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An Effective Biomonitor of Potentially Toxic Elements in Marine Ecosystems: The Brown Alga *Dictyota spiralis*

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Abstract: Coastal marine areas are threatened by different forms of pollution, among which potentially toxic elements (PTEs) represent a primary hazard. In this study, 16 Mediterranean macroalgae colonizing the upper eulittoral and infralittoral zones were studied for their PTE accumulation capabilities in order to identify possible biomonitors that could replace the use of *Posidonia oceanica*, a protected species. To achieve this objective, macronutrients (Ca, K, Mg, P, S), micronutrients (Cr, Cu, Fe, Mn, Na, Ni, Si, V, Zn) and non-essential elements (Cd, Pb) were analyzed in the thalli of different algal species, the leaves of *P. oceanica* and in sediments collected from six sampling sites along the Cilento coast (Campania, Italy), all characterized by different anthropogenic pressures. For sediments, a sequential extraction of PTEs to evaluate their bioavailability profile was also carried out together with the analysis of mineralogical composition, particle size distribution, pH and organic matter content. Macrophytes, belonging to different divisions (six Rhodophyta, four Chlorophyta, six Heterokontophyta, one Embryophyta), are characterized by different PTE concentrations, with a few ones being characterized by an even accumulation response toward the different PTEs. One of these, the brown alga *Dictyota spiralis*, is able to accumulate PTEs in concentrations similar to *P. oceanica* and provides more accurate concentration gradients, highlighting clear pollution scenarios that were overlooked using *P. oceanica* only. Therefore, *D. spiralis* is a useful PTE biomonitor of coastal marine ecosystems and a suitable replacement for *P. oceanica*, also featuring the possibility of being employed in active biomonitoring applications.

Keywords: passive and active biomonitoring; PTE accumulation; marine macroalgae; *Dictyota spiralis*; *Posidonia oceanica*; sediments; coastal marine ecosystems; Mediterranean Sea; marine protected areas

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1. Introduction

In recent decades, coastal marine ecosystems have been increasingly subjected to various forms of chemical pollution [1,2], a stressor with potentially pervasive effects on biodiversity and ecosystem functioning. In spite of the Marine Strategy Framework Directive [3] highlighting marine habitat conservation as the greatest contemporary challenge for the survival of marine ecosystems (and for the trophic webs associated with them), current monitoring activities usually focus on a limited number of contaminants, and several pollutants are only occasionally monitored or properly managed [4,5].

Among the inorganic pollutants, potentially toxic elements (PTEs) heavily affect marine ecosystems due to their persistence in the environment and the ability to bioaccumulate and biomagnify along the food chains, often causing irreparable damage to marine and associated terrestrial environments [6]. Historically, their monitoring in marine ecosystems has mostly focused on water, with comparatively few studies using sediments and biota to evaluate environmental contamination. On the one hand, this strategy can be justified on both technical and theoretical bases, as water analysis is simpler and cheaper than the analysis of other matrices, while water is also the primary exposure route for planktonic organisms and filter-feeders. However, the strategy has important shortcomings, such as high dilution and the temporal/spatial variability in PTEs concentrations [7]. On the other hand, sediments represent long-term reservoirs of PTEs with small temporal fluctuations in concentrations, which are also usually higher than in water due to PTE preferential binding on sediment particles. The analysis of sediments, however, also has drawbacks deriving from their spatial heterogeneity in particle size, mineralogy and organic matter content [8] affecting, together with local ionic conditions and pH [9], the binding of PTEs. Therefore, measurements of PTEs in sediments only are often misleading for the assessment of the risks they actually pose to the marine ecosystem.

A valuable alternative to water and sediment analyses focuses on selected marine organisms (macroalgae, fish, crustaceans, etc.) that are able to accumulate pollutants in relation to the environmental concentrations (biomonitors), and can thus be effectively used to assess the quality of coastal environments, integrating temporal and spatial pollution gradients [10]. Whereas the analysis of the abiotic matrices provides an estimation of the total load of contaminants without any information on the fraction of direct ecotoxicological relevance [11], biomonitors provide information on pollutant bioavailability in the environment over time, as well as on the actual risk for biota deriving from pollutant exposure [8,12–16]. In this context, biomonitoring of PTEs, by directly measuring their concentrations in selected bioaccumulators, is an effective and low-coast way to accurately assess the pollution gradients of PTEs, as well as their possible transfer through food webs [17,18], especially in combination with sediment or water analyses [19].

Due to their bioaccumulation capability, various species of marine plants and algae are commonly used as PTE biomonitors in the Mediterranean Sea. The seagrass most used in this context is *Posidonia oceanica* (L.) Delile [20], an endemic Mediterranean angiosperm colonizing hard and soft bottoms at up to a 40 m depth, a trait responsible for its direct exposure to coastal pollution [21]. In particular, *P. oceanica* leaves provide indications of PTE concentrations over short time spans (months), with a good accuracy [22,23] due to their wide absorption area for seawater pollutants and the contribution of root uptake from sediment interstitial water [24]. Although the conservation status of *P. oceanica* is currently classified as being of Least Concern in the Red List of Threatened Species of the International Union for the Conservation of Nature [25], an ongoing decline in the meadows it forms is being observed throughout the Mediterranean Sea due to anthropogenic activities [26]. The loss of this invaluable ecosystem imposes the adoption of strict conservation measures for this species and, therefore, the timely identification of alternative PTE biomonitors for marine coastal environments.

Marine macroalgae, usually employed as PTE biomonitors, belong to the divisions Chlorophyta (green algae), Rhodophyta (red algae) and Heterokontophyta, primarily to the class Phaeophyceae (brown algae). These multicellular organisms, with complex structures and thalli differentiated into organs akin to those of higher plants [20], are among the most important primary producers in marine coastal waters. Their direct absorption of nutrients from the water column means that they are also able to absorb and potentially accumulate several non-essential elements, constituting a route for the transfer of contaminants through aquatic food webs, along with the associated threats to animal and human health [10]. Indeed, selected macroalgae can accumulate pollutants in concentrations higher than the levels in seawater [27], and they feature other characteristics such as wide geographical distributions, a sessile nature, high biomass, and ease of collection and identification [28], making them ideal candidates to act as effective biomonitors.

Although the Mediterranean coasts are often characterised by a high biodiversity of macroalgae [29], their use as PTE biomonitors is still limited by the scarcity of information on their bioaccumulation responses toward pollution gradients. To fill this gap, the present study aims at ascertaining the PTE accumulation capability of several macroalgae belonging to the three main divisions while using *P. oceanica* as a reference biomonitor, with the goal of identifying species potentially substituting the latter in biomonitoring studies. To this end, the concentrations of 17 PTEs, including macronutrients, micronutrients and non-essential elements, were determined in the thalli of 16 Mediterranean algal species, the leaves of *P. oceanica* and in the sediments of their growing sites (in terms of both total concentrations and bioavailable fractions). The study has been carried out along the Cilento coast (Campania Region, southern Italy), where six sampling sites have been selected for their different anthropogenic pressures.

2. Materials and Methods

2.1. Study Area and Sampling Sites

Macroalgae, *P. oceanica* leaves and sediments were sampled in 6 sites located inside and near the Marine Protected Area (MPA) of "Santa Maria di Castellabate" (Salerno, Italy), in the Mediterranean Sea. The MPA (40°16′ N; 14°55′ E), managed by the "Cilento, Vallo di Diano e Alburni" Italian National Park, extends for 17 km of the coastline and is characterized by a substrate constituted by carbonate and siliciclastic turbidite sequences (Cilento Flysch). Photophilous hard bottoms, dominated by green and brown algae, and by *P. oceanica* seagrass beds, characterize most of the shallow waters of the MPA.

The sampling sites were chosen based on the MPA zonation, defining different protection regimes, and on the proximity to different types of anthropogenic pressures, which are mainly associated with maritime traffic, either professional or recreational (Figure 1):

- CT and CP, in the Full Reserve zone (A—where maritime traffic and any type of professional/recreational activities are strictly forbidden) of the MPA,
- OM, in the General Reserve zone (B—where only swimming and slow navigation, <5 kt, are allowed) of the MPA,
- SM, in the Partial Reserve zone (C—where most of the activities are allowed, if compatible with the general protection of the seafloor) of the MPA and close to a small marina,
- TB, outside the MPA and close to its northern boundary,
- AH, outside the MPA and close to a large harbor.

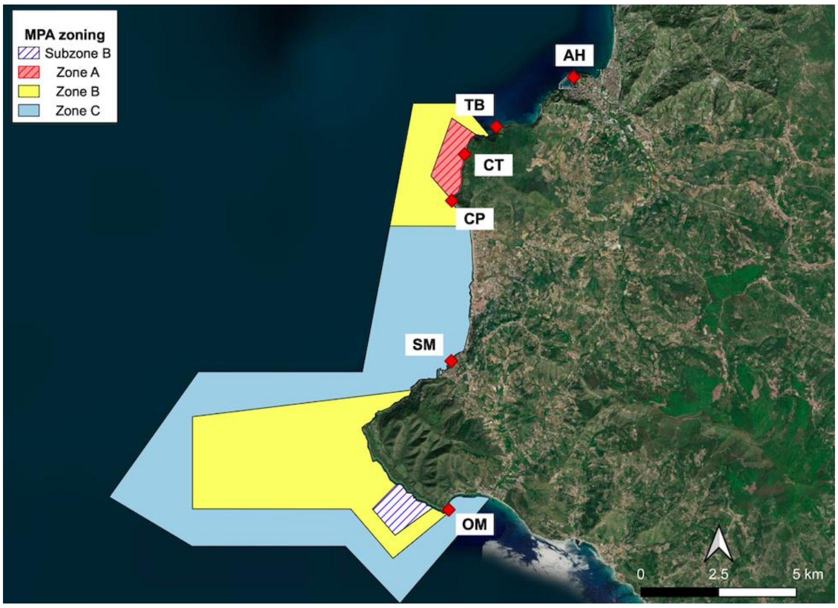

Figure 1. Map of the 6 sampling sites (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina) along the Cilento coast (southern Italy), with the zonation defining areas subjected to different protection regimes in the "Santa Maria di Castellabate" MPA, Salerno, Italy. Background tiles from ESRI maps.

2.2. Sampling and Laboratory Analyses

As reported in Table 1, in February 2023, macroalgae (according to their availability and abundance), *P. oceanica* leaves, and sediments (0–5 cm depth) were handpicked at each site in the sublittoral zone at up to a depth of ~1.5 m.

Table 1. Macrophytes belonging to the different divisions (Rhodophyta, Chlorophyta, Heterokontophyta and Embryophyta), collected in the study area, with indication of the sampling sites (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina).

Division	Species	Site
Rhodophyta	*Corallina elongata* Ellis & Solander, 1786	AH, TB, CT, CP
	Haliptilon virgatum (Zanardini) Garbary & Johansen, 1982	TB
	Jania rubens (L.) Lamouroux, 1816	TB, CP, SM, OM
	Laurencia microcladia Kützing, 1865	CT, CP, SM, OM
	Laurencia obtusa (Hudson) Lamouroux, 1813	CT
	Spyridia filamentosa (Wulfen) Harvey, 1833	CP
Chlorophyta	*Cladophora coelothrix* Kützing, 1843	SM
	Chaetomorpha aerea (Dillwyn) Kützing, 1849	CT
	Ulva clathrata (Roth) Agardh, 1811	TB
	Ulva compressa Forsskål, 1775	AH
Heterokontophyta	*Cystoseira balearica* Sauvageau, 1912	CP
	Cystoseira crinita Duby, 1830	CT
	Cystoseira compressa (Esper) Gerloff & Nizamuddin, 1975	CT, CP, SM
	Cystoseira spinosa var. tenuior (Ercegovic) Cormaci et al., 1992	OM
	Dictyota spiralis Montagne, 1846	AH, TB, CT, CP, SM
	Taonia atomaria (Woodward) Agardh, 1848	SM
Embryophyta	*Posidonia oceanica* (L.) Delile, 1813	AH, TB, CT, CP, SM, OM

In the laboratory, the macrophytes were gently washed with a 35‰ NaCl solution and manually cleaned of exogenous materials using plastic tools. Afterward, algae and leaves, as well as the sediment granulometric fraction (<2 mm particle size), were oven-dried (75 °C to constant weight) and pulverized either by hand or with the aid of a planetary ball mill in agate mortars (PM4, Retsch; Haan, Germany). All the laboratory analyses were carried out in triplicate.

Total PTE concentrations in macrophytes were determined according to the method of Baldantoni et al. [30]. For this purpose, an acid mixture in a microwave oven (Ethos, Milestone; Shelton, CT, USA) was employed to digest each subsample. In detail, 1 mL 49% HF (Merck; Germany) and 2 mL 65% HNO_3 (Carlo Erba; Milano, Italy) were added to 0.125 g of each pulverized sample. After digestion, the solutions were diluted to a final volume of 25 mL using milli-Q water (Elix 10, Millipore; Darmstat, Germany). Macronutrient (Ca, K, Mg, P, S), micronutrient (Cr, Cu, Fe, Mn, Na, Ni, Si, V, Zn), and non-essential element (Cd, Pb) concentrations were quantified by inductively coupled plasma optical emission spectrometry (Optima 7000DV, PerkinElmer; Wellesley, MA, USA), which guaranteed an analytical precision ranging from 2 to 9%, calculated as the standard deviation of $n = 9$ sequential measurements of the same sample for each element. In order to verify the method accuracy, the 1547 peach leaves [31] standard reference material was analyzed, obtaining recoveries, ranging from 87 to 110%, that were used to correct the quantification of the investigated PTEs.

The sequential extraction procedure reported by Rauret et al. [32] was used to derive information on PTE bioavailability profiles in sediment samples (see also Memoli et al. [33]), using BCR-701 [34] as certified reference material (with recoveries for the certified elements in the range 78–122% used to correct the quantification of each PTE). The BCR method allows for obtaining four fractions:

I: Acid-soluble/exchangeable fraction (lightly bound PTEs);
II: Reducible fraction (PTEs associated with Fe and Mn oxides);
III: Oxidizable fraction (PTEs bound to organic matter);
IV: Residual fraction (*aqua regia* extractable PTEs).

The sum of each PTE concentration in the different fractions, also quantified by inductively coupled plasma optical emission spectrometry (Optima 7000DV, PerkinElmer; Wellesley, MA, USA), was employed as a proxy for the total PTE concentration (i.e., pseudo-total, *sensu* Rauret et al. [32]).

On sediments, the particle size distribution, mineralogical composition, pH and organic matter content were also determined. Specifically, particle size distribution was obtained through sequential sieving on an AS200 Basic (Retsch; Haan, Germany) using sieves of 2000, 1000, 500, 250, 125, 63, 38 and 20 µm mesh sizes. Mineralogical composition was investigated through X-ray diffraction analysis on pulverized samples using a Phaser D2 (Bruker; Billerica, MA, USA) diffractometer equipped with a Cu X-ray tube working at 30 kV and 10 mA. Sediment diffractograms were acquired at a step size of 0.016° in the 2θ range of 8.000–50.000°, with 1″ integration per step, rotating the sample at 60 rpm. Finally, the pH was determined via the potentiometric method (HI 4212, Hanna Instruments; Padova, Italy) in a water suspension 1:2.5 *w*:*w*, as well as organic matter content by calcination in muffle (Controller B 170, Nabertherm GmbH; Lilienthal, Germany) at 550 °C for 4 h.

2.3. Data Analysis

Data on sediments were preprocessed in order to summarize their particle size distribution and derive their mineralogical composition. Specifically, sediment particle size distribution of each sample was described using a BEST model [35,36], fitted to the data using a multilevel Bayesian model, with varying intercepts for each site (i.e., random effect), through the functions of the Turing 0.30.1 module for the Julia 1.10 programming language. The mineralogical composition of sediment samples was derived through Rietveld refinement of diffractograms using the Profex 5.2.5 [37] software and data from the Crystallography Open Database [38].

The similarities/dissimilarities among species were evaluated using non-metric multidimensional scaling (NMDS) based on two axes and the χ^2 distance metric, with the superimposition of confidence ellipses (for $\alpha = 0.05$) for the species. Differences in the concentration of each PTE in selected species (*D. spiralis* and *P. oceanica*) at different sites were evaluated using a nested two-way analysis of variance (ANOVA), followed by the Tukey *post hoc* test (for $\alpha = 0.05$), upon checking the assumptions of normality of the residuals and homoscedasticity using the Shapiro–Wilk and the Breuch–Pagan tests, respectively. The same approach (one-way ANOVA) was adopted in evaluating differences in sediment PTE pseudo-total concentrations, whereas the relative similarities among sites in PTE pseudo-total concentration profiles were evaluated using a hierarchical clustering based on the Euclidean distance metric and complete linkage. All the analyses were performed within the R version 4.2.2 programming environment.

3. Results

3.1. Macrophytes

The 17 macrophytes (six Rhodophyta, four Chlorophyta, six Heterokontophyta, one Embryophyta) collected along the Cilento coast are listed in Table 1, whereas the macronutrient, micronutrient and non-essential element concentrations are reported in Tables S1, S2 and S3, respectively.

Overall, macroalgae and *P. oceanica*, the sole Embryophyta analyzed, occupy different areas of the NMDS space (Figure 2A), with the latter characterized by higher average concentrations of Cd, Cu, Mn, Ni, P and Zn. Rhodophyta, Chlorophyta and Heterokontophyta are also differentiated, although to a lesser extent due to species belonging to the genera

Cystoseira, *Laurencia*, *Dictyota*, *Taonia* and *Chaetomorpha* being characterized by similar PTE concentration patterns, despite their phylogenetic distance.

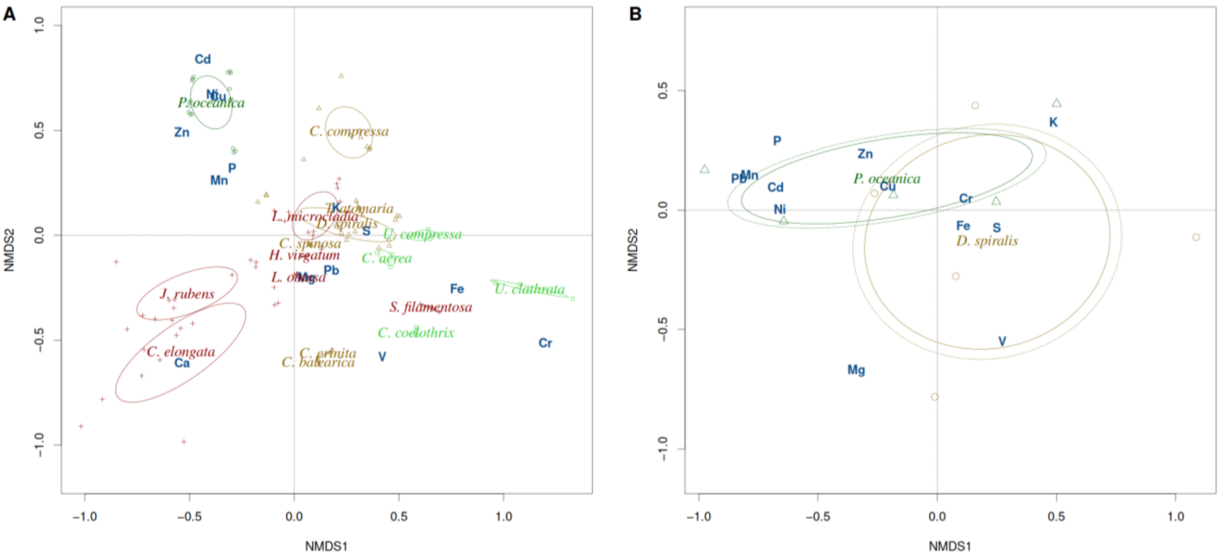

Figure 2. NMDSs on PTE concentrations in macrophytes scaled to unit variance (**A**) and concentrations in *D. spiralis* and *P. oceanica* independently scaled to the unit interval (**B**), with the superimposition of the weighted average mean for each PTE (dark blue). Different divisions are indicated by different symbols and colors (Rhodophyta: plus symbol, red; Chlorophyta: square, light green; Heterokontophyta: triangle, brown; Embryophyta: circle, dark green). Standard deviation ellipses are superimposed as solid lines, while confidence ellipses (for $\alpha = 0.05$) for the position of the group centroids are superimposed as dashed lines.

Among the 17 species analyzed, *L. microcladia* occupies the area around the centroid of the point cloud in the NMDS space, exhibiting PTE concentrations close to the average of all other taxa. In this context, the distance of the coralline red algae *C. elongata* and *J. rubens* from the others belonging to the same division is mainly determined by the high Ca concentrations, which are associated also with average higher concentrations of Mg and Mn than most of the other species. *S. filamentosa* appears close to *C. coelothrix* for their preferential accumulation of V, whereas *U. clathrata*, another Chlorophyte, differentiates from all the other species for its preferential accumulation of Cr. Among the Heterokontophyta, species belonging to the genus *Cystoseira* form two clusters occupying different areas of the NMDS space, with *C. compressa* characterized by lower concentrations of Mg, Pb and V in respect to the others. *D. spiralis* and *T. atomaria*, two species with similar habitus and ecological niche, occupy instead the same area of the NMDS space, close to the centroid of the point cloud and overlapping with the one occupied by *L. microcladia*.

The central position of *D. spiralis*, coupled with its abundance and coverage across the sampling sites, prompted its evaluation in terms of the similarity of the provided gradients to those of *P. oceanica*. In this context, the NMDS projection obtained through the independent scaling of the data provided by the two species highlights a substantial overlap between the respective confidence and standard deviation ellipses (Figure 2B). The ranking of sites in relation to the concentrations of macronutrients (Figure 3), micronutrients (Figure 4) and non-essential elements (Figure 5) in *D. spiralis* and *P. oceanica* also indicates a substantial agreement for elements such as Cu (Figure 4B) and Mg (Figure 3C), but also different gradients in the case of elements such as Mn (Figure 4D) and Ni (Figure 4E).

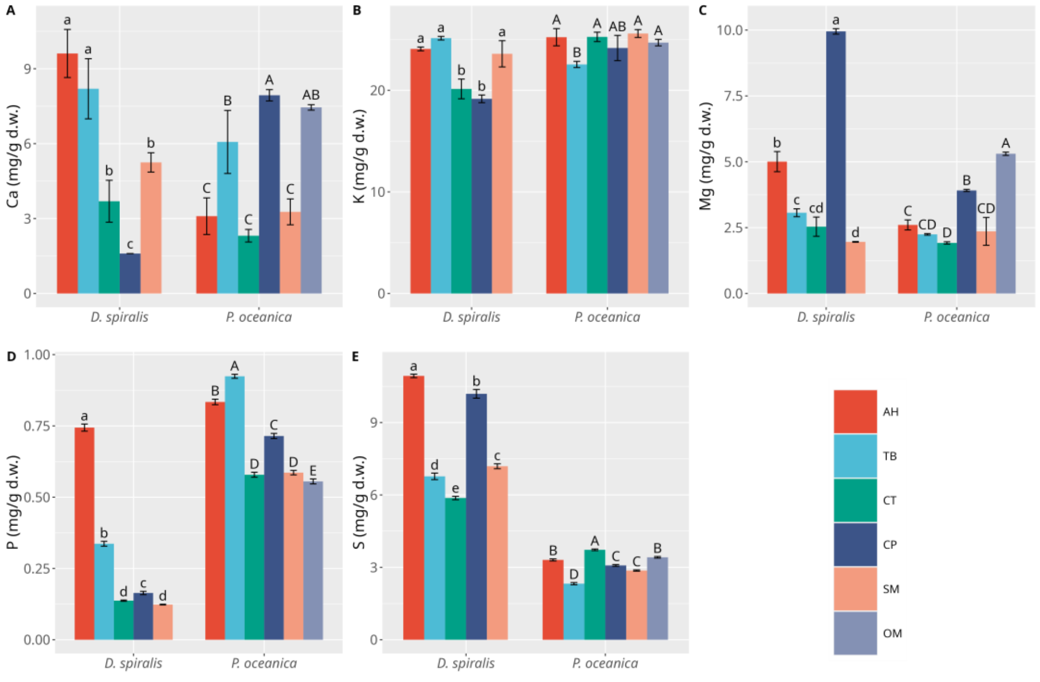

Figure 3. Macronutrient concentrations: Ca (**A**), K (**B**), Mg (**C**), P (**D**), S (**E**), expressed as mean values ± standard deviations, in *D. spiralis* and *P. oceanica* collected from the different sites of the study area (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina). Different letters indicate significant (for α = 0.05) differences among sites for each species (lower-case letters for *D. spiralis* and upper-case letters for *P. oceanica*).

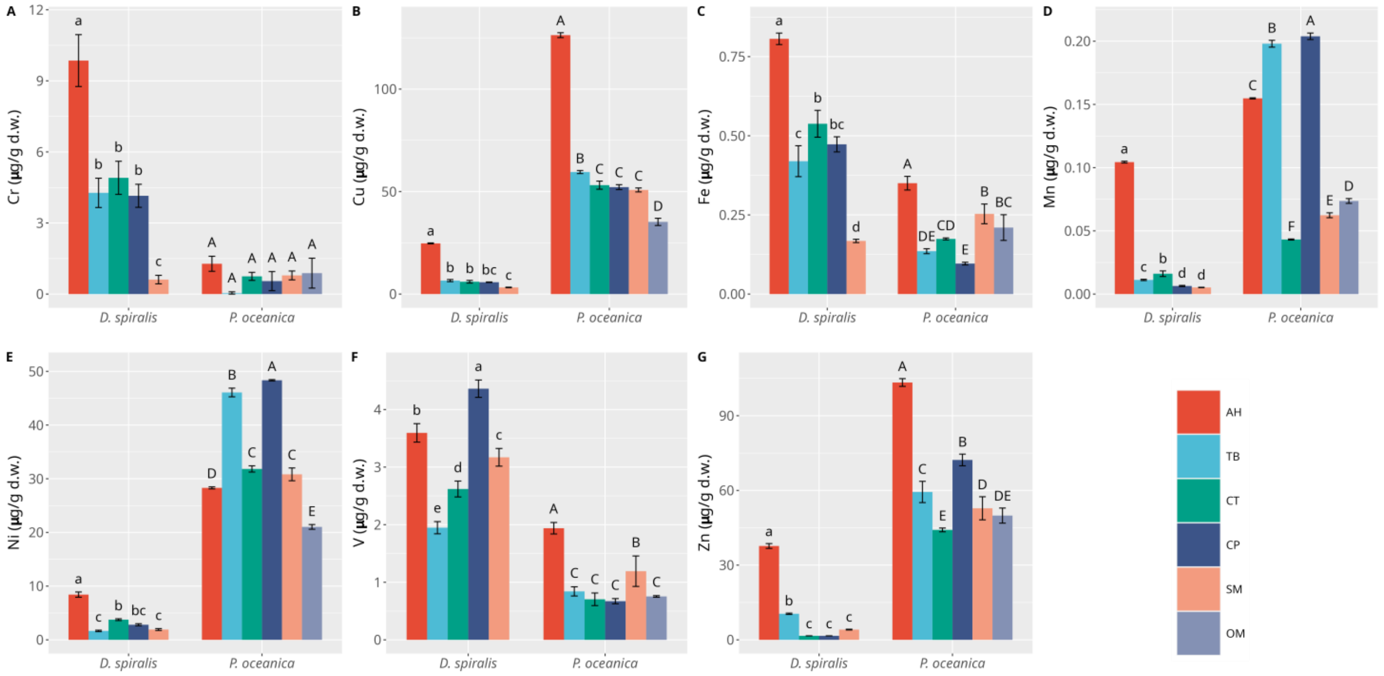

Figure 4. Micronutrient concentrations: Cr (**A**), Cu (**B**), Fe (**C**), Mn (**D**), Ni (**E**), V (**F**), Zn (**G**), expressed as mean values ± standard deviations, in *D. spiralis* and *P. oceanica* collected from the different sites of the study area (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina). Different letters indicate significant (for α = 0.05) differences among sites for each species (lower-case letters for *D. spiralis* and upper-case letters for *P. oceanica*).

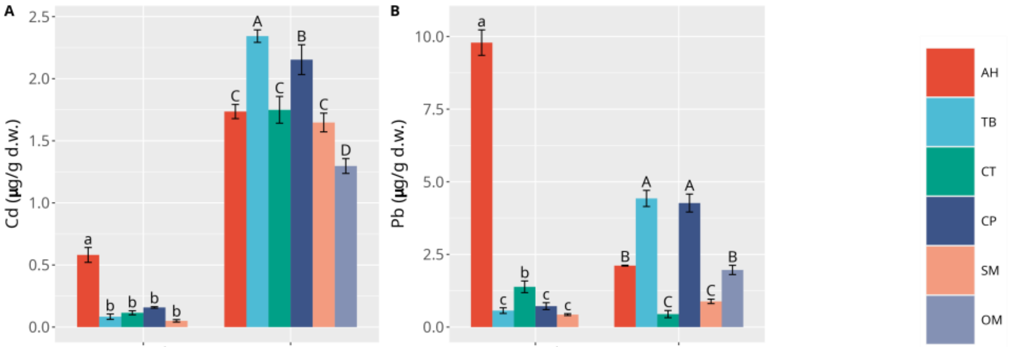

Figure 5. Non-essential element concentrations: Cd (**A**), Pb (**B**), expressed as mean values ± standard deviations, in *D. spiralis* and *P. oceanica* collected from the different sites of the study area (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina). Different letters indicate significant (for $\alpha = 0.05$) differences among sites for each species (lower-case letters for *D. spiralis* and upper-case letters for *P. oceanica*).

In general, *D. spiralis* accumulated the highest concentrations of several micronutrients and non-essential elements in the AH site (Agropoli Harbor), clearly differentiating this site from the others, with similar spatial patterns for most of the investigated PTEs. *P. oceanica*, instead, provided more variable spatial gradients, with only Cu (Figure 4B), Fe (Figure 4C), V (Figure 4F) and Zn (Figure 4G) showing the highest concentrations in the AH site. For macronutrients, the thalli of both species showed concentrations to the same order of magnitude, with variable spatial patterns according to the element.

3.2. Sediments

The mineralogical composition, particle size distribution, organic matter content and pH of the sediments are reported in Figure 6A–C, together with the hierarchical clustering, indicating the relative similarities in PTE pseudo-total concentrations among sites (Figure 6D). Sediments in all the sites are primarily constituted by silicates, such as quartz and feldspars (albite, microcline), with the abundance of the former increasing southward from the AH to the OM site (Figure 6A). The abundance of carbonates (calcite and aragonite) varies from 5 to 17%, which is comparable in a few sites to the abundance of the phyllosilicate minerals (chlorite, phlogopite) (Figure 6A). In this context, AH has, on average, the highest abundance of phyllosilicates, more than 10%, and the finest sediments, with the highest evenness among particle size classes ($d_g = 92 \pm 1$ µm, $b = 5.18 \pm 0.11$ in the BEST model) (Figure 6B). In terms of particle size distribution, the other sites are characterized by sandy sediments, with d_g values ranging from 128 ± 1 µm in CP to 292 ± 5 µm in SM and with b values from 3.31 ± 0.17 (OM) to 8.23 ± 0.25 (TB) (Figure 6B). The pH (8.41–8.72) and organic matter content (0.88%–1.59%) appear to be inversely related across the sites, with the highest values of the latter being observed in the AH site (Figure 6C), which also differentiates from all the other sites in relation to its PTE concentration profile (Figure 6D). In this context, the distance among sites in their PTE concentration profile matches the respective geographical distance (Figure 6D).

The pseudo-total concentration of each PTE and its partitioning into the four bioavailability fractions identified through the BCR sequential extraction technique are reported in Figures 7–9 for macronutrients, micronutrients and non-essential elements, respectively. Overall, pseudo-total concentrations showed comparable concentrations among the sites, with up to 3-fold variations in the case of S (Figure 7E) and most micronutrients (Figure 8A–G). No clear trend was observed among sites in relation to the protection regime and the proximity to the harbors, with concentrations in the AH site similar or lower than in the other sites for most of the PTEs, with the exception of S (Figure 7E) and Zn (Figure 8G). Similarly, the bioavailability profiles for each PTE were comparable among sites, with appreciable variations only in the case of Cd (Figure 9C) and, to a lesser extent, Cu (Figure 8I) and Zn (Figure 8N). In particular, Cd showed on average a 25% reduction in the exchangeable fraction (I) moving south from AH to OM site, with an increase in the fraction bound to the organic matter (III) or, in the case of CT site, to Fe and Mn oxides (II). Notably, the SM site, in proximity to the S. Marco harbor, has similar Cd exchangeable percentages to the AH site (the Agropoli harbor), but a different partitioning between the II and III fractions. In terms of average bioavailability profiles, most of the micronutrients (Figure 8H–N), with the exception of Mn (Figure 8K), showed a dominance of the residual fraction (IV), whereas Cd (Figure 9C), Ca (Figure 7A) and S (Figure 7E) indicated a dominance of the more bioavailable fractions (I, II, III). The other PTEs showed bioavailability profiles in between these extremes, with Pb being characterized by the highest evenness among all the PTEs (Figure 9D).

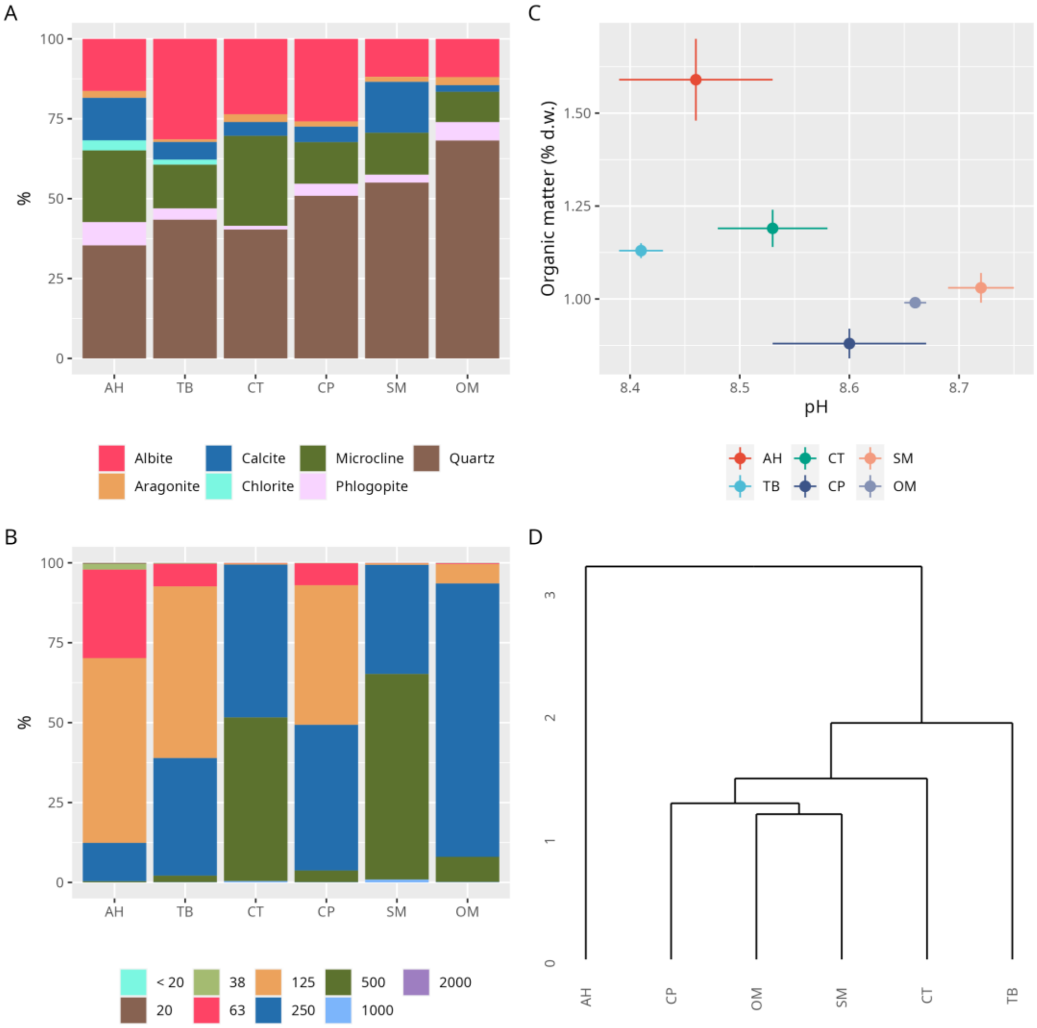

Figure 6. Sediment mineralogical composition (**A**), particle size distribution (**B**), pH and organic matter (**C**), as well as the similarities among sites (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina) in PTE pseudo-total concentrations (**D**).

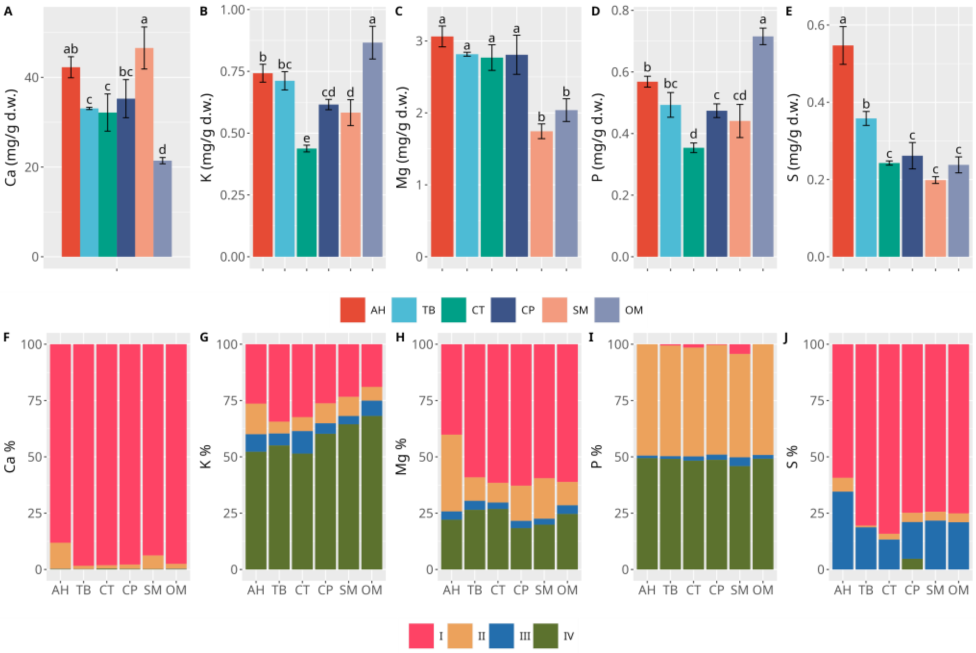

Figure 7. Pseudo-total macronutrient concentrations (**A**–**E**), expressed as mean ± standard deviation, and their relative abundances in the four (I, II, III, IV) bioavailable fractions (**F**–**J**) in the sediments collected from the six sites of the study area (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina). Different letters indicate significant (for α = 0.05) differences among sites.

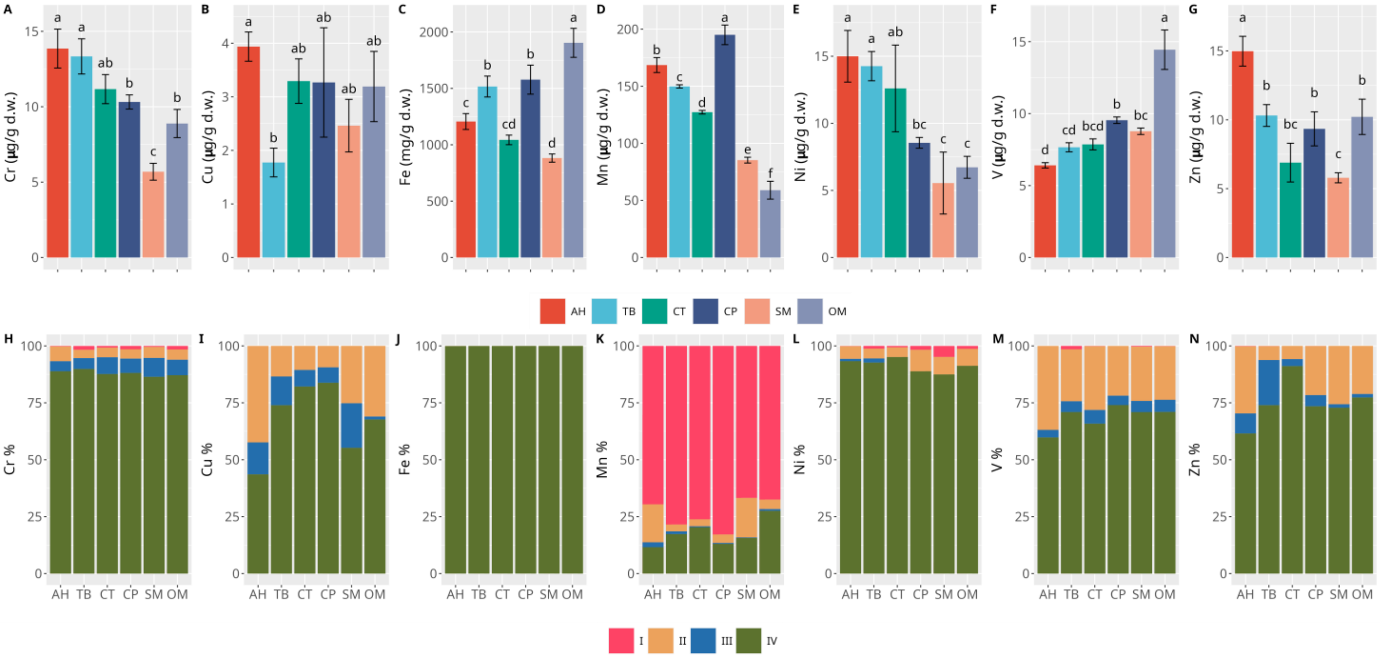

Figure 8. Pseudo-total micronutrient concentrations (**A**–**G**), expressed as mean ± standard deviation, and their relative abundances in the four (I, II, III, IV) bioavailable fractions (**H**–**N**) in the sediments collected from the six sites of the study area (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina). Different letters indicate significant (for α = 0.05) differences among sites.

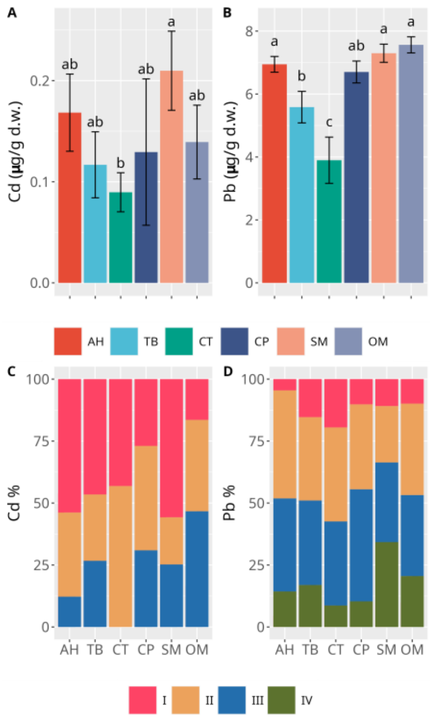

Figure 9. Pseudo-total non-essential element concentrations (**A**,**B**), expressed as mean ± standard deviation, and their relative abundances in the four (I, II, III, IV) bioavailable fractions (**C**,**D**) in the sediments collected from the six sites of the study area (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina). Different letters indicate significant (for α = 0.05) differences among sites.

4. Discussion

The results of this study highlight that macronutrient, micronutrient and non-essential element concentrations measured in different marine macrophytes vary not only among the species, but also within the same species from different sites in relation to their absorption mechanisms and bioaccumulation capabilities. Generally, the accumulation capabilities are similar among macrophytes belonging to the same division, with variations attributable to specific adaptations, such as in the case of coralline red algae (*C. elongata*, *J. rubens*) accumulating the highest concentrations of Ca, or to the micro-environments colonized (e.g., *S. filamentosa* or *C. coelothrix* in tide pools). These adaptations determine a preferential accumulation of specific PTEs in most of the species, with the notable exceptions of *C. spinosa*, *D. spiralis*, *H. virgatum*, *L. microcladia*, *L. obtusa* and *T. atomaria* that, instead, have an even accumulation response toward the different PTEs. Such a neutral response is a desirable property in biomonitoring applications, allowing for the straightforward use of biomonitors in common multi-PTE contamination scenarios, especially when coupled with wide species distribution. In this context, the brown alga *D. spiralis* stands out among the studied macrophytes by combining the even accumulation responses toward different PTEs, a wide spatial coverage and, in addition, rapid growth and large biomass.

In relation to the bioaccumulation of PTEs in *D. spiralis* and *P. oceanica*, the reference biomonitor adopted in the present research, the two species show concentrations to the same order of magnitude and in line with the values reported in other studies for *P. oceanica* leaves from areas characterized by low levels of anthropogenic pressure [21,39–42], coherent with the protection regime offered by the MPA. The unique exception is the concentration of Cu, with values at the AH site higher than most of the reported data, but comparable to the concentrations observed in the Gulf of Corinth [42]. Similarly, PTE pseudo-total concentrations in the studied sites are also comparable to those generally reported for the

Mediterranean area [41,42]. Thus, overall, the study area appears to be characterized by a good environmental status in relation to PTE concentrations.

In terms of concentration gradients, the two species provide, on average, overlapping responses, with similar gradients for several PTEs, but also different accumulation profiles across the sites for specific PTEs. For most of the macro- and micronutrients, *D. spiralis* and *P. oceanica* show similar concentration gradients, pointing out their abundance in the AH site, located at the mouth of a large harbor. Different concentration gradients, instead, are observed in the case of the non-essential elements, with *D. spiralis* showing indications that are largely more coherent than the ones provided by *P. oceanica* in relation to the anthropogenic pressures in the different study sites. Indeed, for Cd and Pb, two PTEs mainly associated with maritime traffic [43], *D. spiralis* clearly differentiates the most affected site (AH) from all the others, whereas *P. oceanica* fails to confirm this result. Moreover, the non-essential element concentration gradients are remarkably similar to the ones of other PTEs primarily associated with marine traffic and other forms of anthropogenic pressures [44], such as Cr, Cu, Fe, Mn, Ni and Zn, in *D. spiralis* but not in *P. oceanica*.

The differences in the concentration gradients provided by *D. spiralis* and *P. oceanica* can be attributed to the different absorption mechanisms and interactions they establish with the substrate [22,43,45]. Indeed, the lack of roots in *D. spiralis* and its strong association to rocky substrates suggest a weak interaction with soft sediments, which affect, instead, the absorption of PTEs by *P. oceanica*. Although PTE concentrations in sediments partially reflect their concentration in the water column [46], sediment characteristics such as mineralogical composition, particle size, pH and organic matter can modulate the availability of PTEs [11,47,48] and their absorption by rooted seagrasses, affecting the gradients they provide [24,49]. For example, the organic matter in surface sediments of coastal marine environments, generally composed of a mixture of terrestrial and marine residues [50], is usually characterized by PTE bound to recalcitrant organic compounds, such as humic molecules [51,52], that are able to modulate their bioavailability.

On the one hand, the similarities among study sites in relation to their sediment characteristics, also in terms of bioavailability profiles, suggest that the contribution of PTE absorption from sediments in *P. oceanica* should be similar among sites. On the other hand, the variations observed in pseudo-total concentrations of several PTEs to the same order of magnitude as those observed in *P. oceanica* suggest that the absorption from sediments may significantly shape the provided PTE gradients. PTE concentration gradients derived using *P. oceanica* do not reflect, thus, the actual gradients in the water column, for which the analysis of *D. spiralis* provides a more accurate proxy, and can be useful primarily in those cases where variations in sediment characteristics can be negligible. Notably, this result highlights not only the usefulness of macroalgae [53–55], and of *D. spiralis* in particular, as biomonitors for PTE concentrations in the water column, but also the contribution that the concurrent analysis of macrophytes and sediments can have in the interpretation of PTE gradients and the identification of anthropogenic pollution. This finding confirms the suitability of brown macroalgae in PTE biomonitoring studies [10,56], which were already considered promising biomonitor candidates for their high absorption potential [57] that the abundance of polysaccharides in the cell wall and of extracellular polymers [8,58] gives them.

5. Conclusions

The clear concentration gradients that *D. spiralis* provide, coherent with the expected anthropogenic pressures, indicate that this species can be a useful tool in PTE biomonitoring in the Mediterranean Sea and a suitable replacement for *P. oceanica*. It has all the prerequisites for its employment as a biomonitor: it is easy to sample and identify, it is available all year round, it has a large biomass and it is present in almost all coastal areas of the Mediterranean Sea. Regarding the spatial contamination gradients, *D. spiralis* has proved to be more effective than *P. oceanica*, highlighting the presence of PTEs in sites with high anthropogenic impact, where *P. oceanica* failed to point out pollution scenarios.

Moreover, *D. spiralis* should also be suitable in active biomonitoring studies by temporarily transplanting it in places where it does not naturally grow, further expanding its usefulness as a marine PTE biomonitor.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments11030051/s1, Table S1. Macronutrient concentrations (mean values ± standard deviations), expressed on a dry weight basis, in the analyzed macrophytes collected from the different sites of the study area (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina). Table S2. Micronutrient concentrations (mean values ± standard deviations), expressed on a dry weight basis, in the analyzed macrophytes collected from the different sites of the study area (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina). Table S3. Non-essential element concentrations (mean values ± standard deviations), expressed on a dry weight basis, in the analyzed macrophytes collected from the different sites of the study area (AH: Agropoli Harbor, TB: Trentova Bay, CT: Cape Tresino, CP: Cape Pagliarolo, SM: San Marco, OM: Ogliastro Marina).

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