground structures or in the sediment surrounding their roots, and played different roles in metals distribution [11]. However, different anthropogenic pressures (different sources and amounts), plant species and seasonality might influence such capacity, being important to assess saltmarsh's role in the long term.

So, a study was conducted over one year to assess the presence of several metals in various saltmarsh plants (*J. maritimus, P. australis,* and *S. maritima*) and sediments (vegetated and non-vegetated) in an estuarine region previously investigated, the Lima River Estuary This study aimed to evaluate if plants potential for metal phytoremediation was still observed as studies on long-term performance are still lacking. We hypothesis that the role of the saltmarsh plants in the retention of these compounds is still be a valuable strategy in the long-term, foreseeing saltmarshes as a nature-based solution to prevent and/or recover impacted environments, if their restoration, creation and management is considered.

Materials and methods

Sample collection and preparation for metals quantification

Juncus maritimus (Lam.), Phragmites australis (Cav.) Trin ex. Steud., and *Spartina maritima (Brongn.)* were collected at low tide in August 2021 (preliminary sampling), November 2021, February 2022, May 2022 and August 2022, at different sites in Lima river estuary (NW Portugal). The sampling campaign in August 2021 was a preliminary sampling to evaluate possible sampling sites. After that, new sites were selected, and four sampling campaigns were carried out, to cover an entire seasonal cycle: November 2021, autumn sampling; February 2022, winter sampling; May 2022, spring sampling; August 2022, summer sampling.

For that cubes, each (ca. $20 \times 20 \times 20$ cm^3) containing ca. 10 plants were retrieved with the sediment around the plants' belowground tissues. On site, from each cube, plants were individually separated, washed in local water and placed in plastic bags. Sediment in contact with their belowground structures (vegetated sediments, rhizosediments) was collected simultaneously into plastic bags. The sampling sites are identified in Fig. 1. At each site, non-vegetated sediment was also collected, with the exception of site L4 in the preliminary sampling for which no non-vegetated sediment was available nearby. This non-vegetated sediment was located 50 m apart for the respective vegetated location. To collect non-vegetated sediments, the first thin layer of sediment was taken out and the sediments on the layer immediately below were collected into a plastic bag.

Each sample was put into an individual plastic bag, using plastic shovels for rhizosediment and non-vegetated sediment or plastic gloves for plants, and was immediately transported to the laboratory.

On arrival at the lab, plants were thoroughly rinsed with deionized water, placed in trays, covered with filtering paper to avoid contamination, and put to dry at room temperature until constant weight. Afterwards, plants were separated into stems/aerial tissues, rhizomes (only for *J. maritimus*) and roots, which were separately cut and homogenized. Plant tissues from each site were combined to form a pooled sample for analysis. Sediments and rhizosediments were placed in individual plastic trays and put to dry at room temperature. Afterwards, sediments and rhizosediments were homogenized and sieved through nylon nets of 2 mm to remove large stones and dead roots, as done previously in other studies for metal analysis in sediments from the area [11]. A portion was then separated for organic matter (OM) determination and another portion separated for metal analysis. Both portions were kept in plastic bags, in the dark until further processing.

Metal content quantification

Aliquots of each specific tissue of the plant (ca. 0.50 g), sediments and rhizosediments (ca. 0.25 g) were digested in closed PTFE vessels at high-pressure, with suitable amounts of concentrated HNO_3 65 % (Merck) (1 mL for the different plant tissues and 5 mL for sediments and rhizosediments), and 5 mL 30 % H_2O_2 (Merck) (only for plant tissues), using a microwave system (ETHOS 1, Milestone), following previously optimized methodologies [11]. The HNO_3 digestion of the sediments and rhizosediments only allows to obtain the total-recoverable metal contents, because it does not provide a complete dissolution of the sample, particularly silicates, but this fraction will be the one environmentally relevant. Triplicates were prepared for each sample.

Total metal content in the different plant tissues, sediments and rhizosediments were determined by atomic absorption spectrophotometry either with flame atomization (AAnalyst 200, Perkin-Elmer), used for Cu, Fe, Mn, and Zn, or with electrothermal atomization (PinAAcle 900Z, Perkin-Elmer coupled to an AS 900 autosampler), used for Cd, Pb, Ni and Cr, accordingly to metal levels. Aqueous matched standard metal solutions were used for external calibrations. All procedures used were previously optimized and validated with the Estuarine Sediment BCR 277 reference material, certified for total metal content, with satisfying values for the triplicate analysis of reference materials being obtained [11]. Blank solutions were prepared using all the microwave digestion and analysis procedures, with no significant metals contents being detected. All material used was previously washed with deionized water, soaked in a 20 % (v/v) nitric acid solution for 12 h and washed again with deionized water to avoid metal contaminations.

Results were normalized by the samples dry weight and were expressed as μg g^{-1}. Metals selected were those that are commonly found and monitor on these areas [11].

Ratios between metals levels in vegetated (rhizosediments) and non-vegetated sediments, as well as between metals levels in roots and non-vegetated sediments, to evaluate metal accumulation in vegetated sediment or plant tissues, were calculated according to the following equation:

$$Ratio = \frac{[Metal\ (Vegetated\ sediment)] or\ [Metal\ (Roots)]}{[Metal\ (Non\ vegetated\ sediment)]}$$

Fig. 1. A: Preliminary sampling locations, L1 colonized by *J. maritimus* and *S. maritima*, L2 colonized by *J. maritimus* and *P. australis*, L3 and L4 colonized by *J. maritimus*. B: Seasonal sampling locations, Salinas and Srª das Areias colonized by *J. maritimus* and *P. australis* and Canoagem colonized by *J. maritimus* and *S. maritima*.

The ratios calculated provide an indication of the role of plants in retaining metals from the estuarine environment. As the rhizosediment is already a consequence of the presence of the plants, non-vegetated sediments are a more suitable parameter for estimating metals bio-retention /bioaccumulation.

Organic matter determination

The determination of OM in sediments followed the loss of ignition method. Vegetated and non-vegetated sediments were placed to dry at 105 °C in an oven for 24 h and then ca. 2 g of sediment were weighed in ceramic coups. Triplicates were performed for each sediment sample. Ceramic cups were placed in a muffle furnace (Carbolite type 301) at 500 °C for 4 h. After the cups cooled down, they were weighed again, and the OM content was calculated considering the mass (M) of sediment before and after furnace according to the following equation:

$$OM\ (\%) = \left[1 - \frac{Msediment_{before\ furnace}}{Msediments_{after\ furnace}}\right] \times\ 100$$

Statistical analysis

Each sediment samples and plant tissue were analyzed in triplicate, the mean and standard deviation being calculated afterwards.

For metals and OM levels significant ($p < 0.05$) differences among samples were evaluated through a parametric one-way analysis of variance (ANOVA).

Results

Metal levels in non-vegetated sediments

Metals' concentrations in non-vegetated sediments varied between 2 and 85 µg g^{-1} for Cu, Zn, Mn, Pb, Cr, and Ni, and between 11 and 20 mg g^{-1} for Fe (Table 1). Cd was detected in some cases but always below the method quantification limit (0.2 µg g^{-1}).

The preliminary sampling campaign indicated that site L4 (see Fig. 1) was not under the direct influence of the river, being in a more restricted area. Besides, the entire area was vegetated and non-vegetated sediment was not collected. Thus, that location was not considered for the seasonal campaign. For the other locations, the metal levels in non-vegetated sediments were of the same order of those of the other locations. Comparison between L2 and L3 showed slightly higher levels in L2 and, thus, this location was selected for the seasonal sampling campaign. Location L2 was colonized by two plant species, *J. maritimus* and *P. australis*. A nearby stream probably contributes, along with the river flooding the saltmarsh at high tide, for the higher metal levels detected in this location. Site L1 was not easily accessible (only by boat) and for

Table 1
Concentrations of Cu, Zn, Mn, Fe, Pb, Cr and Ni (in µg g^{-1}, except Fe which is in mg g^{-1}) observed in non-vegetated sediments (mean ± standard deviation, $n = 3$) over one-year monitoring (C- Canoagem; S- Salinas; SA- Sr[a] das Areias). Data from the preliminary sampling is also included (sampling sites in Fig. 1). Reference values in Portuguese legislation, Norwegian guidelines [12] and concentration ranges associated with adverse toxicity effects to organisms (effect range-low (ERL)) [13] are also indicated.

		Cu	Zn	Mn	Fe	Pb	Cr	Ni
Portuguese legislation for dredging sediments class 1		<35	<100	–	–	<50	<50	<30
Norwegian Reference Values [12]		<35	<150	–	–	<30	<70	<30
Effect range-low (ERL) [19]		34	150	–	–	46.7	81	20.9
Preliminary Sampling	**L1**	14.5 ± 0.7	67 ± 2	52 ± 3	18.6 ± 0.7	10 ± 0.2	18 ± 1	10 ± 2
	L2	16 ± 3	72 ± 10	50 ± 6	20 ± 2	12.1 ± 0.8	16 ± 2	11 ± 1
	L3	12.6 ± 0.9	57 ± 3	47 ± 3	17 ± 2	10.8 ± 0.2	14.3 ± 0.9	10 ± 2
Autumn sampling	**C**	6.9 ± 0.8	33 ± 1	33.1 ± 0.8	10.9 ± 0.6	8 ± 3	10 ± 1	<2 (LOD)
	S	10.2 ± 0.8	36 ± 4	28 ± 2	12 ± 1	11 ± 2	13 ± 2	<2 (LOD)
	SA	10 ± 1	60 ± 8	55 ± 5	18 ± 1	14 ± 4	19 ± 2	4.7 ± 0.6
Winter sampling	**C**	5.1 ± 0.5	47 ± 2	33 ± 2	12.1 ± 0.6	15 ± 5	15.3 ± 0.6	<2 (LOD)
	S	13 ± 1	75 ± 2	33.8 ± 0.6	15.8 ± 0.3	21 ± 6	25 ± 2	3 ± 1
	SA	13.72 ± 0.02	76 ± 2	56 ± 1	19 ± 1	24 ± 7	23.6 ± 0.6	4.6 ± 0.9
Spring Sampling	**C**	8 ± 3	44 ± 4	31 ± 4	12 ± 1	10 ± 1	12 ± 4	3.1 ± 0.4
	S	15.5 ± 0.5	85 ± 2	35.2 ± 0.8	17.3 ± 0.8	28 ± 1	21 ± 1	7.1 ± 0.7
	SA	5.6 ± 0.1	67 ± 2	49 ± 2	17.5 ± 0.8	17 ± 1	17 ± 2	4.66 ± 0.06
Summer Sampling	**C**	5.8 ± 0.2	36 ± 1	64 ± 3	10.3 ± 0.9	6 ± 2	12 ± 1	4.9 ± 0.2
	S	21.1 ± 0.6	83 ± 2	77 ± 3	14 ± 2	14.0 ± 0.7	21.6 ± 0.7	12.2 ± 0.6
	SA	7.9 ± 0.5	54 ± 2	80 ± 3	11.6 ± 0.8	11 ± 1	17 ± 1	8.3 ± 0.3

the seasonal sampling campaign a site in the margin across the river was selected, Canoagem. Nevertheless, levels at this site, L1, were slightly higher than in Canoagem. Sr[a] das Areias was another site added for the seasonal sampling campaign, since it is located at the lower part of the estuary and it is affected by different anthropogenic pressures. A small stream also drains into this location, besides the flooding at high tide by the Lima river. This site has been monitored for its metal levels over the years in the frame of several projects.

For the seasonal sampling campaign, significant differences ($p < 0.05$) among the concentrations of Zn, Mn, Fe, Ni and Cr in non-vegetated sediments of the three locations were in general observed at each season. The lowest levels were in general observed at Canoagem and the highest levels were observed in general at sites Salinas and Sr[a] das Areias. Different sources of contamination can influence the levels registered at the different sites. Comparing the later sites, in the first two seasons, autumn and winter, metal levels in non-vegetated sediments were identical or slightly higher at Sr[a] das Areias. However, in spring and summer metal levels at Salinas were in general higher than those at Sr[a] das Areias.

No significant differences ($p < 0.05$) were observed in general between the metal levels among the seasonal samplings for Canoagem location. The same was in general observed for Sr[a] das Areias site, although there was a tendency for higher metal concentrations in the winter sampling campaign. For Salinas, in general the lowest metals levels were observed in autumn. With the exception of Fe, with values similar among seasons, metal levels in general increased over the year and for most elements, the highest values being observed in summer.

Comparing the values obtained to the values in Portuguese legislation for dredging sediments (Portaria n° 1450/2007 de 12–11–2007, ANEXO III) and those for sediment quality criteria in Norway [12], the levels found in Lima river non-vegetated sediments were clean and in background levels category, respectively. Levels were also below the concentration ranges associated with adverse toxicity effects to organisms (effect range-low (ERL)) [13].

Vegetated versus non-vegetated sediments

The metals levels in rhizosediments, as well as the results for the preliminary sampling are presented in Supplementary material (Table S1).

In general, levels were identical among seasons at each site and similar among sites and among plants.

The ratio between metals' levels in vegetated and in non-vegetated sediments was calculated and is presented in Fig. 2 for the autumn, winter, spring and summer sampling campaigns and in Fig. S1 for the preliminary camping.

For the preliminary sampling campaign, the concentrations of Cu, Pb, Cr and Ni were, in general, higher in vegetated sediments of *J. maritimus* and *P. australis* (Table S1) indicating that the plants were retaining these metals in their rhizosphere. Zn was also slightly higher in vegetated sediment of *P. australis* and the vegetated sediment of *S. maritima* had higher concentrations of Pb and Mn than the respective non-vegetated sediment, showing difference among plant species. *J. maritimus* rhizosediment had higher metal concentrations than non-vegetated sediment in all three locations, concentrations in general similar among sites.

Regarding the seasonal campaign (Fig. 2), most ratios between vegetated and non-vegetated sediments for site Canoagem were higher than 1, meaning that vegetated sediments have a higher metal concentration. This feature is observed for both plants (*P. australis and S. maritima*) contrary to what was observed in the preliminary sampling campaign for *S. maritima*. On the contrary, in Sr[a] das Areias metals were in general identical between vegetated and non-vegetated sediment, for both plants (*J. maritimus and P. australis*) and for all sampling campaigns, indicating that at this location plants were not retaining metals in their rhizophere. For Salinas, results were very variable among seasons, between plants (*J. maritimus and P. australis*) and among metals, with metal concentrations in vegetated sediments either higher or similar to non-vegetated sediment without a clear pattern.

Metal levels in plant tissues

The concentration of metals in plant tissues, as well as the results for the preliminary sampling are presented in Supplementary material (Table S2 and Fig. S1).

As expected, metal levels were higher in plant belowground tissues than in aerial structures, with the ratio between metal level in aerial tissue and metal level in roots being, in general, lower than 1 (results not shown), indicating that there is no bioaccumulation in aerial parts. The only exception was Mn with a ratio >1 for *J. maritimus* in S[a] das Areias. For *J. maritimus* two belowground structures were analysed, roots and rhizomes, due to the plant structure. In general, plant roots had higher metal levels than those in rhizomes, with only a few cases for Zn (autumn sampling), Cu and Ni (winter and summer sampling, Canoagem) for which similar or even slightly higher levels in were observed rhizomes. The later was also observed for the preliminary sampling

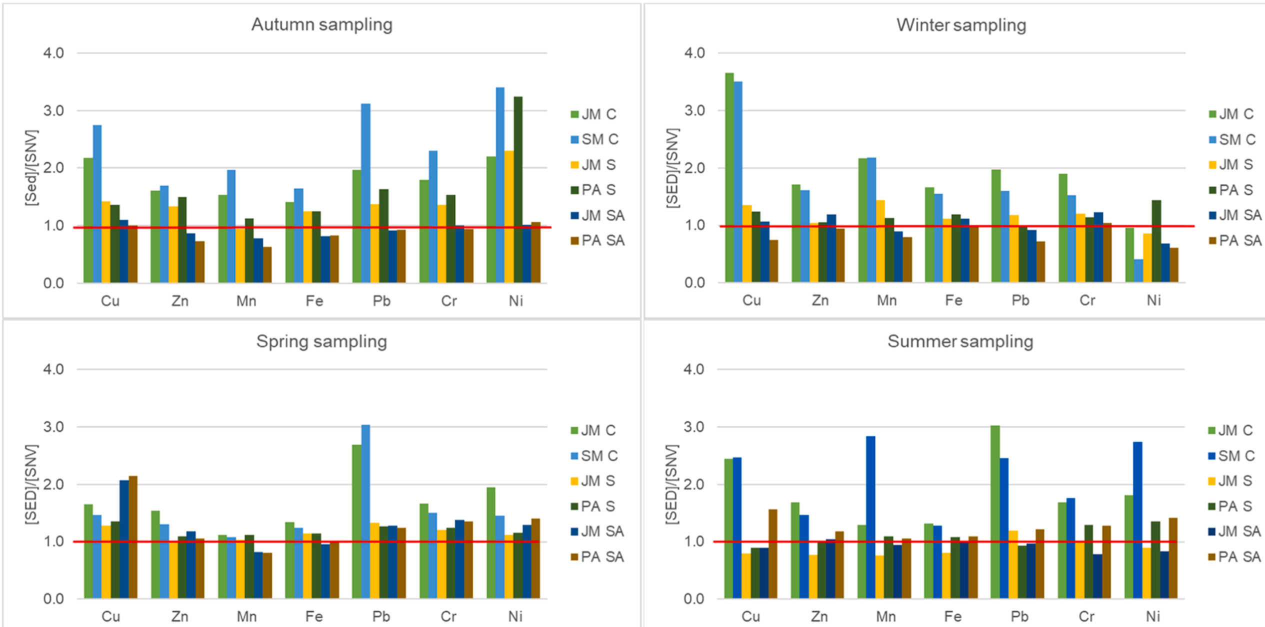

Fig. 2. Ratio between metals' levels in vegetated (Sed) and in non-vegetated sediments (SNV) for the autumn, winter, spring and summer sampling campaigns (C – Canoagem colonized with J. maritimus (JM) and S. maritima (SM); S - Salinas and SA - Sr[a] das Areias colonized with *J. maritimus* (JM) and *P. australis* (PA)).

campaign.

Regarding seasonal variabilities, metal levels varied among seasons, but those variations were dependent on the metal, on the plant and on the location, with no clear pattern being observed.

To evaluate if plants can uptake the metals or just retain them in their sediments, the ratio between the metals' concentration in the roots and in non-vegetated sediments was calculated (Fig. 3).

Regarding the preliminary sampling results clearly show that the plants collected in site L2 have higher levels of Pb in their roots, compared to non-vegetated sediments, as the ratio is equal to 1.5 (Fig. S1). Thus, besides being able to retain this element in their sediments, *J. maritimus* and *P. australis* can uptake it and incorporate it in their roots.

For the autumn sampling results show that plant roots only retained Cu, and only in sites Canoagem and Salinas as well.

For the winter sampling, besides Cu, also Zn, Ni and Cd were retained by plant roots. Once again, this was evident in sites Canoagem and Salinas, contrasting clearly to what is observed in site Sr[a] das Areias, with no accumulation (ratio between the metals' concentration in the roots and in non-vegetated sediments lower than 1).

For the spring sampling, again Cu, Zn and Cd were accumulated in plant roots again in Canoagem and Salinas, although for Zn a slight

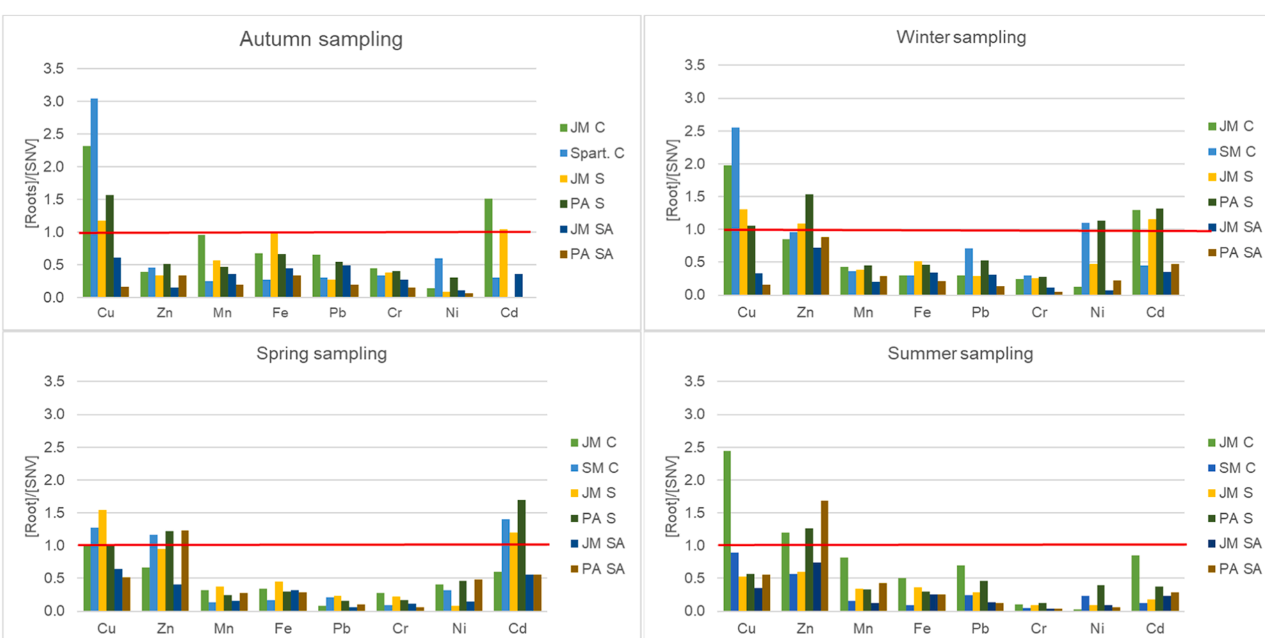

Fig. 3. Ratio between metals' levels in roots (Root) and in non-vegetated sediments (SNV) for autumn, winter, spring and summer sampling (C – Canoagem colonized with J. maritimus (JM) and S. maritima (SM); S - Salinas and SA - Sr[a] das Areias colonized with *J. maritimus* (JM) and *P. australis* (PA)). For Cd, the value of the limit of detection (0.2 μg g^{-1}) was considered for SNV.

accumulation was also observed in Srª das Areias.

For the summer sampling, accumulation of Zn was observed in plants of the three locations, whereas Cu accumulation was only observed for *J. maritimus* in Canoagem.

Thus, clear seasonal variations were observed in metal accumulation, which also varied with the metal, with the plant and with the location. Results indicated that plants have the ability to concentrate metals in their roots, mainly Cu, Zn and Cd.

Organic matter levels

In general, higher levels of OM were observed in vegetated sediment than in non-vegetated ones, with some exceptions at site Srª das Areias (Table 2).

A difference between OM levels in the three locations ($p < 0.05$) was observed among the different seasons. Canoagem and Salinas had, in general, higher levels of OM in its sediments compared to location Srª das Areias. In general, for each location, OM levels were identical between the rhizosediments of the two plants. The exceptions were observed in Srª das Areias that in some seasons (winter and summer) differences between the two plants were observed with no clear trend.

Discussion

Metal concentrations in non-vegetated sediments observed in the three sites in the four sampling campaigns were, in general, within the same range as those observed in previous sampling campaigns carried out in the Lima river at some of the selected sites in the spring of 2009 [11] and spring of 2014 [14]. Only Mn and Ni levels were slightly lower (values previously found in Lima non-vegetated sediments varied between 80 and 104 μg g^{-1} for Mn and between 5 and 17 μg g^{-1} for Ni [11], whereas in the current study values varied between 28 and 77 μg g^{-1} for Mn and between <2 and 12 μg g^{-1} for Ni (Table 1)). Besides, Pb levels were slightly higher (ca. 2 μg g^{-1} [14]) or lower (between 9 and 58 μg g^{-1} [11]) than in the current study (between 6 and 28 μg g^{-1} (Table 1). As mentioned, this area is impacted by diffused pollution and multiple sources can be responsible for pollutants occurrence, including metals. Metals were also detected in the Lima river estuarine water (results not shown), but current results indicate that the system is stable over the years with metal levels not of concern. Metals levels observed in non-vegetated sediments are all considered background levels considering a Norwegian system for the classification of environmental quality of contaminated marine sediments [12], and according to Portuguese legislation for dredging sediments (Portaria n° 1450/2007 de 12–11–2007, ANEXO III), the area can be classify as clean. At all sites, metal levels observed were also lower than their effect range-low (ERL), using the system defined by Long et al. [13] to classify sediments potential toxicity to organisms.

Some seasonal variability in non-vegetated sediment metal levels was observed, namely at Salinas and Srª das Areias, sites more sheltered and confined than the one in Canoagem, which is in an open margin of the river. The latter had also slightly lower metal levels probably do to the low OM content levels. Organic matter can help retain metals in sediments [11]. As indicated before, at Srª das Areias site there was a tendency for higher metal concentrations in the winter sampling campaign. For Salinas, in general the lowest metals levels were observed in autumn. This sampling campaign occurred after 2 months of high precipitation rate as reported by the Instituto Português do Mar e Atmosfera (IPMA) (https://www.ipma.pt/pt/oclima/monitorizacao/index.jsp?selTipo=m&selVar=rr&selAno=-1), which can contribute for some metal washing. With the exception of Fe, with values similar among seasons, metal levels in general increased over the year and for most elements, the highest values were observed in summer, coinciding with the increasing dry weather and low precipitation rates observed over the year 2022 (https://www.ipma.pt/pt/oclima/monitorizacao/index.jsp?selTipo=m&selVar=rr&selAno=-1). So, probably the drier weather facilitated metal incorporation into sediment.

Metal levels were in general lower than those reported in other Northwest Portuguese estuarine areas, such as Douro river estuary [15] and Ave river estuary [16], estuaries with high urban impact and no significant saltmarsh areas.

Saltmarshes can retain pollutants, namely metals, as previously observed [11]. Results of the current study corroborate this, with vegetated sediment showing in general higher levels than those in non-vegetated sediments. The results obtained are in accordance with the OM levels, which were higher in vegetated sediment, and as mentioned high organic matter favors metal retention due to binding of metal on organic matter carbon complexes. In general, vegetated sediment retained more metals at Canoagem site. As mentioned this site is more exposed to the river currents, being on an open margin of the river. So, the presence of plants seems to contribute to retain metals in the sediments of this area. Moreover, metal accumulation in saltmarsh plants at this site also showed plants' capacity to concentrate Cd, Cu, Ni and Zn in their roots, with the ratio between metal levels in roots and metal levels in sediment higher than 1. Regarding the more sheltered sites a similar behavior of metal retention was also observed at Salinas site. However, at Srª das Areias that feature was not so clear. Over the years Srª da Areias has shown a continuous degradation with the loss of vegetation and with smaller plants (ca. 0.5 m height), much smaller than those observed at other locations, namely in Salinas (ca. 1–1.5 m height), which might explain their lower ability to retain metals.

Metal content in different plant tissues showed the plants potential to incorporate some of the elements in their belowground structures, especially their roots. It is noticeable that metals levels are always higher in roots, followed by rhizomes, than in aboveground structures, highlighting the plants' potential to retain these elements in their belowground structures, a feature common of saltmarsh plants [11,17].

Metal levels in plant tissues were lower than those reported for other estuaries with higher amount of metals in their sediments [17], indicating that the plants probably have the capacity to respond to the metal presence in the surrounding environment, accumulating more when the levels are higher.

Plants concentrated in general Cu, Zn and Cd in their roots, contributing to the retention of these metals. Some seasonal variability on plant metal levels was observed, variations that in some cases were correlated with metal levels in rhizosediment as previously observed [18], but metal retention was observed in all seasons, with variabilities being higher among sites than among seasons, showing metal levels in

Table 2
Organic matter content (%) in the collected sediments in the three locations (mean ± standard deviation, $n = 3$). SNV-non vegetated sediment; JM–rhizosediment of *J. maritimus*; SM-rhizosediment of *S. maritima*; PA-rhizosediment of *P. australis*.

	Canoagem			Salinas			Sª das Areias		
	SNV	JM	SM	SNV	JM	PA	SNV	JM	PA
Autumn sampling	2.91 ± 0.07	8.3 ± 0.2	11.1 ± 0.4	7.5 ± 0.2	12.3 ± 0.4	10.4 ± 0.2	5.1 ± 0.1	7.6 ± 0.4	8.3 ± 0.3
Winter sampling	3.3 ± 0.1	11.7 ± 0.2	12.1 ± 0.7	6.3 ± 0.1	13.3 ± 0.1	10.98±0.06	7.2 ± 0.1	9.2 ± 0.2	5.8 ± 0.1
Spring sampling	2.7 ± 0.1	8.9 ± 0.1	8.5 ± 0.4	6.6 ± 0.1	8.7 ± 0.3	9.4 ± 0.2	4.6 ± 0.1	7.7 ± 0.1	9.2 ± 0.1
Summer sampling	2.4 ± 0.2	12.3 ± 0.7	n.d	6.3 ± 0.4	11.3 ± 0.9	10.3 ± 0.6	3.3 ± 0.4	3.2 ± 0.2	10.5 ± 0.5

n.d. – not determined, sample lost during methodological processing.

plant tissues was influenced by sources, locations and concentrations of metals.

In the current study, metals levels in plant tissues of sites Canoagem and Salinas were in general similar to levels previously observed in the Lima river estuary [11]. However, in Srª das Areias metals levels in plant tissues were lower, despite metals levels observed in estuarine sediments being identical or in some cases slightly higher. This site, as indicated above, has shown a continuous degradation with the loss of vegetation over the years, which is being reflected in the lower amount of metals retained either in the rhizosediment or in the plant tissues.

Summarizing, saltmarsh plants have the potential to retain metals in estuarine areas, contributing for reducing metals present in the aquatic environment and preventing them from spreading through the estuarine area and reaching coastal areas. This role seems to be maintained in the long term, with plant metal retention levels similar in 2009 and 2022, indicating plants are probably contributing to remove metals from the surface water and mitigating metals impacts. However, this feature seems to be decreasing in one of the sites, the one with signs of degradation. So, protection of this environment is mandatory and the promotion of re-vegetation of non-vegetated estuarine areas should be carried out to recover impacted estuarine environments.

Saltmarshes are naturally resilient ecosystems, important for the conservation and protection of estuarine environments, that provide a variety of ecosystem services, including coastal protection, water quality improvement, habitat for fish and wildlife, and carbon sequestration [19–23]. Therefore, the protection, restoration, and management of saltmarshes can be an important part of a nature-based solution action. Saltmarshes can be protected and restored using nature-based solutions, such as saltmarsh restoration, saltmarsh creation, and saltmarsh management becoming itself a nature based solution. In fact, saltmarshes can be restored by planting native vegetation and grading the marsh surface to create a suitable tidal regime. This can help to improve coastal protection, water quality, and habitat for fish and wildlife. Moreover, new saltmarshes can be created in areas where they have been lost or degraded. This can help to expand the range of saltmarsh ecosystems and increase the resilience of coastal communities to climate change. In addition, saltmarshes can be managed to protect them from threats such as development, pollution, and climate change. All these features can be considered in the case study of the estuary of the Lima river, as promoting the protection of existing saltmarsh plants and the re-growth/re-plantation of native plants in new areas of the estuary will contribute for the removal and retention of metals spread in the aquatic system. Metals can be toxic, depending on the metal, on its concentration and on its bioavailability so natural wetlands, such as saltmarshes can minimize their negative impact.

All these actions require also a sustainable managing of the saltmarshes that needs to involve the entire local and regional community, including the public sector (e.g., legislators, regulator, policy makers, …), private sector e.g., (industry and enterprises such as touristic ones), academia (researchers) and civil society (citizens, schools and civil society organizations). Being natural, and in general protected areas, saltmarshes intervention will need always permission of public governing bodies. The role of researchers is key to also show all the benefits that these areas have not only for society in general but also for private stakeholders, that benefit from a healthy environment. Saltmarshes can support tourism, recreation, and fisheries industries bringing economic benefits. The civil society has also a key role in the protection of saltmarsh environments. They can volunteer with local saltmarsh restoration or management organizations, support policies and initiatives that protect and restore saltmarshes and educate others about the importance of saltmarshes and how to protect them, as well as raise awareness about the risks of pollutants, namely metals, contamination and encourage responsible disposal of hazardous materials. In fact, they can volunteer for plant native saltmarsh vegetation to help restore degraded saltmarshes and improve their ability to filter pollutants and provide habitat for wildlife. They can also volunteer for helping to remove trash and other debris from saltmarshes, along with collection of plants biomass litter. This can help to improve saltmarsh health and reduce the risk of reintroduction of metals accumulated in plants to return to the aquatic environment, an action that should be coordinated with governing public entities. Moreover, they can volunteer to collect data on saltmarsh health, including water quality, vegetation cover, and wildlife presence in close collaboration with researchers. This data can be used to track the progress of restoration efforts and identify areas where further action is needed

By working together, communities can play a vital role in protecting and sustainably managing saltmarshes, promoting long-term ecological health. This can help to ensure that saltmarshes continue to provide the many benefits that they offer to people and nature alike. Acknowledging saltmarshes pivotal role is imperative for the sustainable management of estuarine ecosystems in the face of ongoing anthropogenic challenges.

Conclusions

Saltmarsh plants have the capability to accumulate metals. The current study confirms the potential of these plants to retain metals either in their rhizosediments or in their tissues, mainly belowground tissues. In fact, in general higher levels were observed in vegetated sediments than in non-vegetated sediments. Moreover, saltmarsh plants were able to retain at least Cd, Cu and Zn, with higher levels in their belowground tissues than in non-vegetated sediments. Using the Lima river estuary as a case study, the current work shows that the potential of saltmarsh to remove metals from the aquatic environment occurs in the long term, preventing metals from spreading in the area and from reaching coastal areas and/or eventually contaminating underlying aquifers. Nevertheless, in a site which shows a continuous degradation with the loss of vegetation, lower metal retention was observed. So, re-vegetation of impacted areas and of non-vegetated areas should be promoted making saltmarshes suitable nature-based solutions to prevent, protect and recover impacted estuarine areas. This approach should involve different actors, from public and private sectors as well as from academia and civil society. Civil society is a key player in the protection of these ecosystems that can be involved for instance in volunteering actions of replantation and biomass litter collection to prevent that accumulated metals are released into the estuarine environment again. All these actions will allow to promote the multiple co-benefits saltmarshes provide as a nature-based solution.

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NBS impacts and implications

Environmental: Current work shows that the potential of saltmarsh to remove metals from the aquatic environment occurs in the long term, preventing metals from spreading in the area and from reaching coastal areas and/or eventually contaminating underlying aquifers. So, re-vegetation of impacted areas and of non-vegetated areas should be promoted, making saltmarshes suitable nature-based solutions to prevent, protect and recover impacted estuarine areas

Economic: Current study shows that saltmarshes are relevant pollutants filters, retaining them, namely metals. By removing these pollutants, the saltmarshes contribute to cleaner water surface allowing for instance the implementation of aquaculture activities in the area, with the production of safe seafood leading to economic gains.

Social: Current study shows that saltmarshes are relevant pollutants filters, retaining them, namely metals. By promoting re-vegetation and protection of saltmarshes, as nature-based solutions, will not only contribute to cleaner water (through pollutants removal/retention) but also to protect natural green areas that can be available to citizens, promoting wellness.

Additional materials

Supplementary material – metal levels in rhizosediments and plant tissues.

CRediT authorship contribution statement

Patrícia Cunha: Formal analysis, Data curation, Methodology, Writing – original draft. **Ana M. Gorito:** Formal analysis, Investigation, Methodology, Data curation, Writing – review & editing. **Joana P. Fernandes:** Supervision, Writing – review & editing. **Ana Paula Mucha:** Funding acquisition, Investigation, Writing – review & editing. **C. Marisa R . Almeida:** Conceptualization, Data curation, Funding acquisition, Investigation, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nbsj.2024.100110.

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