

Trace metals in *Posidonia oceanica* in a coastal area of the Ionian Sea (Calabria, Italy)

Radiana Cozza*, Adele Iaquinta, Davide Cozza, Luisa Ruffolo

Dipartimento di Biologia, Ecologia e Scienze della Terra, Università della Calabria, Arcavacata di Rende, Cosenza, Italy;

*Corresponding Author: radiana.cozza@unical.it

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ABSTRACT

As, Cd, Cu and Pb concentrations were measured in *Posidonia oceanica* sampled from meadows located in two sites along the calabrian coast (Ionian Sea, South-eastern Mediterranean). By dating the scales and the rhizome using retrospective procedures (lepidochronology), a time series over a period of nine years (1995-2004) was analysed. Throughout the whole lepidochronological period, the arsenic content in the scales was tenfold higher than that measured in the rhizome with a peak in the lepidochronological year 1996-1997 and a general decrease in the years after. Pb concentration also showed a tendency to decrease with time, whereas the Cd and Cu concentration were increasing. A comparison of the metal level in dead sheaths (scales) and living tissue (leaves) was also performed. The detailed distribution of the trace metals along the leaf axis and in the leaves at different developmental stages (adult, intermediate and juvenile) showed some differences in the metal content. The results also indicate that the arsenic content measured in the leaf blade reflects the high As content measured at the superficial sediments at one of the two sampled sites, demonstrating, for the first time, that *P. oceanica* would be a good indicator for this element.

Keywords: Biomonitoring; Heavy Metals; Ionian Sea; Lepidochronology; *Posidonia oceanica*

1. INTRODUCTION

Detection and monitoring of environmental contamination in the Mediterranean, an almost closed sea, are matters of concern, particularly in heavily urbanized and

industrialized areas [1]. The major sources of pollution of surface waters include effluent discharges by industries, urban discharge, atmospheric depositions of pollutants and occasional accidental spills of toxic chemicals. Trace metals are regarded as serious pollutants of the aquatic environment because they are persistent, potentially toxic and naturally bioaccumulated [2,3]. There is currently a great interest in the use of living organisms as pollution biomonitors in aquatic ecosystems [4,5]. Among these, the seagrasses *Zostera marina* L. and *Posidonia oceanica* L. (Delile), key species of the coastal ecosystems, have been used as a metal bioindicator for several decades [6-16]. Arsenic can be taken up, bioaccumulated and biotransformed. Its toxic effects depend on the chemical form of its compounds and there is a growing interest in the quantification and identification of the arsenic in matrices related to human and animal food (e.g. sediments, algae, seagrasses, shellfish). Also it has been observed that *P. oceanica* could “record” past metal contamination [17-23]. Annual heavy metal accumulation can be analyzed by dating fragments of rhizomes and scales (dead sheaths of the leaves) according to plant annual life cycle [17]. Therefore, the time course of such a contamination can be reconstructed over a period ranging between few to many years. Since the early 1950's, the area of Crotona (South-Eastern Mediterranean-Ionian Sea-Calabria, Italy), has become one of the most important petrochemical sites in the South of Italy, with several petrochemical plants and many other industrial installations. About the metal contamination of this zone, there are very few published data [24]; in accordance with Italian law 208 of 30/12/2008, the same industrial area was declared an “area at high risk of environment crisis” and must be subjected to environmental restoration for serious contamination by heavy metals (overall As, Zn, Cd, Cr). In this context, we performed a lepidochronological analyses by sampling *P. oceanica* from meadows growing in two different sites

(referred as Disturbed and Control, see section below) in order to detect, for the first time at a local scale, the level of the trace metals As, Cd, Cu, Pb. The distribution of these metals was also verified with respect to the leaf axis (base vs apical portion) as well as the stage of the leaf development (juvenile vs adult leaf), in order to suggest some differences along a gradient of leaf age. The total arsenic occurrence was furthermore analysed in *P. oceanica* during a relatively long period (nine years) demonstrating the capability of the scales as a preferential organs to biotransfer this toxic contaminant.

2. MATERIAL AND METHODS

2.1. Sampling and Tissues Preparation

Plants of *P. oceanica* were collected during the summer of 2004 by scuba diving at 10 ± 1 m depth from meadows growing in two sites of the East coast of Calabria (Ionian Sea, Italy) (**Figure 1**). Probably, the two sampling sites were differently impacted: the first meadow was at Località Tonnara ($38^{\circ}54.881'N$ $16^{\circ}59.845'E$), a site very close to the industrial harbour of Crotona, characterized by petrochemical traffic and a metallurgical plant, therefore this site was considered as disturbed (D); the second meadow was located at Capo Bianco ($38^{\circ}55.014'N$ $17^{\circ}07.857'E$), away from the bay of Crotona, in the Marine Protected Area (MPA) of Capo Rizzuto (**Figure 1**); this area is usually considered a pristine region with widespread *P. oceanica* meadows [25] and was considered as a reference site (control: C). Plants of *P. oceanica* were collected in each meadow along five different transects that were 50 meters separate; twenty plants were collected in each area with orthotropic rhizomes (vertical growth) at a distance of at least 50 - 100 cm apart in order to collect plants belonging to different individuals. The associated superficial sediments were also collected to a depth of about 5 cm.

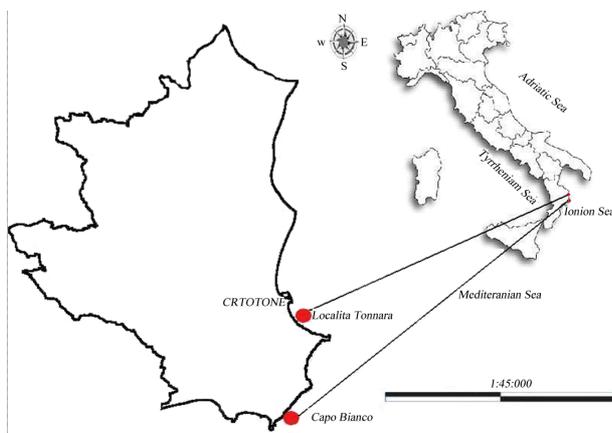


Figure 1. Study area and sampling locations (indicated by circles) along the Ionian coast of Calabria.

The macroscopic epiphytes were then carefully removed by gently scraping and washing and the leaves were separated into adult, intermediate and juvenile leaves according to Giraud (1979) [26]. Each leaf was fractionated in three different portions along the main leaf axis: the tip (last 3 cm), the basal portion (the growing one, including part of the blade and the sheath) and the intermediate one. For the lepidochronological analyses, the dead sheaths (scales) and rhizomes were fractionated according to Pergent (1990) [27]. The collected tissues were dried at $105^{\circ}C$ for 48 h, milled to obtain particles of approximately 100 μm in size and then processed for metal analyses. Also the sediment samples, after sieving on 2-mm meshes, were dried at $105^{\circ}C$ for 48 h.

2.2. Trace Metal Analyses

Three subsamples of the ground material (500 mg-dw) were hot digested in a microwave oven with a mixture of 5 ml of HNO_3 65% and 2 ml of H_2O_2 30%. As, Cd, Cu and Pb concentrations were determined in all samples by an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent Technologies, 7500 CE Octopole Reaction System). The accuracy of the method was evaluated by its calibration versus an international standard (Stock Standard, Environmental Calibration Standard Agilent; Internal Standard Mix Agilent). A parallel analysis of blanks was also carried out to quantify possible contamination. The obtained data were analysed using the Student's t-test for the paired sample and the results expressed as mean \pm S.E. of three independent replicates for every metal analysed. The trace metals content in different tissue, sites and years were compared by 2-way ANOVA using the software package "R" (The R Foundation for Statistical Computing ISBN 3-900051-07-0, version 2.14.1).

3. RESULTS

3.1. Lepidochronological Analyses

The arsenic content detected in the scales was tenfold higher than in the rhizomes, over all sampled years and in both sites (**Figures 2(A)** and **(B)**; **Tables 1, 2**). In particular, the scales sampled at the D site showed a significant higher level of As compared with the C one in the period 1995-2004, with a peak of $22.3 \mu g \cdot g^{-1} \cdot dw^{-1}$ at the D site after which a significantly decreasing trend was detected (**Figure 2(A)**, **Table 1**). In the same lepidochronological year (1997) a peak of As ($2.26 \mu g \cdot g^{-1} \cdot dw^{-1}$) was also detected in the rhizome, after which a significantly downward trend was observed (**Figure 2(B)**, **Table 2**). For Cd, comparable levels between scales and rhizomes were measured (**Figures 2(C)** and **(D)**) with the highest values ($1.89 \mu g \cdot g^{-1} \cdot dw^{-1}$) in the rhizome in 1995 at the D

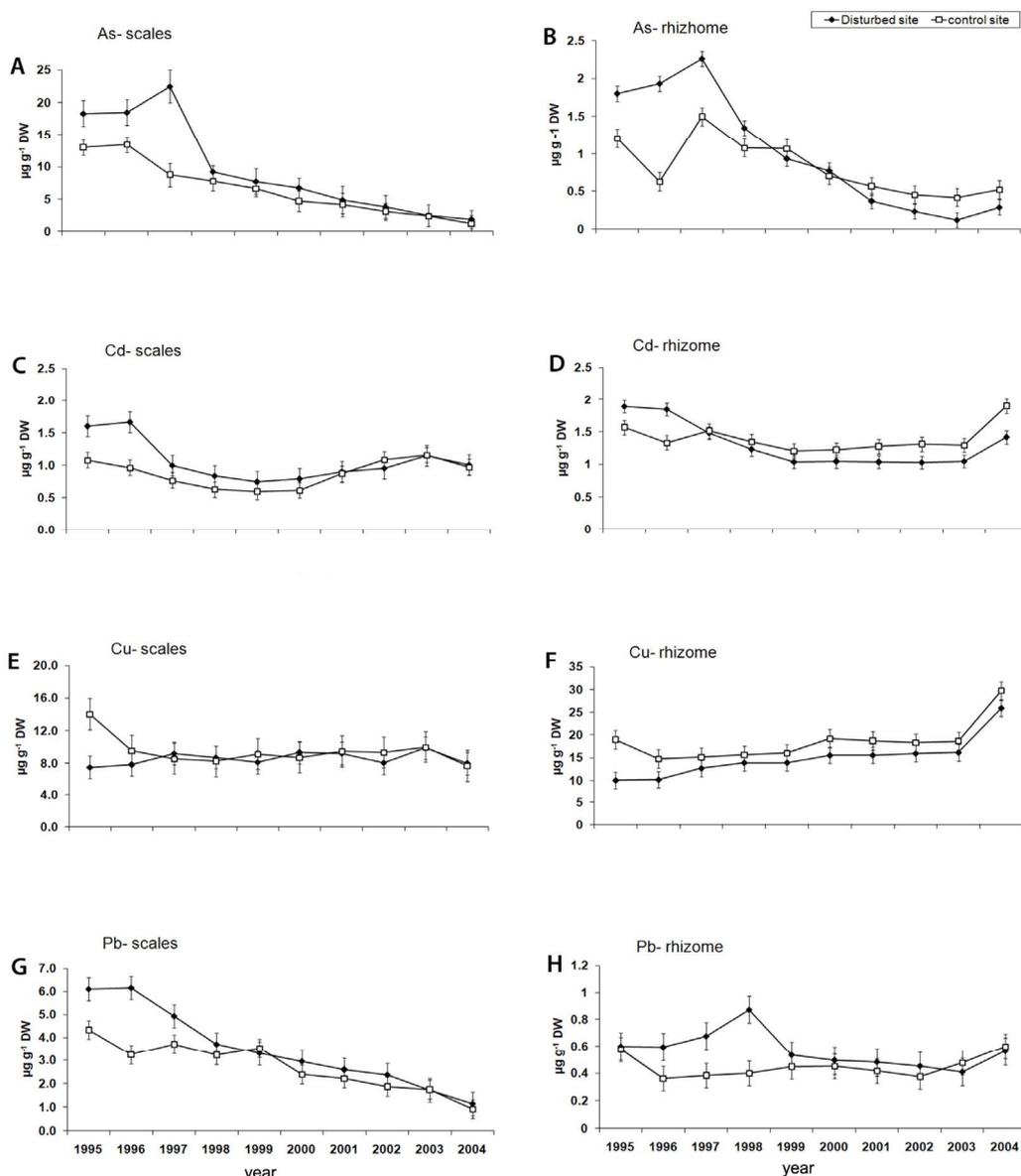


Figure 2. Temporal trend of contamination of heavy metals in *P. oceanica*. Mean (\pm SE, $n = 5$) metal concentration ($\mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$) was measured in scales (A) (C) (E) (G) and rhizomes (B) (D) (F) (H)) sampled at a Disturbed site and a Control site. Scales and rhizomes were dated using the lepidochronological method of Perget-Martini (1998).

site (**Figure 2(D)**); both in the scales as in the rhizome a significant temporal fluctuation was observed (**Figures 2(C) and (D)**); **Tables 1 and 2**). The content of Cu in the rhizomes was higher than in the scales with a clear trend to increase during the whole lepidochronological period reaching the maximum values ($29.71 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$) in 2004 in the rhizome of the both sites (**Figures 2(E) and (F)**); **Table 2**). The concentration of Pb was significantly higher in the scales than the rhizomes (**Figures 2(G) and (H)**); **Tables 1 and 2**). The highest Pb concentration ($6.2 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$) was recorded in the scales in the lepidochronological year 1996 at the D site; after which a constant

significant decline was detected (**Figure 2(G)**); **Table 1**); otherwise, the Pb level measured in the rhizomes was approximately constant along the whole lepidochronological period, except a peak in the D site in the year 1998 (**Figure 2(H)**); **Table 2**).

3.2. Trace Metals in Different Compartments of *P. oceanica* Plants

Table 3 shows the distribution of As, Cd, Cu, Pb in *P. oceanica* specimens and in the upper 5 cm of sediments sampled at the Disturbed and Control site respectively. The distribution of metals in the plant varied considera-

Table 1. Mean metal concentration ($\mu\text{g}\cdot\text{g}^{-1}$) \pm SE and 2-way ANOVA from the scales sampled at the Disturbed and Control site at each time period.

	As		Cd		Pb		Cu									
	Disturbed site	Control site	Disturbed site	Control site	Disturbed site	Control site	Disturbed site	Control site								
1995	18.4 \pm 1.15	13.1 \pm 0.26	1.66 \pm 0.24	1.66 \pm 0.24	1.66 \pm 0.24	1.66 \pm 0.24	1.66 \pm 0.24	1.66 \pm 0.24								
1996	18.5 \pm 0.70	13.6 \pm 0.50	1.66 \pm 0.21	1.66 \pm 0.21	1.66 \pm 0.21	1.66 \pm 0.21	1.66 \pm 0.21	1.66 \pm 0.21								
1997	22.5 \pm 0.89	8.69 \pm 0.70	0.97 \pm 0.08	0.97 \pm 0.08	0.97 \pm 0.08	0.97 \pm 0.08	0.97 \pm 0.08	0.97 \pm 0.08								
1998	9.16 \pm 0.34	7.90 \pm 0.27	0.86 \pm 0.11	0.86 \pm 0.11	0.86 \pm 0.11	0.86 \pm 0.11	0.86 \pm 0.11	0.86 \pm 0.11								
1999	7.85 \pm 0.50	6.79 \pm 0.38	0.74 \pm 0.09	0.74 \pm 0.09	0.74 \pm 0.09	0.74 \pm 0.09	0.74 \pm 0.09	0.74 \pm 0.09								
2000	6.67 \pm 0.70	4.77 \pm 0.25	0.80 \pm 0.06	0.80 \pm 0.06	0.80 \pm 0.06	0.80 \pm 0.06	0.80 \pm 0.06	0.80 \pm 0.06								
2001	4.96 \pm 0.85	4.17 \pm 0.32	0.87 \pm 0.05	0.87 \pm 0.05	0.87 \pm 0.05	0.87 \pm 0.05	0.87 \pm 0.05	0.87 \pm 0.05								
2002	3.88 \pm 0.01	3.12 \pm 0.27	1.03 \pm 0.03	1.03 \pm 0.03	1.03 \pm 0.03	1.03 \pm 0.03	1.03 \pm 0.03	1.03 \pm 0.03								
2003	2.47 \pm 0.46	2.46 \pm 0.48	1.20 \pm 0.12	1.20 \pm 0.12	1.20 \pm 0.12	1.20 \pm 0.12	1.20 \pm 0.12	1.20 \pm 0.12								
2004	1.83 \pm 0.24	1.31 \pm 0.23	1.06 \pm 0.22	1.06 \pm 0.22	1.06 \pm 0.22	1.06 \pm 0.22	1.06 \pm 0.22	1.06 \pm 0.22								
Sites	Analysis of Variance Table Response: As				Analysis of Variance Table Response: Cd				Analysis of Variance Table Response: Pb				Analysis of Variance Table Response: Cu			
Years	Df	Sum Sq	Mean Sq	F value	Df	Sum Sq	Mean Sq	F value	Df	Sum Sq	Mean Sq	F value	Df	Sum Sq	Mean Sq	F value
	1	46.03	46.029	5.197*	1	0.157	0.157	6.159*	1	3.276	3.277	7.846*	1	4.316	4.316	1.857
Residuals	9	585.88	65.098	7.350**	9	1.323	0.148	5.767**	9	33.967	3.774	9.037**	9	13.798	1.533	0.660
	9	79.72	8.857		9	0.229	0.026		9	3.758	0.418		9	20.921	2.325	
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1																

Table 2. Mean metal concentration ($\mu\text{g}\cdot\text{g}^{-1}$) \pm SE and 2 way ANOVA from the rhizomes sampled at the Disturbed and Control site at each time period.

	As		Cd		Pb							
	Disturbed site	Control site	Disturbed site	Control site	Disturbed site	Control site						
1995	1.78 \pm 0.15	1.27 \pm 0.21	1.89 \pm 0.12	1.57 \pm 0.07	0.61 \pm 0.04	0.58 \pm 0.06						
1996	1.93 \pm 0.16	0.63 \pm 0.09	1.84 \pm 0.08	1.33 \pm 0.07	0.61 \pm 0.08	0.35 \pm 0.05						
1997	2.27 \pm 0.46	1.49 \pm 0.25	1.48 \pm 0.07	1.53 \pm 0.07	0.72 \pm 0.03	0.39 \pm 0.04						
1998	1.35 \pm 0.16	1.09 \pm 0.18	1.23 \pm 0.09	1.35 \pm 0.08	0.91 \pm 0.03	0.41 \pm 0.04						
1999	0.93 \pm 0.10	1.10 \pm 0.11	1.03 \pm 0.16	1.25 \pm 0.06	0.52 \pm 0.04	0.40 \pm 0.08						
2000	0.77 \pm 0.11	0.71 \pm 0.04	1.04 \pm 0.25	1.22 \pm 0.05	0.50 \pm 0.02	0.45 \pm 0.06						
2001	0.38 \pm 0.03	0.57 \pm 0.06	1.04 \pm 0.07	1.27 \pm 0.05	0.49 \pm 0.06	0.42 \pm 0.09						
2002	0.24 \pm 0.05	0.44 \pm 0.05	1.03 \pm 0.01	1.33 \pm 0.05	0.44 \pm 0.05	0.37 \pm 0.05						
2003	0.12 \pm 0.02	0.43 \pm 0.05	1.05 \pm 0.02	1.30 \pm 0.09	0.41 \pm 0.03	0.49 \pm 0.04						
2004	0.29 \pm 0.01	0.52 \pm 0.04	1.42 \pm 0.07	1.88 \pm 0.09	0.56 \pm 0.04	0.60 \pm 0.04						
Site	Analysis of Variance Table Response: As				Analysis of Variance Table Response: Cd				Analysis of Variance Table Response: Pb			
Years	Df	Sum Sq	Mean Sq	F value	Df	Sum Sq	Mean Sq	F value	Df	Sum Sq	Mean Sq	F value
	1	0.162	0.162	1.138	1	0.051	0.051	1.166	1	0.088	0.088	5.414*
Residuals	9	5.502	0.611	4.299*	9	1.013	0.113	2.597	9	0.120	0.013	0.822
	9	1.280	0.142		9	0.390	0.043		9	0.146	0.016	
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1												

Table 3. Trace metal concentration ($\mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$) in leaves, scales, rhizomes of *Posidonia oceanica* collected from meadows at disturbed site control site. Values are means ($\pm\text{SE}$, $n = 3$) of samples collected along a transect in five different areas at the same depth. The leaves were considered at different developmental stage (adult, intermediate and juvenile) and fractionated along the leaf axis in a tip, middle and basal portion. For each trace metal, the maximum values measured in the tissues of *P. oceanica*, are in bold.

	Disturbed site				Control site			
	As	Cd	Cu	Pb	As	Cd	Cu	Pb
Adult leaves Tip	0.41 \pm 0.04	7.28 \pm 0.03	7.71 \pm 0.03	2.29 \pm 0.02	3.91 \pm 0.02	7.78 \pm 0.05	5.58 \pm 0.02	0.95 \pm 0.01
Middle	0.30 \pm 0.03	4.92 \pm 0.04	11.26 \pm 0.05	1.61 \pm 0.01	0.75 \pm 0.10	4.74 \pm 0.03	10.58 \pm 0.03	0.97 \pm 0.01
Base	0.15 \pm 0.01	0.71 \pm 0.02	12.40 \pm 0.03	0.79 \pm 0.01	0.20 \pm 0.01	1.32 \pm 0.01	16.68 \pm 0.06	0.42 \pm 0.01
Intermediate leaves Tip	0.36 \pm 0.01	6.28 \pm 0.06	8.33 \pm 0.02	2.52 \pm 0.01	1.90 \pm 0.10	5.35 \pm 0.05	9.25 \pm 0.02	1.19 \pm 0.01
Middle	0.26 \pm 0.01	2.46 \pm 0.01	10.86 \pm 0.03	1.01 \pm 0.02	0.71 \pm 0.02	2.75 \pm 0.01	14.03 \pm 0.04	0.76 \pm 0.01
Base	0.36 \pm 0.01	1.71 \pm 0.02	34.16 \pm 0.01	2.50 \pm 0.01	0.22 \pm 0.10	5.35 \pm 0.01	34.20 \pm 0.10	0.32 \pm 0.01
Juvenile leaves	0.20 \pm 0.01	1.41 \pm 0.01	22.01 \pm 0.07	0.70 \pm 0.01	0.54 \pm 0.03	2.01 \pm 0.01	26.32 \pm 0.05	0.60 \pm 0.01
Scales	1.83 \pm 0.30	1.01 \pm 0.01	7.96 \pm 0.01	1.15 \pm 0.01	1.25 \pm 0.10	0.97 \pm 0.02	7.68 \pm 0.05	0.93 \pm 0.01
Rhizomes	0.29 \pm 0.20	0.42 \pm 0.03	25.86 \pm 0.05	0.56 \pm 0.01	0.53 \pm 0.10	1.90 \pm 0.20	29.71 \pm 0.20	0.60 \pm 0.01
Sediments	15.5 \pm 0.50	0.12 \pm 0.02	2.17 \pm 0.50	6.63 \pm 0.50	31.75 \pm 0.19	0.06 \pm 0.01	2.03 \pm 0.14	4.57 \pm 0.35

bly depending on the organ and/or the tissue examined. In the case of As, the concentration measured in leaves as well as scales and rhizomes, differed significantly at the two sampling station with the highest value ($3.91 \pm 0.02 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$) in the tip of the adult leaf sampled at the C site; interestingly at the same site the As content in the sediments was higher ($31.75 \pm 0.19 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$) (Table 3) than the contamination limit of $20 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$, according to Legislative Italian Decree 152/06 of the Environmental Ministry. For the Cd, despite that the sediment of the D site showed a value of Cd twice compared to the C one (1.12 ± 0.02 vs $0.06 \pm 0.01 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$; low contamination limit of $2 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$) its concentration in the plants varied, ranging from $0.42 \pm 0.03 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$ in the rhizome of the D site, to $7.78 \pm 0.05 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$ in the tip the adult leaf sampled at the C one (Table 3). The Cu level measured in the sediments showed comparable values at the two sampling sites (2.17 ± 0.19 vs $2.03 \pm 0.14 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$), values relatively low if compared with the contamination limit of $120 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$; in the *P. oceanica* tissues the highest values were detected in the basal portion of intermediate leaves in both the sample sites, with a maximum of 34.2 ± 0.10 in the D site, followed by rhizome and juvenile leaves sampled in the C site (Table 3). Regarding the Pb, the superficial sediments at the D site showed an higher level than the C one ($6.63 \pm 0.48 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$ vs $4.57 \pm 0.35 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$) but still well below the limit of contamination ($100 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$); notwithstanding in the tissue of *P. oceanica* the highest concentration ($2.52 \pm 0.01 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$) was detected in the intermediate leaves (tip and base) sampled in D site (Table 3).

4. DISCUSSION

The lepidochronological data show a relatively high level of As in the scales suggesting that the scales could be an elective compartments to biotransfer this element and also an useful organ for recording the past arsenic contamination. The concentration of As is consistent with the range of $14 - 21 \mu\text{g}\cdot\text{g}\cdot\text{dw}^{-1}$ reported by Gosselin *et al.* (2006) [14] in the sheaths of *P. oceanica* but is higher compared to the levels reported for other seagrasses [28-32]. Interestingly, the trend of As is similar both in the scales and in the rhizomes, with the highest values until 1999 followed by a falling trend; this could be related to peak of the industrial activity (overall metallurgical) developed around Crotona city during the decades before 1996-1997; afterwards, some important metallurgical plants were closed. For Cd, Cu and Pb, the level reported in *P. oceanica* in this study falls in the lower half of the range reported at the scale of the Mediterranean [15], highlighting a trend that is consistent with the global dynamics of contamination for these elements. Regarding the distribution of these trace metals in the different compartments of the plants; it has been observed differences in the different developmental stage of the leaf and also along the leaf axis, confirming that the metabolic condition and the lifecycle stage of the organ may influence the metal uptake and accumulation. As general role, the data highlighted that the apical tissues of mature leaves showed the highest levels of trace metals except for Cu, confirming its metabolic role during growth and lignifications [33,34]. However, the levels of Cu observed in this study are higher compared to previous studies on *P. oceanica* [14] suggesting that our sites

are exposed to these metal. Regarding the As, our results suggest that total As was mainly concentrated at the tip of mature leaves in the site with high As content in the sediments (Control site). Although the heavy metal contents of submerged macrophytes are often found to be correlated to the levels observed in the sediment, [35,36], the physico-chemical characteristics of sediment may greatly influence this pattern [37,38]. This study, even if at a local scale, confirms that the lepidochronology is a useful tool to provide evidence of past metal contamination in marine environment. Finally, taking into account the level of As measured in the blade and/or in the scales, we suggest that *P. oceanica* would be a suitable biomonitor to assess the present and past As contamination.

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