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Is the Plant *Bolboschoenus maritimus* an Adequate Biomonitor for Trace Metal Contamination in Saltmarshes? A Field Study from the Óbidos Lagoon (Portugal)

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Abstract: Monitoring the negative impacts of trace metals is crucial to assess the health and stability of ecosystems. In salt marshes, halophyte plants were reported as possible bioaccumulators of these elements. The aim of this work was to explore the bioaccumulation potential of *Bolboschoenus maritimus* as a tool for monitoring the presence of metals in coastal environments. *Bolboschoenus maritimus* were collected from a brackish water lagoon, and the presence of the trace metals lead, cadmium, and nickel were seasonally evaluated in distinct parts of the plants, and in water and sediment samples. Lead was the trace metal with the highest concentration detected in water and sediments of the sampling site. The highest lead concentrations in *B. maritimus* were recorded in the spring season. The transport index indicated an accumulation of lead in the leaves of around 70% in the spring of 2009. Cadmium in leaves in spring and summer of 2009 reached values above 5 mg Cd. kg^{-1}. Nickel was not detected in most samples collected. *Bolboschoenus maritimus* was considered an adequate biomonitor for lead and cadmium, since it bioaccumulates both metals with seasonally distinct results, as the bioaccumulation factor results indicated.

Keywords: halophyte plants; transport index; monitoring; pollution; bioaccumulation factor

1. Introduction

Trace metals are metallic elements found in low concentrations in aqueous environments and geological samples [1]. These elements are also observed in ground water, soil, plants, animals, etc., resulting in persistence, bioaccumulation, non-degradability and other harmful effects. The environmental behavior of metals such as cadmium (Cd), lead (Pb) and nickel (Ni) depends on their chemical form. Trace metals can be transported through rivers and streams both in dissolved and suspended forms or accumulated in aquatic organisms and in suspended sediments, with repercussions on their mobility, bioavailability, and toxicity [2]. The importance and magnitude of the problem of contamination by trace metals in estuarine environments is investigated and monitored worldwide. Estuarine and coastal areas are regions affected by intense human activity. Several industries are located close to estuaries, where growth in urbanization, an increase in effluent disposal, intensive agriculture and aquaculture are responsible for an increase in pollution [3]. Because they are exposed to low turbulent mixing and to limited exchange with the ocean, coastal lagoons are susceptible environments that accumulate pollutants provided by the adjoining watershed. Understanding the effects of the distribution of trace metals in those environments and their potential impacts is important for managing the health and stability of estuarine ecosystems.

Salt marsh habitats are often present in estuarine ecosystems such as lagoons. They are restricted to sheltered areas that protect the coastline from erosion [4]. Salt marshes are classified as sensitive habitats that need to be protected according to several regulations [5–7]. Besides being habitats with a high diversity of fauna and flora, with renowned tasks such as nursery areas and breeding sites, salt marshes play a central role in nutrient cycling, transforming and sequestering nutrients, and are also important in phytoremediation [8]. Industrial wastes and sewage sludge, as well as extractive activities such as mining, are potential sources of trace metal pollutants that affect different ecosystems [9]. Substantial amounts of municipal sewage discharges have caused detrimental effects in saltmarshes worldwide, increasing eutrophication, and decreasing biodiversity. As stated in the literature, the pollutants are conveyed in shallow waters by resuspension and deposition, and their fate is highly dependent on tidal currents, a factor with low intensity in several estuarine environments [10]. The contamination of the soil [11] and water resources [12] with trace metals reduces agricultural productivity, deteriorates food chains, and poses health problems to humans and other organisms [10,12–14].

Coastal lagoons often have important economic, social, and ecological roles in the region where they are inserted, and that is a major reason why lagoons must be preserved and protected. In coastal lagoons in North Africa, the main economic sectors include fishing, shellfish harvesting, salt and sand extraction, and maritime transport [15,16]. On the American Continent nurseries, coastal protection and carbon storage are important, namely for tourism and for the energy sector [17]. Therefore, the potential and sensitivities of coastal lagoons should be acknowledged, and it is vital to monitor these ecosystems.

In Europe, one of the most extensive coastal lagoons of the Portuguese coast, the Óbidos Lagoon, is also affected by human activities [18–21] similar to other coastal lagoons worldwide. This lagoon is influenced by agriculture, livestock farming, and several discharges of domestic and industrial sewage from the towns nearby [22]. Although monitoring programs with the aim of assessing the concentrations of trace metals in Óbidos Lagoon were conducted already [22–24], these often relied on sediment and water samples, while the impact of trace metals in salt marsh plants is still poorly understood.

Halophyte plants present a wide geographical distribution [25,26]. *Salicornia ramosissima* J. Woods and *Bolboschoenus maritimus* (L.) Palla which have the capacity to accumulate trace metals in their roots [26–30] are common halophytes in the salt marsh habitats of Óbidos Lagoon. *Bolboschoenus maritimus* has been reported as a bioaccumulator of trace metals [27–29] and can provide insight into the incidence, concentration values, and the consequences of the exposure to a pollutant [31–35]. This plant belongs to the Cyperaceae family and is common in Europe, North America and South Africa, in habitats such as wet marsh flats, pond margins and estuaries, but also in seasonal and permanent wetlands. These plants are widely spread in brackish water bodies of shallow depth in temperate regions, reaching a height of 1.2 m [35,36]. This perennial plant has a rhizome system bearing underground elliptical to spherical tubers, erect stems, and is mostly robust with an upper leafless part of flowering shoots [36,37]. Their shoots are photosynthetically active for only one growing season and similar dense monospecific stands of this species were already recorded in shallow brackish marshes during the spring and summer seasons [37,38].

The aim of the present work was to explore the presence of metals in coastal environments by using *B. maritimus* plants as potential biomonitors. Additionally, the concentrations of the trace elements Cd, Pb and Ni, hazardous metals of large concern in the aquatic environment, were analyzed in samples of water, sediments, and *B. maritimus* collected seasonally from saltmarshes of Óbidos Lagoon during one year of study.

2. Materials and Methods

2.1. Study Area

The study area was at Óbidos Lagoon (39°24′ N, 9°17′ W), on the western coast of Portugal (Figure 1). This coastal ecosystem has an area of 17 km² and a mean depth of 3 m. In this system, longitudinal variations of salinity or stratification are not observed and

the semidiurnal tides, which range between 0.5 m and 4.0 m, spread their influence to the entire lagoon [21]. This lagoon is attached to the Atlantic Ocean by a small inlet (<100 m wide) in which the morphological transformations were reported on monthly time scales.

Figure 1. Location of the sampling station (S1) at the Óbidos Lagoon (Portugal).

Due to distinct hydromorphological and sedimentary characteristics, two separate regions are observed in the lagoon: an upper lagoon, with low water velocities, high residence time and muddy bottoms [21], and a lower lagoon, with various sand banks and channels, with strong water velocities and low residence time. Two inner branches are distinguished in the upper lagoon, the Barrosa arm at the east side and the Bom-Sucesso arm at the west side (Figure 1). The Barrosa arm is the shallower arm of the lagoon, with a mean depth of 0.5–1 m. Here, the water circulation is mainly propelled by the tides and by the small freshwater tributary, the Cal River. Freshwater discharge is negligible in summer (<0.05 $m^3 \cdot s^{-1}$) [3] and averages 3 $m^3 \cdot s^{-1}$ per year [39]. The sampling point, hereafter called S1, was selected in between those arms, at the vicinity of the Arnóia River mouth (Figure 1). This location is an area that drains the main agricultural fields of the region. This narrowed area was reported as an eutrophic zone by [26]. *Bolboschoenus maritimus* are found in the saltmarshes near the mouths of rivers that run into the Óbidos Lagoon. Those plants are exposed to periodic submersions by highly saline brackish waters (>30.0 in the summer). Several plants of *B. maritimus* are grown namely in S1, which allows us to easily collect samples for analyses.

2.2. Water, Sediments, and <u>B. maritimus</u> Plant Collection

Water and sediment samples were collected on a seasonal basis between 2009 and 2010, at S1, in the following months: May (spring), July (summer), September (autumn) of 2009, and in January (winter), April (spring) and July (summer) of 2010 (Figure 1). The sampling took place during low tides and the samples were immediately transported to the laboratory for treatment. To determine the concentration of trace metals in water (dissolved fraction and suspended fraction), surface water (at <0.5 m depth) was sampled in polypropylene bottles. Surface sediments were also collected by hand for the quantification of organic matter content and trace metal concentration. During the collection of the samples, environmental, hydrological and hydro chemical parameters such as Dissolved Oxygen (DO, %), salinity, water temperature and pH, were monitored in situ with a portable multiparameter probe (HANNA HI9828). Precipitation data were obtained from SNIRH, the National Water Resources Information System, as presented in the Supplementary Material.

At the same collection point (S1) and in the same months of 2009 and 2010, a minimum of 7 plants and a maximum of 29 plants were carefully collected, depending on their

abundance. This occurred at the marshes of the upper intertidal areas, where the salinity in low water tidal pools varied from 0.5 in winter to 31.0 in summer. Green plants of *B. maritimus*, with equivalent size and age, and without a senescent appearance were collected. To remove attached sediments, the plants were cleaned in the water from the lagoon and were afterwards transported in plastic containers to the laboratory.

2.3. Sample Treatment (Water, Sediment, and Plants)

Previous to the trace metal determination analyses, the glass and plastic materials were prepared and cleaned as in [39]. All the standard solutions were prepared daily from stock solutions with ultra-pure water.

At the laboratory, the water samples were filtered, using a Whatman GF/C glass-fiber filter, to extract the dissolved and suspended particulate matter. The dissolved trace metals analysis was performed starting with filtered water that was acidified (69% HNO_3), to a pH < 2, transferred to 50 mL plastic containers and stored frozen, at $-18\,°C$, until further analyses. For the determination of trace metal concentration in the suspended particulate matter of the water, the glass-fiber filter of each water sample was prepared for trace metals analysis following the digestion methods described in [29], and afterwards stored at $-18\,°C$, until further analyses.

Sediment mean grain size of the sampling stations was evaluated according to the method described in [39]. To evaluate the organic matter content in sediments a loss ignition at 450 °C, for 8 h, was performed and then the sediments were dried for 48 h, at 60 °C. The dried sediment samples were also prepared by digestion methods for trace metal analysis (as described in [39]) and stored at $-18\,°C$, until further analyses.

All plants were washed with ultrapure water, to remove slurry and epiphytic algae, and finally dried with paper towels. The plants were then fresh weighed (FW), measured for their length, and split into their parts: underground organs (roots, rhizomes and tubers) and aerial parts (stems and leaves), using a ceramic knife. The various parts of the plants were afterwards dried at 60 °C, during 48 h, dry weighed (DW) and powdered in a glass mortar. An acid digestion of the ground samples was performed: 0.1 g of dried material with two times 3 mL of 69% HNO_3 were dried on a hot plate at 150 °C, until 1 mL solution remained [40,41]. The 3 mL of 1% HNO_3 was added to the samples at room temperature, and finally these were filtered with ashless quantitative filter papers Whatman grade no. 41 (0.45 μm pore size, 55 mm Ø, Whatman GmbH, Dassel, Germany. The samples were then diluted with ultrapure water to make up the final volume of 50 mL, transferred to plastic containers and frozen ($-18\,°C$) until further analysis. After all the preparation, three independent replicates of each part of each individual plant sample were used for the trace metal analyses.

2.4. Trace Metal Analyses

Cd, Pb and Ni were the trace metals analyzed in all the samples collected because of their toxicity and persistence in coastal environments, and particularly in Óbidos Lagoon, where those elements were already reported in halophyte plants, such as *S. ramosissima* [29,30]. Atomic Absorption Spectrometry (AAS) (Thermo Scientific iCE3500 Atomic Absorption Spectrometer), with a graphite furnace (SOLAAR FS95 Furnace auto sampler) was the analytical technique used to quantify the concentrations of Cd, Pb and Ni in water (dissolved fraction and suspended particles), sediments and *B. maritimus* samples. Hollow cathode lamps were used for each metal (Cd: 10 mA; Pb: 10 mA; Ni: 15 mA; Thermo Electron Corporation). Argon was the carrier gas (Praxair Portugal Gases S.A., Portugal). According to the equipment specifications, 20 μL of matrix modifier (magnesium nitrate) was added in 1 mL of water, sediment and plants samples used for the detection of cadmium and lead. All the standard solutions were prepared daily for trace metal analysis, using the cadmium, lead and nickel stock solutions presented in [39]. Blank solutions were prepared for each type of trace metal and external calibrations were performed using aqueous matched standards. The detection limits of this technique for each metal are the

following: cadmium (0.2 µg·L^{-1}), lead (0.1 µg·L^{-1}) and nickel (10 µg·L^{-1}, as described in [41].

2.5. Data Analyses

Data normality and homoscedasticity were evaluated prior to analysis and variables were transformed whenever necessary. The effects of the year of sampling and of seasons on the trace metal concentrations of the water and sediment samples, as well as on the plant samples, were evaluated with one-Way ANOVA procedures, with post-hoc tests (Tukey HSD and LSD tests), using the SPSS Software package. Additionally, the Pearson correlation analysis was performed to analyze if there was any correlation between: (1) the concentration of metals in the environmental samples (water and sediments); (2) the concentration of metals detected in environmental samples and their concentration in the various parts of the plants (underground organs, stems and leaves) and in the whole plant which refers to the sum of the underground organs, and aerial parts (stems and leaves) (significance level *p*-value < 0.05). The concentrations in whole plants were only determined when the plants presented leaves.

To better understand the bioaccumulation ability of *B. maritimus* for the analyzed trace metals, several calculations were performed. The transportation index (Ti) was calculated to evaluate the proportion between leaf and rhizomes' metal concentrations as in [42], that depicts the plants' ability to translocate the metal ion form from rhizomes to leaves. The Bioaccumulation Factor (BAF), as previously used by [29] was estimated, and corresponds to the ratio between a contaminant concentration in the organism tissues and its concentration in the ambient water. Additionally, the plant/soil concentration ratio was measured to evaluate the amount of contaminant uptake [43].

3. Results

3.1. Physicochemical Parameters

At the sampling site, the highest water temperatures were observed in the summer of 2009 while the lowest temperatures were recorded in the winter of 2010 (Table 1). The highest pH values were registered during the autumn of 2009 and the summer of 2009, while the lowest were recorded in the winter of 2010 and in the spring of 2010. The highest salinity results were measured in the summer of 2009 and in the autumn of 2009, while the lowest salinities were measured in the winter and spring of 2010. The DO (%) showed the lowest values in both spring seasons and was highest in both summer seasons.

Table 1. Environmental parameters observed at the sampling station S1 for the seasons studied at the Óbidos Lagoon (Portugal) during 2009 and 2010. For quantitative features, the averages and deviation error recorded in the period under study were calculated and reported.

Station S1	Environmental Parameters		
Season/Year	Temperature (°C)	pH	Salinity	Dissolved Oxygen (%)
Spring/2009	18.37 ± 1.46	8.46 ± 0.24	16.29 ± 10.31	64.21 ± 10.62
Summer/2009	26.20 ± 1.01	8.56 ± 0.03	31.16 ± 0.43	91.52 ± 11.48
Autumn/2009	21.42 ± 0.00	8.73 ± 0.00	30.03 ± 0.00	84.8 ± 0.00
Winter/2010	12.97 ± 2.12	8.22 ± 0.28	1.29 ± 1.15	77.88 ± 12.38
Spring/2010	18.29 ± 4.41	7.95 ± 0.22	1.20 ± 0.73	64.74 ± 27.24
Summer/2010	20.27 ± 0.96	8.43 ± 0.09	5.99 ± 3.88	94.19 ± 4.48

3.2. Concentrations of Trace Metals in Water and Sediment

Lead was the metal with highest concentrations in water dissolved fractions, especially in the samples collected during the spring of 2009 (0.03 ± 0.00 mg·L^{-1}) (Figure 2a). Cadmium was not detected in these samples (Figure 2a). The highest concentrations of Ni quantified in the samples collected were values lower than 0.01 ± 0.00 mg·L^{-1} in the spring of 2009 and the winter of 2010.
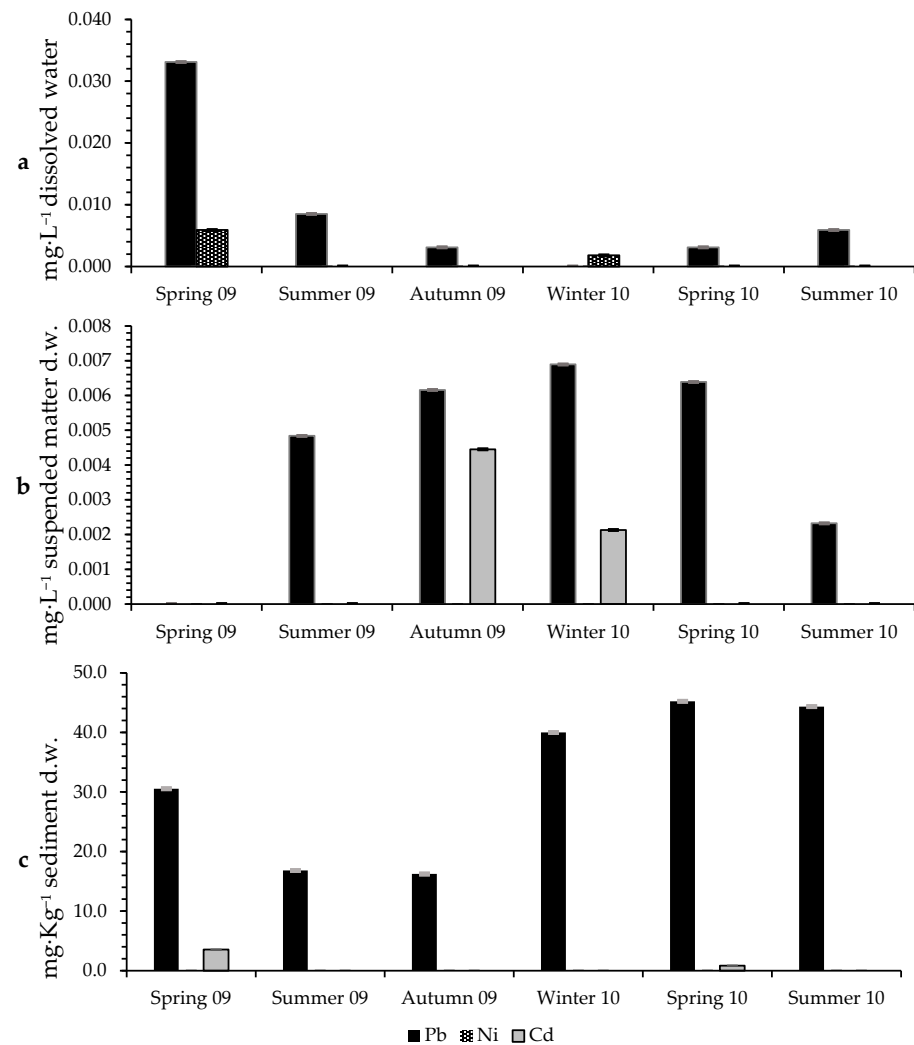
Figure 2. Final concentrations of Cd, Pb and Ni measured in the dissolved fractions of water samples (**a**), final concentrations of the suspended forms of Cd, Pb and Ni measured in water samples (**b**) and the final concentrations of Cd, Pb and Ni measured in sediment samples (**c**) collected seasonally on S1 at the Óbidos Lagoon (Portugal) during 2009 and 2010. d.w.—dry weight.

As for the trace metals determined in the suspended fraction of water (Figure 2b), Pb was, once again, the most abundant element recorded, with the highest concentrations obtained in samples from the autumn of 2009 (0.01 ± 00 mg·L^{-1}), and the winter and spring of 2010 (both 0.01 ± 00 mg·L^{-1}). The highest concentrations of Cd were reported in the autumn of 2009 (0.01 ± 0.00 mg·L^{-1}) and the winter of 2010 (0.01 ± 0.00 mg·L^{-1}), but Ni was not detected in the suspended fraction of the water samples collected, in any of the two years studied.

As for sediment samples, Pb was the most persistent trace metal, having been found in all samples, and with the highest concentrations (the lowest value was 16.23 ± 0.00 mg·kg^{-1} and the highest was 45.22 ± 0.00 mg·kg^{-1}) (Figure 2c), especially in both the 2009 and 2010 winter samples (43.06 ± 0.00 mg·kg^{-1} and 39.99 ± 0.00 mg·kg^{-1}, respectively) collected, and in the spring, and summer of the 2010 samples (45.22 ± 0.00 mg·kg^{-1} and 44.33 ± 0.00 mg·kg^{-1}, respectively). Concentrations of Cd recorded were also highest in the sediment samples of the winter and spring of 2009 (2.44 ± 0.00 mg·kg^{-1} and 5.54 ± 0.00 mg·kg^{-1}, respectively), compared with the results of the dissolved and suspended water fractions. Nickel was never detected in the sediment samples collected in the four seasons during 2009 and 2010, since their measurements were always below the detection limits of the analytical equipment. Results of the one-way ANOVA conducted, showed that neither the year of sampling nor

the seasons of the year influenced the presence of the studied metals in the environmental samples ($p > 0.05$). The Pearson correlation analysis between the concentrations of Cd, Pb and Ni in water (suspended and dissolved fractions) and in the sediments, on the other hand, demonstrated the existence of a strong positive correlation between the concentrations of Ni and Pb present in the dissolved fraction of water samples (Table 2).

Table 2. Pearson's correlation coefficients between the concentrations of metals (Cd, Pb and Ni) in the environmental samples of water and sediments collected and evaluated at the Óbidos Lagoon (Portugal). Significant correlations with $p < 0.05$ are highlighted in bold. susp frac—suspended fraction; dissol frac—dissolved fraction.

Pearson Correlation	Cd Water (susp frac)	Cd Sediments	Pb Water (dissol frac)	Pb Water (susp frac)	Pb Sediment	Ni Water (Dissol frac)
Cd water (susp frac)	1					
Cd sediments	−0.417	1				
Pb water (dissol frac)	−0.310	0.669	1			
Pb water (susp frac)	0.481	0.062	−0.552	1		
Pb sediments	−0.482	0.191	−0.232	−0.157	1	
Ni water (dissol frac)	−0.152	0.694	**0.880**	0.478	−0.045	1

3.3. Trace Metal Concentrations in B. maritimus Plants

The plants presented their highest lengths in the spring of 2010 (87.83 ± 22.43 cm), while the lowest lengths were obtained in plants collected in the spring of 2009 (31.78 ± 13.70 cm) (Figure 3). Additionally, measurements of the total length of the plants in the autumn of 2009 and the winter of 2010 were not possible since the plants did not present leaves in those seasons (the resulting measurements were exclusively of underground organs collected) (Figure 3).
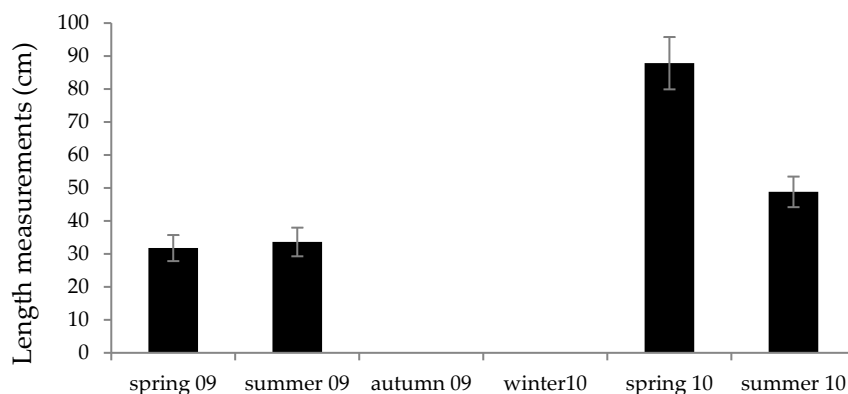

Figure 3. Total length measurements of *B. maritimus* plants collected at S1 at the Óbidos Lagoon (Portugal), across the seasons of 2009 and 2010. The concentrations in whole plants were only determined when the plants presented leaves. Therefore, data for autumn 2009 and winter 2010 are not shown to avoid inadequate comparisons. Values are means ($\pm$standard deviation).

Cd and Pb were the only trace elements detected in the samples of *B. maritimus* during this study (Figure 4), while the presence of Ni was not detected by the atomic absorption spectrometry instrument in any of the samples of *B. maritimus* (underground organs, stems, and leaves) that were analyzed.
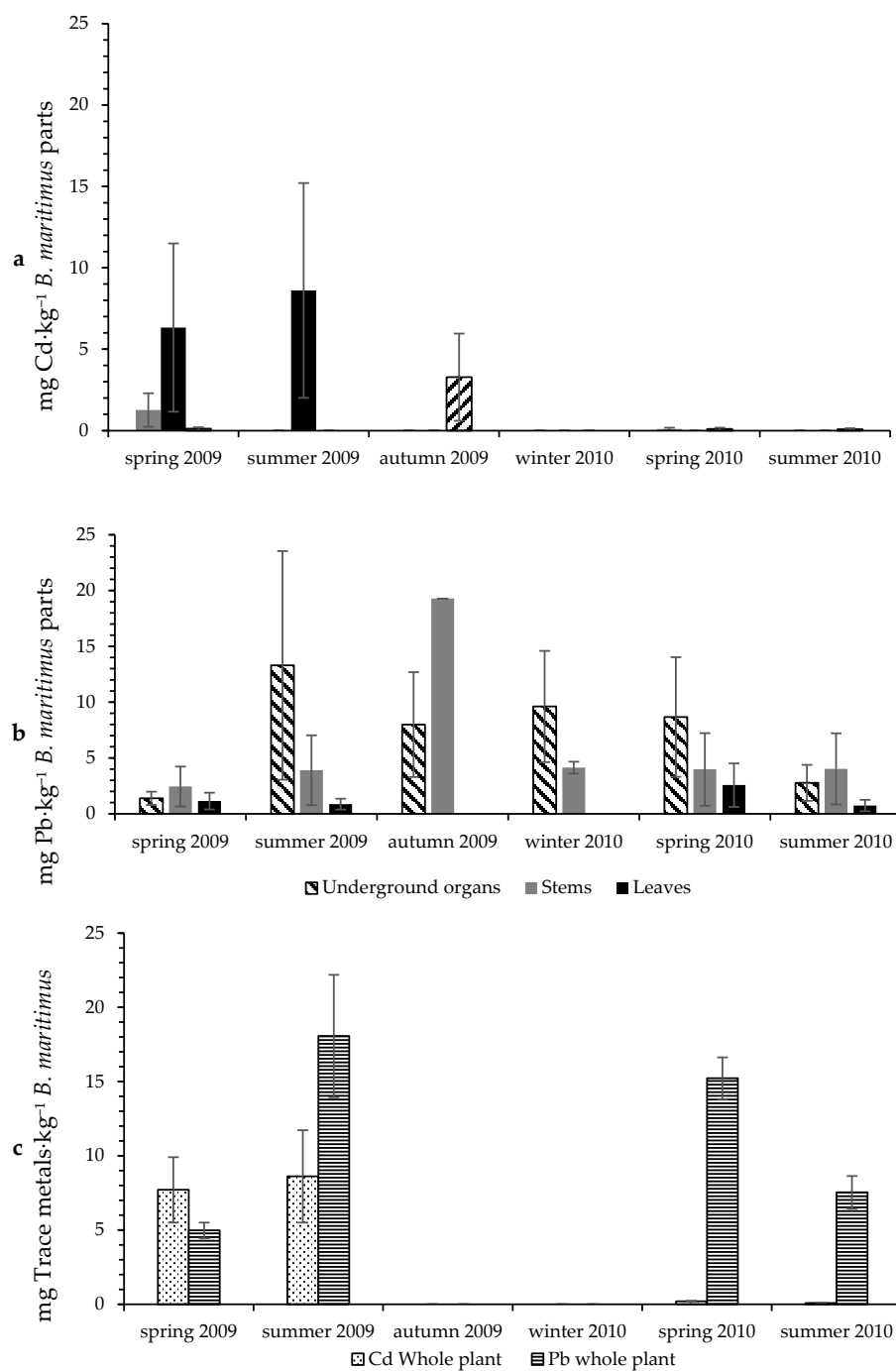
Figure 4. Final concentrations of Cd (mg·kg^{-1}) (**a**) and Pb (mg·kg^{-1}) (**b**) in underground organs, stems and leaves and final concentrations of Cd and Pb measured in plants overall (**c**) seasonally collected at S1 sampling station at the Óbidos Lagoon (Portugal) in the years of 2009 and 2010. Values are means (±standard deviation).

The leaves were the part of the plant where Cd concentrations were highest as opposed to underground organs and stems, particularly in plants collected in the spring and summer of 2009 (concentration in leaves: 6.33 ± 8.95 mg Cd kg^{-1} in spring and 8.61 ± 11.43 mg Cd kg^{-1} in the summer of 2009) (Figure 4a).

As for the lowest Cd concentrations recorded, these were detected in stems collected in the winter and spring of 2010 (concentration in stems: 1.26 ± 1.78 mg Cd kg^{-1} in winter, 0.10 ± 0.14 mg Cd kg^{-1} in the spring of 2010). In the remainder of samples from the underground organs, the metal Cd was not detected. The ANOVA analysis performed for

the underground organs, showed that Cd concentrations in the stems and in the leaves did not present significant statistical differences between the years of study, or between the seasons analyzed ($p > 0.05$).

Pb was detected in several parts of *B. maritimus* plants, particularly in the underground organs and in the stems (Figure 4b). The highest concentrations of Pb in the underground organs were registered in the summer of 2009 (13.29 ± 17.74 mg Pb kg^{-1}) and the lowest in the spring of 2009 (1.38 ± 1.03 mg Pb kg^{-1}). The stems presented the highest Pb concentrations in the autumn of 2009 (19.28 ± 0.00 mg Pb kg^{-1}) and the lowest in the spring of 2009 (2.45 ± 3.09 mg Pb kg^{-1}). As for the leaves of *B. maritimus*, the Pb concentration was highest in samples collected in the spring of 2010 (2.57 ± 3.36 mg Pb kg^{-1}) and lowest in samples collected in the summer of 2009 (0.86 ± 0.84 mg Pb kg^{-1}) and the summer of 2010 (0.73 ± 0.88 mg Pb kg^{-1}). The ANOVA analysis of Pb concentrations in the underground organs per year and per season did not present significant differences ($p > 0.05$).

Considering the whole plants of *B. maritimus*, Pb was the metal present in highest concentrations, in comparison with Cd (Figure 4c). The plants presented higher concentrations of Pb in the summer of 2009 (18.07 ± 7.14 mg Pb kg^{-1} *B. maritimus*) and the spring of 2010 (15.22 ± 2.44 mg Pb kg^{-1} *B. maritimus*). As for Cd, the highest concentrations were observed in the summer of 2009 (8.61 ± 5.38 mg Cd kg^{-1} *B. maritimus*) and the spring of 2009 (7.71 ± 3.82 mg Cd kg^{-1} *B. maritimus*).

Pearson correlation analysis between the concentrations of Cd and Pb, both in the environmental and in the *B. maritimus* samples, revealed that there were some significant correlations ($p < 0.05$) between the presence of these elements in the environmental samples and their presence in the distinct parts of the tested plants, especially for Cd (Table 3). A strong positive correlation was observed between the Cd present in the suspended fraction of water samples and the Cd present in underground organs, while a very strong positive correlation was obtained between the Cd present in the sediments and the Cd measured in the stems of the plants. Additionally, the concentrations of Cd measured in the whole plants were very strongly and positively correlated with the concentrations of this metal in the leaves. The environmental concentrations of Pb did not correlate significantly with any of the Pb concentrations observed in the several parts of the plants tested, but the Pb concentrations in the whole plants were strongly and positively correlated with the Pb concentrations in the stems.

Table 3. Pearson's correlation coefficients between the concentrations of metals (Cd and Pb) in the environmental samples of water and sediments and the concentrations of those metals in *Bolboschoenus maritimus* plants (underground organs, stems, leaves and in whole plant) collected and measured at the Óbidos Lagoon (Portugal). Significant correlations with $p < 0.05$ are highlighted in bold. susp frac—suspended fraction; dissol frac—dissolved fraction.

Pearson Correlation	Cd Water (susp frac)	Cd Sediments	Cd Underground Organs	Cd Stems	Cd Leaves	Cd Whole Plants
Cd water (susp frac)	1					
Cd sediments	−0.417	1				
Cd underground organs	**0.877**	−0.227	1			
Cd stems	−0.317	**0.987**	−0.196	1		
Cd leaves	−0.451	0.413	−0.317	0.460	1	
Cd whole plants	−0.196	0.461	−0.006	0.519	**0.944**	1

Pearson correlation	Pb water (dissol frac)	Pb water (susp frac)	Pb sediments	Pb underground organs	Pb stems	Pb leaves	Pb whole plants
Pb water (dissol frac)	1						
Pb water (susp frac)	−0.552	1					

Table 3. *Cont.*

Pearson Correlation	Cd Water (susp frac)	Cd Sediments	Cd Underground Organs	Cd Stems	Cd Leaves	Cd Whole Plants	
Pearson correlation	Pb water (dissol frac)	Pb water (susp frac)	Pb sediments	Pb underground organs	Pb stems	Pb leaves	Pb whole plants
Pb sediments	−0.232	−0.157	1				
Pb underground organs	−0.607	0.587	−0.383	1			
Pb stems	−0.329	0.258	−0.566	0.137	1		
Pb leaves	−0.281	0.827	0.484	0.195	0.152	1	
Pb whole plants	−0.584	0.555	−0.617	0.663	**0.825**	0.328	1

3.4. Bioaccumulation Ability of *B. maritimus*

Calculations of the Ti (%) of *B. maritimus* revealed that there was a higher translocation of Pb into the leaves in the samples collected in the spring of 2009, although this process was also observed in the spring and summer of 2010 on a much smaller scale (Figure 5a). In the summer and autumn of 2009 and the winter of 2010 the plants did not present leaves, and therefore Ti calculations were not possible to be performed.
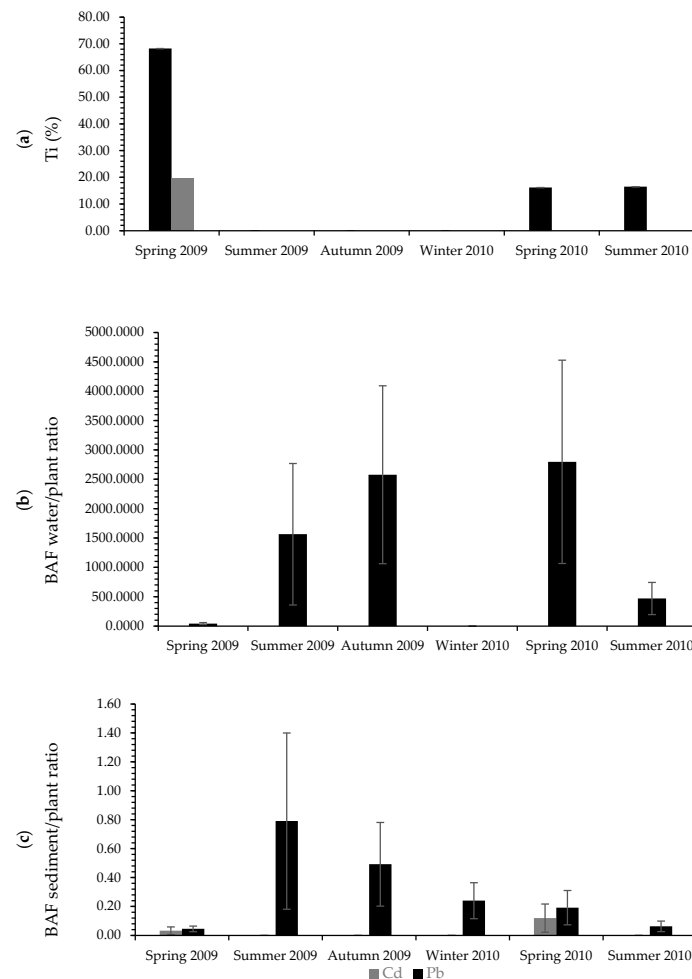

Figure 5. Cd and Pb transport index (Ti) in *B. maritimus* plants (**a**) Cd and Pb bioaccumulation factor (BAF) representing the water/plant ratio of *B. maritimus* (**b**) and the bioaccumulation factor (BAF) representing the sediment/plant ratio (**c**) seasonally collected in the Óbidos Lagoon (Portugal) in the years of 2009 and 2010. Values are means (±standard deviation).

To evaluate the bioaccumulation capacity of *B. maritimus* to retain Cd and Pb, BAF calculations were performed (Figure 5b). Concerning this ratio, the results indicated an

accumulation of Pb, while Cd was not accumulated. During the autumn of 2009 and spring 2010 the highest accumulations of Pb were measured. As for the plant/soil concentration ratios, Cd accumulation was observed during both the spring seasons of 2009 and 2010, while for Pb higher values were obtained in the summer and autumn of 2009 (Figure 5c).

4. Discussion

4.1. Physicochemical Parameters

At Óbidos lagoon, spatial heterogeneity of water quality and a progressive decline in water quality were previously reported in the literature [23]. The highest mean water temperatures were registered in the summer seasons of the current study, especially in 2009. It was also in summer 2009 that the remaining physicochemical parameters (pH, salinity, and DO) presented the highest values measured. The limited tidal waves seen in the lagoon induced the recorded temperatures, which may have been reinforced by solar radiation and convection processes in the homogeneous water column.

Another physical parameter analyzed was salinity, which depends on meteorological conditions (rains and temperatures), but also on the proximity of tributaries and on the hydrographic circulation of the system. This was seen between the collection point S1 and the proximity to the tributaries, where salinity decreases from the mouth to the upstream region. High salinities monitored in the summer and autumn of 2009 may be related to the low precipitation and the reduction of the freshwater inputs [25,44]. As expounded by [45], salt stress affected the trace metal bioaccumulation capacity of the plants.

At S1, the elevated dissolved oxygen measured over the different months was similar to previously reported results, such as in [22–24]. Sewage and domestic discharges foster an increase in nutrients in the water and potentially, the inception of eutrophication.

4.2. Trace Metal Concentrations in Water and Sediment

At Óbidos Lagoon, trace metal pollution is a significant environmental stressor factor, given that its watershed drains into an agricultural area. The presence of trace metals in water (dissolved and suspended fractions) was measured in samples collected at S1, a site where disposal is frequent. According to [46], 50 µg L^{-1} of Cd is the maximum allowed concentration (MAC) for irrigation waters, while for residual water discharges 200 µg L^{-1} of Cd is the emission limit value (ELV). The [13] regulated the Environmental Quality Standard Arithmetic Mean (EQS-AM) of Cd and their compounds in surface waters. For freshwater, the values depend on water hardness and are ≤0.08 and 0.25 µg L^{-1}, while for transitional, coastal, and territorial waters the value is 0.2 µg L^{-1}. Cadmium amounts present in water (dissolved and suspended fractions) (Figure 2a) were below those values, but the concentrations of this metal in the suspended fraction were higher as opposed to the dissolved fraction (Figure 2b). This metal is resistant to corrosion, however, as a salt, Cd combines with sulphates and chlorides and is one of the most soluble trace metals in water and is biopersistent in most organisms [30]. Cadmium solubility is also influenced by the pH, not being soluble above 7.5. In our study, pH values were above 7 (Table 1), suggesting that effluent discharges might have been responsible for the water contamination with this metal, and therefore, for the contamination of sediments. Despite the Cd concentrations measured being below the detection limits, which was also indicated for Cd in sediments from other reports [11,47], 2–2.5 mg kg^{-1} of Cd is pointed out as a maximum allowed concentration for sediments, according to the European Union regulations [48,49].

The organic matter content of the sediments quantified in this study were similar to the results reported in [22]. Infaunal communities directly contact the sediment, and in the sediment organic matter and other pollutants are easily accumulated [22], being afterwards bioaccumulated by the plants in saltmarshes. Lead was the trace metal with the highest concentration detected both in water and sediment samples collected at S1. Portuguese Decree Law [48] states that 50 µg L^{-1} of Pb is the maximum allowed concentration (MAC) for superficial waters. More recently, the [13] established for Pb and their compounds in surface waters, the value of 7.2 µg L^{-1} for fresh, transitional, coastal, and territorial waters.

Pb present in the dissolved fraction and suspended matter is related to their solubility and translocation (Figure 2). Under standard conditions (T = 20 °C and P = 1 bar), Pb is insoluble in water, but it can also appear in its ionic form (Pb^{2+}), which is highly mobile and bioavailable. The highest concentrations of this metal were reported in the suspended fraction of water in the seasons of 2009 and 2010, except in the spring of 2009. Dissolved Pb concentrations were reported in the range of 50 ng L^{-1} to 300 ng L^{-1} in coastal waters of the Atlantic ocean affected by anthropogenic activities [49,50]. Wastewater streams, pipes and soils are sources of suspended forms of Pb, which may then ascend to higher trophic levels via food chains [50]. This is in agreement with the results obtained in this work at Óbidos Lagoon.

Ni was detected in water samples in the spring of 2009, but it was not detected in a suspended form or in sediment samples. The [13] has defined the value of EQS-AM for Ni as 20×10^{-3} mg L^{-1} for surface water. The results obtained in the present study were within this range of values. This is seen because some Ni compounds are water soluble, with Ni chloride the most soluble form of this metal in water (553 g L^{-1} at 20 °C to 880 g L^{-1} at 99.9 °C) or Ni carbonate (90 mg L^{-1}). Others are water insoluble, such as Ni oxide, Ni sulfide and Ni tetra-carbonyl as previously reported by [51].

4.3. Trace Metal Concentrations in *B. maritimus*

The abundance of the plants collected in the different seasons (results not shown) were in accordance with the life cycle of *B. maritimus*, with higher abundances in spring and summer, since *B. maritimus* rhizomes present tubers that are reserves of carbohydrates and allow plant growth; these germinate in autumn, emerging from the sediment in winter. At spring, these monoic plants form flowers and fruits due to sexual reproduction [29]. Therefore, total length measurements were only possible in spring and summer seasons, with the plants reaching higher lengths in 2010, where the samples are indicated as whole plants (underground organs, stems and leaves).

The amount of elements absorbed by macrophytes in shallow coastal ecosystems is related to the content of those elements in the abiotic compartment, but also to the life-form of the plants [52,53]. Therefore, trace metals present in water and sediment samples affected the presence of trace metals in *B. maritimus*. The translocation of trace metals through the plants is not restricted to underground organs, stems or leaves and there are different protective mechanisms against the uptake of trace metals to restrain their transport within the body of the plant [28]. During growth, the absorption of essential elements increases, and higher concentrations of trace metals may be obtained at the mature stage of the plant. Metal uptake occurs most probably through the root cells, using similar uptake processes as the ionic metals that are essential micronutrients for the growth of plants [54]. Nevertheless, different apoplastic structures, together with cell walls, work as filters, restricting substance movements inside the symplast and in the aerial parts. The cell vacuoles of most hyperaccumulator plants store metals, setting them apart from the cytosol [27,28].

In accordance with previous studies [29,30,55], underground organs of halophyte plants, such as *B. maritimus* and *S. ramosissima* present the highest concentrations of trace metals, such as cadmium and lead, as seen in Figure 4. Since the stems of those plants have vascular tissues with lower metabolic activity, less accumulation of metals than in underground organs is expected [29,56].

Some species of halophyte plants, such as *Salicornia europea*, are capable of absorbing Cd in the rhizomes and accumulate it in the aerial parts [56]. In the autumn of 2009, the highest concentration of Cd present in underground organs may be a result of the nutrient uptake that occurs in the senescent aerial part. This may be achieved because of translocation to the underground over wintering tissues [28,29]. The leaves evidenced a higher accumulation of Cd during the spring and summer of 2009 (Figure 4b), showing that cadmium content in leaves was linked to the plants' life-cycle. In accordance with [28], Cd does not influence plant growth, particularly in rhizomatous plants, which agrees with

the present results (Figure 3). The pH of the sediments influences the capacity of plants to absorb Cd, since when pH increases, Cd mobility decreases and the dissolved form of this metal decreases accordingly [57].

Due to the presence of Cd in water and in sediments, the correlation obtained in the present work between Cd in the suspended fraction of water samples and Cd in the underground organs of the plants was expected, because the biochemical form of Cd present in the water allows the metal to be bioavailable for translocation [56–62]. Additionally, since Cd is a predominant trace metal found in the dissolved form which, as previously reported by the literature, was detected in sediments of this lagoon, there is an increased possibility of translocation of this metal in the plants [59–62]. This is supported by the very strong positive correlation found between the concentrations of Cd in sediments and in *B. maritimus*' stems.

Pb was observed in the distinct parts of *B. maritimus* in the four seasons of 2009 and 2010, especially in stems and underground organs. Additionally, the concentrations of Pb in water and sediment samples were also the highest, with a high availability of this trace metal able to be translocated and accumulated in several parts of the plant, revealing the capacity of *B. maritimus* to accumulate Pb in its tissues. In fact, the strong positive Pearson correlation observed between the Pb concentration in the stems of the plants and the Pb concentration in whole plants suggests that most of the Pb translocated by the plants was accumulated in their stems. This is in accordance with works performed with *Scirpus grossus*, where higher concentrations of Pb were observed in the upper parts of the plant rather than in the roots [63]. Following the previous results reported in [29,30], as well as in the work of [58] where Cd and Pb were detected in distinct parts of *B. maritimus* plants collected in the lower Diep River (South Africa), *B. maritimus* can actually be used as a biomonitor for trace metals.

4.4. Bioaccumulation Ability of B. maritimus

Biomonitors are organisms that hold information on quantitative aspects of the quality of their environment [64]. For *B. maritimus* to be seen as an adequate biomonitor for trace metal contamination in saltmarshes, the plants must be able to bioaccumulate these pollutants from their surrounding environment.

For the trace metal Cd, higher concentrations of this metal were found in leaves of *B. maritimus*, especially in those collected in the spring and summer of 2009, leading to the observed reported results in the Ti (%) and BAF depicted in Figure 5. Ti (%) values confirm that Cd uptake follows the life cycle of the plant and is related to the uptake of trace metals through the roots, in stems up through the leaves (up to 20%) (Figure 5b). *B. maritimus* Ti analyses for the absence of Cd were below 20%, as similarly reported in a previous work by [55]. Cadmium's Tis (%) in *B. maritimus* were higher than 1 and are in agreement with the results of Figure 4, where plants were able to retain higher concentrations in their tissues during the spring of 2009, when full plants were collected and measured. Similar Ti (%) values were already reported in other plants, such as *T. repens* in polluted soils growing in a heavy metal-contaminated field near a closed smelter in France, which accumulated Cd in both roots and shoots [59].

As for BAF water/plant ratio (Figure 5b) and for sediment/plant ratio (Figure 5c), it was not possible to calculate those ratios for Cd, except in the case of sediment/plant ratio in both spring seasons. However, Ti and BAF results reported for Cd indicated that the presence of this trace metal in the plant was higher than from non-polluted environments, classifying *B. maritimus* as a biomonitor of the presence of this metal at the Óbidos Lagoon, in the warmer seasons.

In terms of Pb, Ti (%) values were always higher than 1 and reached the highest values also in the spring of 2009 (68.22%), spring of 2010 (16.16%) and summer of 2010 (16.48%), seasons in which *B. maritimus* was collected as a full plant (Figure 5a). The trace metal Pb, reached the highest results for most of the seasons for the plants collected, demonstrating that *B. maritimus* is a suitable biomonitor for this metal, namely at the

Óbidos Lagoon. The highest values of BAF water/plant ratio were obtained in the spring of 2010, reaching 2797 (Figure 5b), but for BAF sediment/plant ratio the value obtained in this season was only 0.19, one of the lowest results (Figure 5c). The remaining results were similar, suggesting that plants can transport and accumulate Pb in their tissues during the four seasons, except in the winter of 2010. As explained by [27], the trace metal content of the shoots was not affected by the distance from the inlet, but it was easier for the plant to translocate trace metals closer to the underground organs proficiently, than from the water to the soil, where trace metals mostly exist as insoluble forms and are not bioavailable to plants [61]. The present results therefore demonstrate that the uptake of Pb in *B. maritimus* plants is an important factor, and that this plant efficiently transports this metal from the underground organs to the shoots, also suggesting the development of tolerance mechanisms to endure high concentrations of this element [44]. As stated in [62], the BAF value for other halophytes such as *Scirpus mucronatus* was >1 for Pb, where the authors also concluded that Pb bioaccumulates when contaminants carried by the plant are not degraded rapidly. In their study, the concentration of Pb was not higher than $1000 \, \text{mg} \cdot \text{kg}^{-1}$ in the shoot, though the ability of S. *mucronatus* to endure and store heavy metals was beneficial for phytostabilization. Other works such as [62], indicated that *Scirpus americanus* and *T. latifolia* could extract Pb, from their water surroundings, as demonstrated by the higher concentrations of heavy metals in roots as opposed to stem and leaves. This agrees with the results obtained in the present work. The last authors also stated that the uptake of Pb from root to stem was likely to occur in both species and assumed that, because of their capacity to extract Pb from their lagoon and their capability to accumulate this metal in their roots, *S. americanus* and *T. latifolia* are interesting alternatives to restore polluted water systems. Following this reasoning, *B. maritimus* can be considered both as a biomonitor and as a bioindicator for Pb and Cd at the Óbidos Lagoon. The plant *B. maritimus* can indicate the presence of trace metals in the ecosystem in all seasons, due to the accumulation of these pollutants in their tissues as demonstrated by the Ti and BAF results, making them suitable for seasonal monitoring.

5. Conclusions

The results obtained in this work increase the understanding of the accumulation and translocation capacity of *B. maritimus* in coastal lagoons regarding contamination by trace metals and may support coastal management actions for trace metal bioaccumulation and pollution monitoring. The concentrations of the trace metals, Cd, Pb and Ni recorded as present in water and sediment samples in Óbidos Lagoon were a result of local disposals and seasonal variation. The accumulation of Cd and Pb in the various parts of these plants suggests that *B. maritimus* is capable of retaining those trace metals in their tissues and able to translocate them to the leaves. The plant *B. maritimus* can be considered an adequate biomonitor for cadmium and lead, especially in spring and summer, where the highest concentrations, Ti and BAF results were reported. Additionally, this halophyte plant can be used as a phytoremediator for these pollutants, to remove those from water and sediments or to render them innocuous. This is an approach that provides an appealing alternative, with minimal disturbance to the ecosystem's efficiency due to reduced levels of associated contamination and the potential for recovery of trace metals, using plants such as *B. maritimus* as bioaccumulators.

Phytoremediation techniques in marine environments, particularly in coastal lagoons, will help to identify new strategies to monitor trace metal pollution, using plants as biomonitors in situ and to identify pollution specific points that can be strategically used for more political regulation and environmental control.

References

1. Robbins, L.J.; Mänd, K.; Planavsky, N.J.; Alessi, D.S.; Konhauser, K.O. Trace metals. In *Encyclopedia of Astrobiology*; Gargaud, M., Irvine, W.M., Amils, R., Claeys, P., Cleaves, H.J., Gerin, M., Rouan, D., Spohn, T., Tirard, S., Viso, M., Eds.; Springer: Berlin/Heidelberg, Germany, 2020.
2. Namieśnik, J.; Rabajczyk, A. The speciation and physicochemical forms of metals in surface waters and sediments. *Chem. Spec. Bioavailab.* **2010**, *22*, 1–24. [CrossRef]
3. Malhadas, M.S.; Neves, R.J.; Leitão, P.C.; Silva, A. Influence of tide and waves on water renewal in Óbidos Lagoon, Portugal. *Ocean. Dynam* **2010**, *60*, 41–55. [CrossRef]
4. Duarte, B.; Carreiras, J.; Caçador, I. Climate change impacts on salt marsh blue carbon, nitrogen and phosphorous stocks and ecosystem services. *Appl. Sci.* **2021**, *11*, 1969. [CrossRef]
5. The Habitats Directive. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. *Off. J. Eur. Communities* **1992**, *206*, 50.
6. Portuguese Decree of Law 149/2004, of 22 June 2004, approving a list of identification of sensitive areas and zones less sensitive, amending Directive 91/492/EC of the European Parliament and of the Council of 21 May 1991. *Port. Repub. J. Gov. "Diário Da República"* **2004**, *145*, 3380–3805.
7. Council of the European Communities. Directive 2009/147/EEC of the European Parliament and of the Council of 30 November 2009 on the conservation of wild birds. *Off. J. Eur. Communities* **2010**, *20*, 7–25.
8. Sousa, A.I.; Caçador, I.; Lillebø, A.I.; Pardal, M.A. Heavy metal accumulation in *Halimione portulacoides*: Intra- and extra-cellular metal binding sites. *Chemosphere* **2008**, *70*, 850–857. [CrossRef]
9. Pan, K.; Wang, W.-X. Trace metal contamination in estuarine and coastal environments in China. *Sci. Total Environ.* **2012**, *421–422*, 3–16. [CrossRef]
10. Chapman, D. *Water Quality Assessments—A Guide to Use of Biota, Sediments and Water in Environmental Monitoring*, 2nd ed.; UNESCO/WHO/UNEP: Cambridge, UK, 1992.
11. Wang, P.; Chen, H.; Kopittke, P.M.; Zhao, F.-J. Cadmium contamination in agricultural soils of China and the impact on food safety. *Environ. Pollut.* **2019**, *249*, 1038–1048. [CrossRef]
12. He, Y.; Xiang, Y.; Zhou, Y.; Yang, Y.; Zhang, J.; Huang, H.; Shang, C.; Luo, L.; Gao, J.; Tang, L. Selenium contamination, consequences and remediation techniques in water and soils: A review. *Environ. Res.* **2018**, *164*, 288–301. [CrossRef]
13. Portuguese Decree of Law, no 103/2010, of 24 September, approving the environmental quality standards in the field of water policy and implementing Directive 2008/105/EC and in part Directive 2009/90/EC. *Port. Repub. J. Gov. "Diário Da República"* **2010**, *187*, 4289–4296.

14. Portuguese Decree of Law 76/2016, of 31 May, approving the national plan for water. *Port. Repub. J. Gov. "Diário Da República"* **2016**, *215*, 3951–4007.

15. El Mahrad, B.; Abalansa, S.; Newton, A.; Icely, J.D.; Snoussi, M.; Kacimi, I. Social-environmental analysis for the management of coastal lagoons in north Africa. *Front. Environ. Sci.* **2020**, *8*, 37. [CrossRef]

16. D'Alpaos, C.; D'Alpaos, A. The valuation of ecosystem services in the Venice Lagoon: A multicriteria approach. *Sustainability* **2021**, *13*, 9485. [CrossRef]

17. Rebelo, C.F.C.; Alves, C.P.F.; Moiteiro, G.C.; Ezequiel, G.M.G.; Brasão, I.P.C.; Vasconcelos, J.V.; Carvalho, M.J.P.J. Tourism through the gaze of stakeholders: The case of Óbidos Lagoon in Portugal. *Tour. Plan. Dev.* **2015**, *12*, 447–462. [CrossRef]

18. Willaert, T.; García-Alegre, A.; Queiroga, H.; Cunha-e-Sá, M.A.; Lillebø, A.I. Measuring vulnerability of marine and coastal habitats' potential to deliver ecosystem services: Complex Atlantic Region as case study. *Front. Mar. Sci.* **2019**, *6*, 2296–2774. [CrossRef]

19. Silva, C.V.; Ortigão, M.; Willaert, T.; Rosa, R.; Nunes, L.C.; Cunha-e-Sá, M.A. Participatory Geographic Information Systems (PGIS): Alternative approaches to identify potential conflicts and positional accuracy in marine and coastal ecosystem services. *Mar. Pol.* **2021**, *131*, 104650. [CrossRef]

20. Malhadas, M.S.; Nunes, S.A.; Neves, R.; Carvalho, S.M.; Couto, C.F.; Zenha, H.S. Impact of Casalito wastewater treatment plant discharge on Óbidos Lagoon water quality. In Proceedings of the 11th Conference on Environmental Science and Technology, Chania, Greece, 3–5 September 2009.

21. Carvalho, S.; Gaspar, M.B.; Moura, A.; Vale, C.; Antunes, P.; Gil, O.; da Fonseca, L.C.; Falcão, M. The use of the marine biotic index AMBI in the assessment of the ecological status of the Óbidos lagoon (Portugal). *Mar. Pollut. Bull.* **2006**, *52*, 1414–1424. [CrossRef]

22. Pereira, P.; de Pablo, H.; Vale, C.; Rosa-Santos, F.; Cesário, R. Metal and nutrient dynamics in a eutrophic coastal lagoon (Óbidos, Portugal): The importance of observations at different time scales. *Environ. Monit. Assess.* **2009**, *158*, 405–418. [CrossRef]

23. Pereira, P.; de Pablo, H.; Vale, C.; Franco, V.; Nogueira, M. Spatial and seasonal variation of water quality in an impacted coastal lagoon (Óbidos Lagoon, Portugal). *Environ. Monit. Assess.* **2009**, *153*, 281–292. [CrossRef]

24. Pereira, P.; de Pablo, H.; Dulce Subida, M.; Vale, C.; Pacheco, M. Biochemical responses of the shore crab (*Carcinus maenas*) in a eutrophic and metal-contaminated coastal system (Óbidos lagoon, Portugal). *Ecotox Environ. Safe* **2009**, *72*, 1471–1480. [CrossRef]

25. O'Leary, J.W.; Glenn, E.P. Global distribution and potential for halophytes. In *Halophytes as a Resource for Livestock and for Rehabilitation of Degraded Lands. Tasks for Vegetation Science Book Series*; Squires, V.R., Ayoub, A.T., Eds.; Springer: Dordrecht, The Netherlands, 1994; Volume 32.

26. Almeida, C.M.R.; Mucha, A.P.; Vasconcelos, M.T.S.D. Comparison of the role of the sea club-rush *Scirpus maritimus* and the sea rush *Juncus maritimus* in terms of concentration, speciation and bioaccumulation of metals in the estuarine sediment. *Environ. Pollut.* **2006**, *142*, 151–159. [CrossRef]

27. Bragato, C.; Brix, H.; Malagoli, M. Accumulation of nutrients and heavy metals in *Phragmites australis* (Cav.) Trin. Ex. Steudel and *Bolboschoenus maritimus* (L.) Palla in a constructed wetland of the Venice Lagoon watershed. *Environ. Pollut.* **2006**, *144*, 967–975. [CrossRef]

28. Madejón, P.; Murillo, J.M.; Marañón, T.; Espinar, J.L.; Cabrera, F. Accumulation of As, Cd and selected trace elements in tubers of *Scirpus maritimus* L. from Doñana marshes (South Spain). *Chemosphere* **2006**, *64*, 742–748. [CrossRef]

29. Pedro, C.A.; Santos, M.S.S.; Ferreira, S.M.F.; Gonçalves, S.C. The influence of cadmium contamination and salinity on the survival, growth and phytoremediation capacity of the saltmarsh plant *Salicornia ramosissima*. *Mar. Environ. Res.* **2013**, *92*, 197–205. [CrossRef]

30. Pérez-Romero, J.A.; Redondo-Gómez, S.; Mateos-Naranjo, E. Growth and photosynthetic limitation analysis of the Cd-accumulator *Salicornia ramosissima* under excessive cadmium concentrations and optimum salinity conditions. *Plant Physiol. Biochem.* **2016**, *109*, 103–113. [CrossRef]

31. Milić, D.; Bubanja, N.; Ninkov, J.; Milić, S.; Vasin, J.; Luković, J. Phytoremediation potential of the naturally occurring wetland species in protected Long Beach in Ulcinj, Montenegro. *Sci. Total Environ.* **2021**, *797*, 148995. [CrossRef]

32. Couto, C.M.C.M.; Ribeiro, C. Pollution status and risk assessment of trace elements in Portuguese water, soils, sediments, and associated biota: A trend analysis from the 80s to 2021. *Environ. Sci. Pollut. Res.* **2022**, *29*, 48057–48087. [CrossRef]

33. Agoramoorthy, G.; Chen, F.A.; Hsu, M.J. Threat of heavy metal pollution in halophytic and mangrove plants of Tamil Nadu, India. *Environ. Pollut.* **2008**, *155*, 320–326. [CrossRef]

34. Shuping, L.S. Biomonitoring of Metal Contamination in the Lower Diep River, Milnerton, Western Cape. Master's Thesis, Cape Peninsula University of Technology, Cape Town, South Africa, 2008.

35. Marques, B.; Lillebø, A.I.; Pereira, E.; Duarte, A.C. Mercury cycling and sequestration in salt marshes sediments: An ecosystem service provided by *Juncus maritimus* and *Scirpus maritimus*. *Environ. Pollut.* **2011**, *159*, 1869–1876. [CrossRef]

36. Hroudová, Z.; Zákravský, P.; Flegrová., M. The tolerance to salinity and nutrient supply in four European Bolboschoenus species (*B. maritimus*, *B. laticarpus*, *B. planiculmis* and *B. yagara*) affects their vulnerability or expansiveness. *Aquat. Bot.* **2014**, *112*, 66–75.

37. Lillebø, A.I.; Pardal, M.A.; Neto, J.M.; Marques, J.C. Salinity as the major factor affecting *Scirpus maritimus* annual dynamics: Evidence from field data and greenhouse experiment. *Aquat. Bot.* **2003**, *77*, 111–120. [CrossRef]

38. Oliveira, A.; Fortunato, A.B.; Rego, J.R.L. Effect of morphological changes on the hydrodynamics and flushing properties of the Óbidos Lagoon (Portugal). *Cont. Shelf Res.* **2006**, *26*, 917–942. [CrossRef]

39. Pedro, C.A.; Santos, M.S.S.; Ferreira, S.M.F.; Gonçalves, S.C. The presence of cadmium in the intertidal environments of a moderately impacted coastal lagoon in western Portugal (Óbidos Lagoon): Spatial and seasonal evaluations. *Environ. Sci. Pollut. R.* **2016**, *23*, 1960–1969. [CrossRef]

40. Sharma, A.; Gontia, I.; Agarwal, P.K.; Jha, B. Accumulation of heavy metals and its biochemical responses in *Salicornia brachiata*, an extreme halophyte. *Mar. Biol. Res.* **2010**, *6*, 511–518. [CrossRef]

41. Barzev, A.; Dobreva, D.; Futekov, L.; Rusev, V.; Bekjarov, G.; Toneva, G. Determination of detection limits in graphite furnace atomic absorption spectrometry by using ensemble summation of signals. *Fresenius Z. Anal. Chem.* **1986**, *325*, 255–257. [CrossRef]

42. Ghosh, M.; Singh, S.P. A review on phytoremediation of heavy metals and utilization of its byproducts. *Asian J. Energy Environ.* **2005**, *6*, 214–231.

43. Cheraghi, M.; Lorestani, B.; Yousefi, N. Introduction of hyperaccumulator plants with phytoremediation potential of a lead- zinc mine in Iran. *Int. J. Geol. Environ. Eng.* **2011**, *5*, 289–294.

44. Crivelli, A.J.; Ximenes, M.-C.; Gout, B.; Lassere, G.; Freon, P.; Do Chi, T. Causes and effects of terrestrial runoff and riverine outflow on brackish/coastal marine fisheries ecosystems in the northern Mediterranean region. *FAO Fish. Tech. Pap.* **1995**, *349*, 59–88.

45. Santos, M.S.S.; Pedro, C.A.; Gonçalves, S.C.; Ferreira, S.M.F. Phytoremediation of cadmium by the facultative halophyte plant *Bolboschoenus maritimus* (L.) Palla, at different salinities. *Environ. Sci. Pollut. Res.* **2015**, *22*, 15598–15609. [CrossRef]

46. Decree Law n.º 236/98, 1 de Agosto, Diário da República n.º 176/1998, Série I-A de 1998-08-01; pp. 3676–3722. Available online: https://diariodarepublica.pt/dr/detalhe/decreto-lei/236-1998-430457 (accessed on 1 June 2023).

47. Pradit, S.; Pattarathomrong, M.S.; Panutrakul, S. Arsenic, cadmium and lead concentrations in sediment and biota from Songkhla Lake: A review. *Procedia—Social. Behav. Sci.* **2013**, *91*, 573–580. [CrossRef]

48. Commission Regulation (EC). No 466/2001 of 8 March 2001 Setting Maximum Levels for Certain Contaminants in Foodstuffs (Text with EEA Relevance). *Off. J. L* **2001**, *42*, 16–22. Available online: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2001R0466:20060701:EN:PDF (accessed on 1 June 2023).

49. Davis, W.J. Contamination of coastal versus open ocean surface waters: A brief meta-analysis. *Mar. Pollut. Bull.* **1993**, *26*, 128–134. [CrossRef]

50. Angel, B.M.; Apte, S.C.; Batley, G.E.; Raven, M.D. Lead solubility in seawater: An experimental study. *Environ. Chem.* **2015**, *13*, 489–495. [CrossRef]

51. Von Burg, R. Nickel and some nickel compounds. *J. Appl. Toxicol.* **1997**, *17*, 425–431. [CrossRef]

52. Mazej, Z.; Germ, M. Trace element accumulation and distribution in four aquatic macrophytes. *Chemosphere* **2009**, *74*, 642–647. [CrossRef]

53. Bonanno, G.; Borgb, J.A.; Di Martino, V. Levels of heavy metals in wetland and marine vascular plants and their biomonitoring potential: A comparative assessment. *Sci. Total Environ.* **2017**, *576*, 796–806. [CrossRef]

54. Ross, S.M.; Kaye, K.J. The meaning of metal toxicity in soil and plant system. In *Toxic Metals in Soil and Plant System*; Ross, S.M., Ed.; John Wiley & Sons: New York, NY, USA, 1994; pp. 27–61.

55. Santos, M.S.S. Phytoremediation of Cadmium at Different Salinities by *Scirpus maritimus* from the Óbidos Lagoon (Portugal). Master's Thesis, School of Tourism and Maritime Technology, Olytechnic Institute of Leiria, PPeniche, Portugal, 2011.

56. Ozawa, T.; Miura, M.; Fukuda, M.; Kakuta, S. Cadmium tolerance and accumulation in a halophyte *Salicornia europaea* as a new candidate for phytoremediation of saline soils. *Sci. Rep. Grad. Sch. Life Environ. Sci. Osaka Prefect. Univ.* **2009**, *60*, 1–8.

57. Sintorini, M.M.; Widyatmoko, H.; Sinaga, E.; Aliyah, N. Effect of pH on metal mobility in the soil. In Proceedings of the 5th International Seminar on Sustainable Urban Development, Jakarta, Indonesia, 5 August 2020; Volume 737, IOP Conference Series: Earth and Environmental Science.

58. Shuping, L.S.; Snyman, R.G.; Odendaal, J.P.; Ndakidemi, P.A. Accumulation and distribution of metals in *Bolboschoenus maritimus* (Cyperaceae), from a south African river. *Water Air Soil. Pollut.* **2011**, *216*, 319–328. [CrossRef]

59. Bidar, G.; Pruvot, C.; Garçon, G.; Verdin, A.; Shirali, P.; Douay, F. Seasonal and annual variations of metal uptake, bioaccumulation, and toxicity in *Trifolium repens* and *Lolium perenne* growing in a heavy metal-contaminated field. *Environ. Sci. Pollut. Res.* **2009**, *16*, 42–53. [CrossRef]

60. Yan, A.; Wang, Y.; Tan, S.N.; Yusof, M.L.M.; Ghosh, S.; Chen, Z. Phytoremediation: A promising approach for revegetation of heavy metal polluted land. *Front. Plant Sci.* **2020**, *11*, 359. [CrossRef]

61. Hamzah, A.; Sarmani, S.B.; Yatim, N.I. Phytoremediation of Pb and Hg by using *Scirpus mucronatus* with addition of bacterial inoculums. *J. Radioanal. Nucl. Chem.* **2015**, *304*, 151–155. [CrossRef]

62. Carranza-Álvarez, C.; Alonso-Castro, A.; Alfaro-De La Torre, M.C.; García-De La Cruz, R.F. Accumulation and distribution of heavy metals in *Scirpus americanus* and *Typha latifolia* from an artificial lagoon in San Luis Potosí, México. *Water Air Soil. Poll.* **2008**, *188*, 297–309. [CrossRef]

63. Tangahu, B.V.; Abdullah, S.R.S.; Basri, H.; Idris, M.; Anuar, N.; Mukhlisin, M. Phytotoxicity of wastewater containing lead (Pb) effects *Scirpus grossus*. *IJP* **2013**, *15*, 814–826.

64. Markert, B.A.; Breure, A.M.; Zechmeister, H.G. (Eds.) *Bioindicators and Biomonitors—Principles, Concepts and Applications*; Elsevier: Amsterdam, The Netherlands, 2003; pp. 3–21.