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Seagrass *Cymodocea nodosa* as a trace element biomonitor: Bioaccumulation patterns and biomonitoring uses

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ABSTRACT

This study aimed to investigate the bioaccumulation patterns of As, Cd, Cr, Cu, Hg, Ni, Pb and Zn in the seagrass *Cymodocea nodosa*. Results showed that roots and leaves of *C. nodosa* tend to accumulate similar values of trace elements but significantly greater than rhizomes, which seem to act as transit organs. Cu was the only element with similar concentrations in the three organs. *C. nodosa* showed no correlation with trace elements in water, whereas Cd, Cu, Ni and Zn in sediments were correlated with *C. nodosa*. This study showed also that *C. nodosa* tends to adopt an exclusion strategy for some toxic elements like Cr except As. In general, the roots and leaves of *C. nodosa* acted as bioindicators of several trace elements in sediments. Leaves are easier to sample and are suggested for short-term monitoring, given their periodic turnover. In turn, roots are more suitable for collecting historical information on past environmental conditions.

1. Introduction

Coastal ecosystems are affected by a wide range of pollutants among which trace elements are particularly widespread and increasingly reaching marine habitats (Faganelli et al., 1997; Ralph et al., 2006; Halpern et al., 2008; Boudouresque et al., 2009). The risks of trace element pollution are of great concern, and difficult to assess because of element complex behavior and interactions in water ecosystems (Guilizzoni, 1991; Greger, 2004). Unlike organic pollutants, trace elements are typically not removed from aquatic ecosystems by natural processes (Bargagli, 1998). Once accumulated in bottom sediments, they begin to move up the food chain, often biomagnifying at higher trophic levels and ultimately causing potential disorders in humans and animals (Barwick and Maher, 2003; Roberts et al., 2008). As a consequence of the worldwide alteration and deterioration of coastal areas, and the need to assess and monitor the environmental quality, scientific community is increasingly focused on testing biological indicators for marine waters (Martínez-Crego et al., 2008).

Although numerous attempts have been made to assess the environmental quality of coastal marine ecosystems by quantifying the trace element content in water and sediment, these approaches do not provide information about the element fraction that is biologically available or ecotoxicologically relevant (Chaphekar, 1991; Fränzle, 2006). A suitable alternative would be to measure trace element concentrations in marine organisms. Plant species used as biomonitors can indeed provide not only a measure of element bioavailability in the environment but also historical information about past environmental conditions in a more cost-effective way compared to water and sediment whose contamination patterns require periodic analyses to be defined (Bargagli, 1998; Fränzle, 2006). Through the monitoring of trace element concentrations in the tissues of living organisms (biomonitoring), we gain direct information on the fraction of contaminants with direct ecotoxicological relevance (i.e., the bioavailable forms), but also we detect early signs of environmental disturbance (Rainbow, 1995). In coastal waters, marine organisms, like algae and mussels, have been regularly used as bioindicators of trace element contamination (Conti and Cecchetti, 2003). Similarly, seagrasses are often heralded as sentinel or indicator species because they are long-lived and integrate biological, physical and chemical parameters (Schlacher-Hoenlinger and Schlacher, 1998; Pergent-Martini and Pergent, 2000; Orth et al., 2006; Orlando-Bonaca et al., 2015). Various authors, in particular, showed that several marine phanerogams are suitable for trace element biomonitoring (Carter and Eriksson, 1992; Romeo et al., 1995; Prange and Dennison, 2000; Ferrat et al., 2003; Kaldy, 2006; Lewis and Devereux, 2009).

The marine angiosperm *Cymodocea nodosa* (Ucria) Asch., known as Lesser Neptune Grass, is a coastal seagrass of tropical origin, nowadays restricted to the Mediterranean Sea and some locations in the North Atlantic, from southern Portugal and Spain to Senegal, including the Canary Islands and Madeira (Green and Short, 2003; OSPAR, 2010). Generally, it forms mono-specific meadows, and can be found in deep

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waters (40 m) (Mazzella et al., 1993). *C. nodosa* is considered a pioneer species that can quickly colonize bare areas of the sea floor, with its rhizomes growing several meters per years (Duarte and Sand-Jensen, 1990; Borum and Greve, 2004). *C. nodosa* has several characteristics that make it a suitable bioindicator since it is abundant, widely distributed, long-lived, sensitive to certain natural and anthropogenic stresses, easy to identify and sample (Reizopoulou and Nicolaidou, 2004). Consequently, *C. nodosa* has been the focus of several studies on trace element biomonitoring (Nicolaidou and Nott, 1998; Prange and Dennison, 2000; Marín-Guirao et al., 2005; Malea and Kevrekidis, 2013). However, despite various studies, standardized protocols of environmental monitoring are generally lacking, thus, *C. nodosa* is usually not considered in monitoring programs of trace element pollution.

This study aimed to shed further light on the correlation of trace element content between water, bottom sediments and *C. nodosa* organs. In particular, the uptake of toxic elements like As has not been sufficiently considered in seagrass literature, and to our knowledge, no previous study has investigated *C. nodosa* populations around Sicily for purposes of trace element biomonitoring. The aim is also to corroborate the use of *C. nodosa* as a trace element bioindicator, and identify those aspects that need further investigation before *C. nodosa* can be used for routine activities of monitoring. Specifically, this study investigated to what extent *C. nodosa* reflects trace element concentrations in water, whether *C. nodosa* reflects the concentrations of the most toxic elements in sediments, and what organs of *C. nodosa* are more suitable as bioindicators of trace elements.

2. Materials and methods

2.1. Study area

This study was carried out in six locations of Sicily (Italy), which included two protected areas and four urban sites. (Fig. 1; Table 1). The protected areas are important marine reserves that were chosen as control sites. Trace element inputs of anthropogenic origin should be considered negligible in the control sites given the absence of nearby impacting human activities. The urban sites are seaside resorts that may be affected by trace element inputs associated with untreated municipal wastewaters and polluting spills from marine traffic. In general, the study areas did not show any sign of eutrophication. The climate of the study areas is characterized by wet and mild winters, and dry summers. The annual mean temperature is 18 °C, and precipitation ranges between 400 and 600 mm annually. *C. nodosa* meadows were significantly abundant in the study sites, and formed thick monospecific populations distributed up to a depth of 10 m within 100 m from the coastline.

2.2. Sampling

The sampling of *C. nodosa* was carried out every two months during 2014 and 2015. During sampling, weather conditions were regular; specifically, days were with smooth sea, sunny, not windy and without recent rains. Sampling activities were carried out in a once-off trip per site,

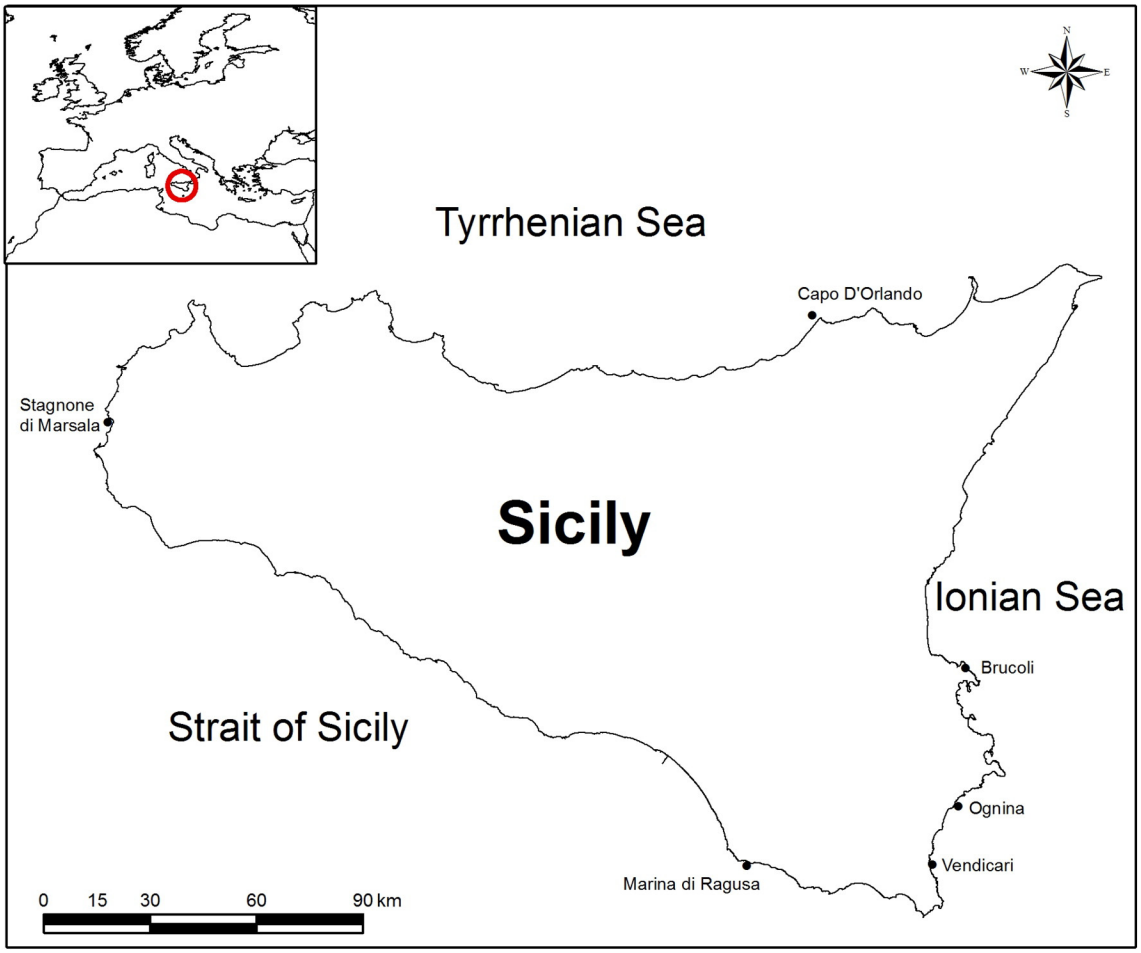

Fig. 1. Sampling sites where *Cymodocea nodosa* was collected.

Table 1
Sampling sites, degree of human pressure and coordinates.

Site name	Site type	Number of inhabitants	Maritime traffic	Human impact	Coordinates
Brucoli	Seaside resort	1000	High	High	37°16′56″N 15°11′34″E
Ognina	Seaside resort	1000	Moderate	Moderate	36°58′48″N 15°15′37″E
Vendicari	Nature reserve	Negligible	Negligible	Negligible	36°47′33″N 15°06′09″E
Marina di Ragusa	Seaside resort	3500	High	High	36°47′02″N 14°31′31″E
Stagnone di Marsala	Nature reserve	Negligible	Negligible	Negligible	37°51′27″N 12°28′29″E
Capo d'Orlando	Seaside resort	13,000	Negligible	High	38°09′25″N 14°45′54″N

and all samples were transported to laboratory on the same day of collection. In each collection site, sampling was carried out within the area occupied by dense meadows of *C. nodosa*, which were far from the coast from 1 to 100 m, and with a sea depth of 1 ÷ 10 m. The size of *C. nodosa* meadows was moderately variable, ranging from 5 × 5 m to 20 × 20 m. The collected samples included water, sediment and individuals of *C. nodosa*. Specifically, plant individuals consisted of roots, rhizomes and leaves. In each sampling site, 20 samples per typology were collected. Each of these 20 samples was obtained by mixing subsamples. Regarding *C. nodosa*, the generic analytical sample was obtained by mixing 10 individuals with similar biomass size randomly and manually collected within a subplot of average size of 5 × 5 m. In the same subplot, 10 subsamples of sediment and 10 of water were also randomly collected. The generic analytical sample of sediment was obtained by mixing such subsamples into a representative composite sample. Water sampling was the same as sediments. This protocol was repeated twenty times for every kind of sample in each collection site (N = 20). Once ashore, plant individuals were carefully shaken to remove gross attached material, rinsed with distilled water to remove minor sediment particles, and dried with a clean linen cloth. After that, the 10 plant individuals of each subplot were sealed in one sterilized and airtight plastic bag. Sediment subsamples were collected from the top 5 cm of the upper layer (affected by the rhizosphere) through a Plexiglas corer (internal diameter 10 cm). Regarding water, subsamples were collected at mid-height between sea bottom and water surface. Sediment and water samples were finally put in 0.5-L polyethylene bottles. All samples were gathered in PVC containers and kept at 4 ± 1 °C until laboratory analysis.

2.3. Chemical analysis

This study analyzed the concentrations of one metalloid (As) and seven metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn) in water, sediment and *C. nodosa* organs. Ultra clean conditions were kept during all stages of sample collection, transport, handling, processing and analysis. Once in the laboratory, plant samples were preliminary washed through running tap water to remove gross superficial particles, and then rinsed with bidistilled water to remove possible residual materials attached to the surface. After dissecting *C. nodosa* organs into roots, rhizomes and leaves, plant and sediment samples were stored at 4 °C and preserved until analysis. Plant organs and sediment were then dried to constant weight at ambient temperature because higher temperatures (>60 °C) may interfere in some element analyses (Llagostera et al., 2011). Once dry, plant samples were ground and homogenized in an agate mortar, whereas sediment samples were passed through a 1 mm diameter sieve. After that, such samples were weighed at 0.1 ± 0.05 g, and oven-digested at 90 °C overnight (microwave oven Mars 6, CEM Corporation) in an acid solution (H_2O_2/HNO_3, 2:3 ratio; Carlo Erba). Water samples were acidified with 63% HNO_3 to pH ≤ 2, before being filtered through a filter paper 2.0 μm (Whatman® GF/A glass microfiber filters). After digestion, plant and sediment samples were diluted with ultrapure Milli-Q water to a final volume of 25 mL and analyzed via ICP-MS (Cd, Cr, Cu, Ni, Pb, Zn), and FAAS (As and Hg) (respectively through PerkinElmer Elan® 6000 PerkinElmer® AAnalyst™ 400 AA Spectrometer). The element rhodium (Rh) was used as internal standard. Quality control was performed through stability of instrumental recalibration and using analytical blanks. The instruments were regularly checked against the low level standards (once every five samples) and recalibrated either when signs of drift were noted or after every 10 samples. The standard reference material *Ulva lactuca* (B.C.R. reference material No. 279/504) was analyzed with the same protocol of field-collected samples in order to assess the validity and accuracy of the analytical procedures. Student's *t*-test ($\alpha = 0.05$) was performed to ascertain the good agreement between analyzed values for the reference material and certified values. The percent recovery ranges were 95 ÷ 100, 91 ÷ 100 and 85 ÷ 100% in water, sediment and plant samples respectively. All the analyses were performed in three replicates to evaluate the reproducibility of measurements. Instrument detection limits were expressed as three times the standard deviation from the mean blank. Specifically, the detection limits for sediment and plant analysis were: 0.255 μg/g (As), 0.325 μg/g (Cd), 0.442 μg/g (Cr), 0.245 μg/g (Cu), 0.189 μg/g (Hg), 0.165 μg/g (Ni), 276 μg/g (Pb), and 567 μg/g (Zn); the detection limits for water analysis were: 0.076 μg/L (As), 0.088 μg/L (Cd), 0.110 μg/L (Cr), 0.064 μg/L (Cu), 0.050 μg/L (Hg), 0.124 μg/L (Ni), 0.077 μg/L (Pb), and 0.145 μg/L (Zn). Element concentrations were not reported if below detection limits.

2.4. Statistical processing

Translocation and bioaccumulation factors were calculated to assess element mobility:

- Translocation factors (TF):

$$[X]_{rhizome}/[X]_{root}$$

$$[X]_{leaf}/[X]_{root}$$

$$[X]_{leaf}/[X]_{rhizome}$$

where $[X]_{root}$, $[X]_{rhizome}$ and $[X]_{leaf}$ are, respectively, the concentrations (mg/kg DW) of the specific X element in roots, rhizomes and leaves of *C. nodosa*. TF expresses the mobility of the X element within the plant species, and higher TF values result in a greater translocation capability (Deng et al., 2004).

- Bioconcentration Factor (BCF):

$$[X]_{root}/[X]_{sediment}$$

where $[X]_{sediment}$ and $[X]_{root}$ are respectively the concentrations (mg/kg DW) of the specific X element in sediment and roots of *C. nodosa*. BCF expresses the efficiency of a plant species to uptake from sediment and accumulate a specific X element in its tissues. Higher BCF values result in a greater bioaccumulation capability (EPA, 2007).

The Kruskal-Wallis *H*-test was used to find a possible relationship between trace elements in water, sediment and plant organs. To identify significant differences between sample pairs, contrasts were carried out with the Mann-Whitney *U* test. When performing multiple sample contrasts, the Type I error rate may become inflated. Therefore, the initial level of risk (or significance), $\alpha = 0.05$, was adjusted according to

the Bonferroni formula $\alpha_B = \alpha/k$, where α_B is the adjusted level of risk, and k is the number of comparisons. The Spearman correlation coefficient was calculated to assess the relationship between element concentrations in the organs of *C. nodosa* and element concentrations in water and sediments. Statistical processing was performed with the statistical package IBM SPSS Version 22.0.

3. Results and discussion

Trace elements were found in detectable concentrations in the organs of *C. nodosa* in all study sites, with the exception of Hg that was under detection limits in water, sediment and plant organs. The element uptake of *C. nodosa* showed a mean BCF > 1, implying that this seagrass tends to accumulate greater trace element concentrations than sediment (Table 2). However, this was true only for 4/7 elements, specifically in As (BCF = 2.61), Cd (BCF = 1.30), Cu (BCF = 2.44), and Zn (BCF = 2.25). In turn, relatively low BCF values were found in Cr (0.20) and Ni (0.27). Besides Cd, Pb was the only element with a BCF close to the unit (0.88). Internal element mobility (TF) showed instead a more homogeneous trend. In particular, rhizome/root and leaf/rhizome translocations were respectively <1 and >1 for all elements. Leaf/root translocation was <1 in all elements except in Cu and Zn, but in general, close to the unit (mean TF = 0.86). Regarding the values of trace element concentrations (Table 3), results showed these trends:

- Cu > Ni = Zn > Cr > As > Cd = Pb in water;
- Cr > Ni = Zn > Cu = As = Pb > Cd in sediment;
- Zn > As > Cu > Cr = Ni = Pb > Cd in roots;
- Zn > Cu > As = Cr = Ni > Pb > Cd in rhizomes;
- Zn > As = Cu > Cr = Ni > Pb > Cd in leaves;

where "=" indicates statistically similar concentrations.

The organs of *C. nodosa* showed similar bioaccumulation trends but significantly different from water and sediment. The element decreasing trends in *C. nodosa* organs were in general agreement with several authors who studied accumulation patterns in *C. nodosa* under different environmental conditions (e.g., Nicolaidou and Nott, 1998; Llagostera et al., 2011). In addition, results showed a marked variation in the element content of roots and leaves compared to rhizomes. Specifically, roots and leaves accumulated greater and comparable concentrations, whereas rhizomes showed the lowest trace element content for all elements (except Cu). In particular, the element concentrations decreased in the various organs as follows:

- roots > leaves > rhizomes for As, Pb
- leaves > roots > rhizomes for Zn
- leaves = roots > rhizomes for Cd, Cr, Ni
- leaves = roots = rhizomes for Cu

Results in Table 3 highlight also that, with the exception of Cu, rhizomes may act as transit organs because of the high translocation (TF) from roots to leaves, which showed mean element concentrations from 2 to 10 times as high as in rhizomes. In particular, this study found $TF_{leaf/rhizome} > 1$ in all elements, with a range from 1.18 (Cu) to 5.30 (As). Our findings agree with several studies that reported *C. nodosa* rhizomes displaying significantly lower trace element concentrations compared to roots and leaves (e.g. Marín-Guirao et al., 2005; Malea and Kevrekidis, 2013).

Given the similar concentrations between roots and leaves, the results of this study suggest also that *C. nodosa* may apply a mixed tolerance strategy based both on root accumulation and on losing temporary organs (leaves). The former mechanism is relatively common in rooted species, both terrestrial and aquatic, and aims to store the bulk of trace elements in underground organs such as roots, as a way of protection against the adverse effects of toxic concentrations in photosynthetic processes (Williams et al., 1994; Caçador et al., 2000; Cardwell et al., 2002; Stoltz and Greger, 2002; Fitzgerald et al., 2003; Matthews et al., 2004; Fritioff and Greger, 2006; Reboreda and Caçador, 2007; Willis et al., 2010). The other mechanism may rely on the strategy that a species accumulates toxic element levels in some of its temporary organs in order to remove dangerously high concentrations. This possible tolerance mechanism relying on a "removal strategy" has been observed in *C. nodosa* by various authors (e.g., Malea and Haritonidis, 1999), who pointed out that active mobilization of toxic metals, such as Pb, Cd and Ni, from roots to shoots in the seagrass *C. nodosa*, may facilitate metal loss due to the high turnover rates of leaves. Uptake kinetics and passive absorption properties of leaves may also differ from those of roots, and elements absorbed can be internally redistributed to a variable extent through active or passive transport mechanisms (Llagostera et al., 2011).

Table 4 shows trace element concentrations in each study area. Results indicate that there is a tendency for sediments and *C. nodosa* to accumulate some elements at greater concentrations in the highly man-impacted areas. Specifically, the element concentrations showed this general decreasing trend in the study areas: Marina di Ragusa > Brucoli > Capo d'Orlando > Ognina > Vendicari > Stagnone di Marsala. Concentrations in water were statistically similar for all trace elements in all control and impacted sites. Regarding sediments, only the concentrations of As, Cd and Pb were similar in all study areas. In turn, Cr, Ni and Zn in sediments seemed to reflect the degree of human impact since they reported the highest values in the two most impacted sites (Brucoli and Marina di Ragusa). Cu showed the highest concentrations in sediments only in one impacted site (Marina di Ragusa). Regarding the possible relationship of water and sediment with *C. nodosa* organs, no element showed a significant correlation between water and seagrass. In general, the possible use of seagrasses as bioindicators of trace elements in water is a controversial issue. First, trace elements tend to accumulate in bottom sediments that act as sinks, consequently, the real risk of an important trace element contamination in marine waters becomes open to question. Second, although submerged plant species can absorb elements from water through their leaves (e.g., Demirezen and Aksoy, 2006; Harguinteguy et al., 2016), a significant trace element uptake from the water column seems generally unlikely to occur because sediments have a greater potential accumulation of contaminants than the overlying water column, and rooted submerged plants with well-developed root systems, would primarily extract elements from sediments with subsequent translocation to above-ground tissues (Jackson, 1998). In turn, Cd, Cu, Ni and Zn concentrations in sediment showed a positive relationship with *C. nodosa*. However, with the exception of Zn, a positive correlation between sediment and *C. nodosa* was found only in some sites, and not always for all three organs. Specifically, only rhizomes did not show any significant correlation with sediment in some cases. As pointed out, rhizomes act as transit organs, and thus follow different bioaccumulation patterns from roots and leaves. Consequently, roots and leaves tend to reflect more faithfully the trace element content in bottom sediments, and this should be considered as an important operational criterion for the organ choice in a biomonitoring program. In general, this study

Table 2
Translocation and bioconcentration factors in the organs of *C. nodosa*.

Element	BCF	TF		
	$[X]_{root}/[X]_{sediment}$	$[X]_{rhizome}/[X]_{root}$	$[X]_{leaf}/[X]_{rhizome}$	$[X]_{leaf}/[X]_{root}$
As	2.61	0.10	5.30	0.54
Cd	1.30	0.23	3.71	0.87
Cr	0.20	0.26	2.87	0.76
Cu	2.44	0.91	1.18	1.07
Ni	0.27	0.44	2.07	0.92
Pb	0.88	0.13	3.45	0.45
Zn	2.25	0.59	2.32	1.38
Mean	1.42	0.38	2.98	0.86

Table 3
Mean concentrations of trace elements in water, sediment and organs of *C. nodosa*.

	As	Cd	Cr	Cu	Ni	Pb	Zn
Water [μg/L]	2.48 ± 0.31	0.83 ± 0.01	5.79 ± 0.75	71.9 ± 10.2	15.1 ± 2.95	0.72 ± 0.10	12.0 ± 1.66
Sediment [mg/kg]	5.89 ± 0.65	0.23 ± 0.03	28.0 ± 4.15	4.22 ± 0.68	18.3 ± 3.26	6.04 ± 0.81	17.8 ± 3.02
Root [mg/kg]	15.4 ± 2.13	0.30 ± 0.04	5.72 ± 0.81	10.3 ± 2.35	4.89 ± 0.88	5.31 ± 0.93	40.0 ± 6.78
Rhizome [mg/kg]	1.57 ± 0.25	0.07 ± 0.01	1.51 ± 0.22	9.33 ± 1.35	2.17 ± 0.35	0.69 ± 0.08	23.7 ± 4.15
Leaf [mg/kg]	8.32 ± 0.97	0.26 ± 0.03	4.34 ± 0.52	11.0 ± 2.08	4.49 ± 0.73	2.38 ± 0.45	55.0 ± 7.52

showed that trace element bioaccumulation in living organisms is mainly element-, organ-, and site-dependent. Llagostera et al. (2011), for example, showed that organs explain element variability in *C. nodosa* up to 80%. Element characteristics, instead, influence the rate of absorption, accumulation and translocation (Fitzgerald et al., 2003; Deng et al., 2004). The specificity of a site is also important because it affects contamination level and soil and water properties such pH, temperature, salinity, redox conditions, organic matter, nutrients or presence of other metals (Fritioff et al., 2005; Sousa et al., 2008).

Arsenic is a non-essential element, ranked as one of the most toxic pollutants due to its persistent nature and tendency to bioaccumulation (Kapaj et al., 2006). Results showed that As values in water and sediment were below the quality limits of 5 μg/L and 12 mg/kg respectively in all study sites, according to the Italian Guidelines (GURI, 2011). However, the roots and leaves of *C. nodosa* contained significantly higher concentrations of As in the most impacted sites (Brucoli and Marina di Ragusa) compared to the other study areas. Despite the similar values of As in water and sediment in all study sites, the fact that only the organs of *C. nodosa* from the impacted sites registered the highest concentrations fits a basic aspect of bioindicators according to which species used as biomonitors can provide historical information regarding past environmental conditions (Bargagli, 1998). Indeed, As content in *C. nodosa* is presumably the result of the cumulative effects of past environmental pollution from water and soil, in agreement with several authors who showed that bioindicator species can register the temporal fluctuations of trace elements (Bargagli, 1998; Žáková and Kočková, 1999; Vardanyan and Ingole, 2006). The high values of As in *C. nodosa* may therefore reflect the increased concentrations of this element in the environment, supporting Jones (1985) who stated that aquatic species concentrate elements and integrate temporal fluctuations in water chemistry, thus being useful for monitoring purposes in addition to the chemical analyses of water and bottom sediments. The use of good bioindicators results in a cost-effective approach for monitoring long-term impacts compared to the mere analysis of water and sediment

Table 4
Concentrations in samples of water, sediment and organs of *C. nodosa*.

		Sampling sites					
Element	Kind of sample	Brucoli	Ognina	Vendicari	Marina di Ragusa	Stagnone di Marsala	Capo d'Orlando
As	Water [μg/L]	3.04 ± 0.25	1.56 ± 0.12	3.10 ± 0.20	3.03 ± 0.41	1.25 ± 0.15	2.90 ± 0.31
	Sediment [mg/kg]	5.85 ± 0.42	5.59 ± 0.62	6.03 ± 0.51	7.23 ± 1.21	4.33 ± 0.52	6.31 ± 0.72
	Root [mg/kg]	$28.5 \pm 3.85^{a,b}$	$8.52 \pm 1.10^{a,b,B}$	$5.66 \pm 0.55^{a,b,B}$	$34.8 \pm 4.45^{a,b}$	$4.15 \pm 0.35^{a,B}$	$10.7 \pm 2.10^{a,b,B}$
	Rhizome [mg/kg]	$1.10 \pm 0.15^{a,b}$	$1.73 \pm 0.21^{a,b}$	$1.25 \pm 0.18^{a,b}$	$2.74 \pm 0.38^{a,b}$	$1.12 \pm 0.17^{a,b}$	$1.47 \pm 0.22^{a,b}$
	Leaf [mg/kg]	$14.6 \pm 1.77^{A,b}$	$4.22 \pm 0.45^{A,b,B}$	$3.15 \pm 0.25^{A,b}$	$20.5 \pm 3.52^{A,b}$	$2.36 \pm 0.31^{A,b}$	$5.08 \pm 0.63^{A,B}$
Cd	Water [μg/L]	0.90 ± 0.01	–	0.74 ± 0.08	0.85 ± 0.09	–	0.81 ± 0.10
	Sediment [mg/kg]	0.15 ± 0.02	–	0.15 ± 0.02	0.35 ± 0.05	–	0.25 ± 0.03
	Root [mg/kg]	$0.38 \pm 0.04^{a,b,B,1}$	$0.27 \pm 0.03^{a,b,b}$	$0.21 \pm 0.02^{a,b,B,1}$	$0.45 \pm 0.05^{a,B,1}$	$0.15 \pm 0.02^{a,B}$	$0.34 \pm 0.05^{a,b,1}$
	Rhizome [mg/kg]	$0.05 \pm 0.01^{a,b}$	$0.09 \pm 0.01^{a,b,b}$	$0.10 \pm 0.01^{a,b,1}$	$0.09 \pm 0.01^{a,b,b}$	$0.04 \pm 0.01^{a,b}$	$0.07 \pm 0.01^{a,b}$
	Leaf [mg/kg]	$0.25 \pm 0.02^{a,b,1}$	$0.20 \pm 0.02^{a,b}$	$0.55 \pm 0.06^{A,b,1}$	$0.22 \pm 0.03^{A,b}$	$0.10 \pm 0.01^{A,B}$	$0.24 \pm 0.03^{a,b,1}$
Cr	Water [μg/L]	4.74 ± 0.55	5.30 ± 0.60	6.85 ± 0.54	6.75 ± 0.82	5.25 ± 0.53	5.87 ± 0.82
	Sediment [mg/kg]	74.5 ± 8.55	4.45 ± 0.55	6.68 ± 0.72	70.3 ± 9.68	5.41 ± 0.62	6.42 ± 0.83
	Root [mg/kg]	$6.90 \pm 0.81^{a,b}$	$4.88 \pm 0.52^{a,b}$	$4.56 \pm 0.47^{a,b}$	$6.85 \pm 0.83^{a,b}$	$5.31 \pm 0.73^{a,b}$	$5.79 \pm 0.70^{a,b}$
	Rhizome [mg/kg]	$1.25 \pm 0.15^{a,b}$	$1.53 \pm 0.22^{a,b}$	$1.85 \pm 0.22^{a,b}$	$1.46 \pm 0.20^{a,b}$	$1.90 \pm 0.25^{a,b}$	$1.05 \pm 0.18^{a,b}$
	Leaf [mg/kg]	$5.30 \pm 0.65^{a,b,b}$	$3.50 \pm 0.42^{a,b}$	$3.45 \pm 0.41^{a,b}$	$6.21 \pm 0.85^{a,b}$	$4.22 \pm 0.45^{a,b}$	$3.35 \pm 0.45^{A,b}$
Cu	Water [μg/L]	70.6 ± 8.37	87.4 ± 10.6	63.5 ± 7.50	75.3 ± 9.21	65.9 ± 5.85	68.8 ± 8.65
	Sediment [mg/kg]	5.82 ± 0.68	0.30 ± 0.05	2.21 ± 0.23	10.8 ± 1.88	3.04 ± 0.38	3.15 ± 0.28
	Root [mg/kg]	$17.3 \pm 2.24^{a,b,1}$	$5.84 \pm 0.63^{a,b,B}$	$3.35 \pm 0.42^{a,a,b,B,1}$	$25.8 \pm 3.85^{a,b,1}$	$2.85 \pm 0.32^{a,B,1}$	$6.64 \pm 0.83^{a,B,1}$
	Rhizome [mg/kg]	$23.1 \pm 3.12^{a,b,1}$	$2.92 \pm 0.45^{a,b,B}$	$2.06 \pm 0.25^{a,b,1}$	$20.8 \pm 2.95^{a,b,1}$	$2.55 \pm 0.24^{a,b,1}$	$4.56 \pm 0.37^{a,B,1}$
	Leaf [mg/kg]	$18.5 \pm 2.15^{a,b,1}$	$6.41 \pm 0.72^{a,b}$	$3.90 \pm 0.31^{a,B,1}$	$28.8 \pm 4.02^{a,B,1}$	$3.61 \pm 0.52^{a,B,1}$	$4.98 \pm 0.58^{a,b,B,1}$
Ni	Water [μg/L]	15.2 ± 2.21	19.0 ± 2.44	13.6 ± 1.95	15.9 ± 2.12	14.2 ± 1.78	12.6 ± 2.25
	Sediment [mg/kg]	33.6 ± 4.05	4.79 ± 0.54	13.5 ± 1.58	45.4 ± 5.65	5.40 ± 0.62	6.85 ± 0.86
	Root [mg/kg]	$7.89 \pm 0.82^{a,b,1}$	$3.32 \pm 0.41^{a,b}$	$3.45 \pm 0.50^{a,b}$	$6.21 \pm 0.85^{a,b,1}$	$4.32 \pm 0.55^{a,b,b}$	$4.15 \pm 0.52^{a,b,b}$
	Rhizome [mg/kg]	$0.88 \pm 0.10^{a,b}$	$1.42 \pm 0.23^{a,b,B}$	$1.15 \pm 0.18^{a,b,B}$	$5.34 \pm 0.42^{a,b,1}$	$1.65 \pm 0.21^{a,B}$	$2.55 \pm 0.35^{a,B}$
	Leaf [mg/kg]	$4.12 \pm 0.51^{A,b,1}$	$3.20 \pm 0.34^{a,b}$	$5.57 \pm 0.66^{A,b,b}$	$6.65 \pm 0.88^{a,b,1}$	$3.55 \pm 0.46^{a,b}$	$3.83 \pm 0.41^{a,b}$
Pb	Water [μg/L]	0.80 ± 0.09	–	0.69 ± 0.09	0.75 ± 0.08	–	0.64 ± 0.07
	Sediment [mg/kg]	4.85 ± 0.61	4.66 ± 0.72	7.35 ± 0.82	7.89 ± 1.24	6.22 ± 0.52	5.25 ± 0.63
	Root [mg/kg]	$6.25 \pm 0.82^{a,b}$	$3.85 \pm 0.41^{a,b}$	$4.56 \pm 0.38^{a,b,b}$	$6.99 \pm 0.83^{a,b}$	$4.55 \pm 0.60^{a,b,b}$	$5.64 \pm 0.75^{a,b}$
	Rhizome [mg/kg]	$0.85 \pm 0.08^{a,b}$	$0.75 \pm 0.08^{a,b}$	$0.38 \pm 0.04^{a,b}$	$1.07 \pm 0.15^{a,b}$	$0.21 \pm 0.02^{a,B}$	$0.89 \pm 0.08^{a,b}$
	Leaf [mg/kg]	$3.85 \pm 0.44^{A,b}$	$1.42 \pm 0.19^{A,b}$	$1.85 \pm 0.22^{A,b}$	$3.55 \pm 0.46^{A,b}$	$2.05 \pm 0.23^{A,b}$	$1.58 \pm 0.17^{A,b}$
Zn	Water [μg/L]	15.2 ± 2.85	10.2 ± 2.04	13.6 ± 2.05	12.7 ± 2.25	10.5 ± 1.98	9.65 ± 1.24
	Sediment [mg/kg]	25.3 ± 3.54	13.9 ± 1.75	12.5 ± 1.67	32.3 ± 4.15	11.4 ± 1.44	10.9 ± 1.25
	Root [mg/kg]	$38.3 \pm 5.42^{a,b,1}$	$45.4 \pm 6.21^{a,b,1}$	$35.3 \pm 4.71^{a,b,1}$	$45.8 \pm 5.21^{a,b,1}$	$34.5 \pm 4.21^{a,b,1}$	$40.9 \pm 5.55^{a,b,1}$
	Rhizome [mg/kg]	$18.6 \pm 3.12^{a,b,1}$	$22.4 \pm 3.37^{a,b,1}$	$24.2 \pm 3.65^{a,b,1}$	$25.3 \pm 2.01^{a,b,1}$	$21.7 \pm 3.67^{a,b,1}$	$30.1 \pm 4.68^{a,b,1}$
	Leaf [mg/kg]	$62.7 \pm 8.52^{A,b,1}$	$65.2 \pm 8.62^{A,b,1}$	$43.4 \pm 6.41^{a,b,1}$	$65.8 \pm 7.21^{A,b,1}$	$42.6 \pm 5.23^{a,b,1}$	$50.4 \pm 6.21^{A,b,b,1}$

Different forms of letter "a" mean significant differences between organs for one specific element in the same site.
Different forms of letter "b" mean significant differences between the same kinds of organ for one specific element in all sampling sites.
The number "1" means positive correlation between organs and sediment for one specific element in the same site.
Values under detection limits were indicated with "–".

whose contamination patterns require periodic investigation to be defined. In this study, the fact that As showed similar values in water and sediment in all sites but high concentrations in the organs of *C. nodosa* in the most impacted areas, may suggest that a past polluting event disappeared without a trace in sediments but not in *C. nodosa* organs, which registered the impact of anthropogenic stressors. Cd is another non-essential, toxic element whose detected values in water were higher than the Italian quality limits (0.20 μg/L) (GURI, 2011). However, Cd mobilization in water is likely due to natural causes given the relatively constant concentrations in all study sites, both pristine and impacted. Cd values in sediments were, instead, lower or around the quality limits (0.30 mg/kg). Results showed also that Cd concentrations in *C. nodosa* organs were generally in line with values from non-impacted areas (Sanchiz et al., 2000). Ralph and Burchett (1998) showed that in presence of high concentrations of toxic, non-essential elements like Cd in sediments, plant species tend to adopt exclusion mechanisms that may affect bioindication capability. In this study, Cd concentrations in *C. nodosa* were correlated with values in sediment, in agreement with literature data showing that *C. nodosa* may reflect Cd content in sediments (e.g., Malea and Haritonidis, 1999; Marín-Guirao et al., 2005). Regarding Cr, values in water were moderately higher in all sites compared to the Italian quality limits (4 μg/L) (GURI, 2011). In sediments, although Cr quality limits (50 mg/kg) were passed abundantly in the most impacted sites, *C. nodosa* organs (especially roots) did not reflect the Cr content in sediments. This pattern found confirmation in Nicolaidou and Nott (1998) who reported high Cr concentrations in polluted sediments in contrast to the much lower values in *C. nodosa*. In particular, Wasserman et al. (1992), who studied 25 metals in sediments and *Zostera noltii*, consider Cr as one of the metals concentrating mostly in sediments, as opposed to those that accumulate preferentially in plant tissues or those that are indifferent. Cr concentrations in *C. nodosa* seem thus not to increase proportionally to those in sediment, implying that maybe this seagrass is not a good bioindicator of Cr in sediments.

Cu was the only element whose concentrations were similar in roots, rhizomes and leaves, and this pattern was consistent in all study areas, regardless of their impact, and in general agreement with previous studies (e.g. Nicolaidou and Nott, 1998). This is probably a consequence of uptake mechanisms of Cu, an essential micronutrient, which, however, can be potentially toxic at high concentrations in surface waters (Kabata-Pendias and Pendias, 2001). Given the consistent correlation with Cu content in sediment, *C. nodosa* acted as a promising bioindicator of Cu in sediments. Regarding Ni, concentrations in *C. nodosa* organs showed a positive correlation only with sediments in the most impacted sites, where the quality limit of 30 mg/kg was also passed (significantly in the site "Marina di Ragusa"). *C. nodosa* seems thus to reflect Ni content in sediments in case of high accumulation as found in previous studies (e.g., Catsiki and Panayotidis, 1993; Lewis and Devereux, 2009). However, exclusion mechanisms, which can affect bioindication capacity, may be activated as a consequence of Ni toxicity.

Another non-essential element is Pb, considered among the most toxic metals even at low concentrations (Prasad, 2004). This study, in particular, found Pb values much lower than the Italian quality limits (GURI, 2011), both in water and sediment (respectively, 7.20 μg/L and 30 mg/kg). Pb is relatively immobile in soil, and tends to accumulate in roots with a scarce translocation into aboveground organs (Siedlecka et al., 2001). Most studies showed that plant species generally limit Pb uptake by adopting exclusion mechanisms in case of high contamination (e.g., Sharma and Dubey, 2005; Nagajyoti et al., 2010). Consequently, Pb uptake is generally limited both for its intrinsic immobility and for species tolerance strategies. Although our results cannot support this because the study areas did not show high concentrations of Pb, previous studies showed that *C. nodosa* responds to Pb inputs in line with most vascular plants (terrestrial and marine), by accumulating Pb preferentially in roots in case of high contamination (Llagostera et al., 2011). Zn is instead an important micronutrient for vegetation that can prove toxic at excessive concentrations (Kabata-Pendias and Pendias, 2001). In this study, *C. nodosa* showed a significant tendency to accumulate Zn in leaves, in line with previous findings (e.g., Nicolaidou and Nott, 1998; Llagostera et al., 2011). This study found also a consistent correlation between all organs of *C. nodosa* and sediments, thus implying that any organ can be used for Zn biomonitoring.

We should bear in mind that trace element contamination in marine coastal ecosystems may cause an ecological domino effect with serious consequences also for human health and economy. Seagrasses communities like *C. nodosa* meadows are, indeed, a key trophic resource for many primary consumers in the coastal environments (Hemminga and Duarte, 2000). Specialized marine grazers can bioaccumulate high values of trace elements with the associated risk of transferring toxic concentrations from seagrasses to higher trophic level consumers (Romero et al., 2006). Specifically, these consumers have the potential to sequester metals from leaf, root-rhizome and detrital material, posing threats to coastal resources and affecting trophic relationships (Ralph et al., 2006). Industrial and urban development in coastal environments can exacerbate the impact on marine ecosystems by increasing the quantity of metals available to seagrasses. Management strategies should focus on these issues in order to minimize trace element contamination in coastal environments. The use of seagrasses as a first level measurement of trace elements may thus prove a viable management tool to assess the potential anthropogenic effects on the coastal marine environment.

4. Conclusions

The seagrass *C. nodosa* acts as a promising bioindicator of several trace elements in sediments. The element compartmentation, in particular, with high concentrations in roots and leaves, may contribute to, and may be crucial to the survival of *C. nodosa* in highly contaminated environments. Further investigation is, however, needed to show whether *C. nodosa* can effectively bioindicate the presence of some elements, like Cd and Pb, against which this seagrass tends to adopt exclusion mechanisms. This study showed also that *C. nodosa* does not reflect significantly the trace element content in water. Seagrass communities may prove useful not only as bioindicators of trace elements but also as early detectors of contamination across trophic levels that may lead to cautionary concentrations for human consumption. Despite generally lacking in the protocols of trace element biomonitoring, the use of seagrasses could instead provide valuable models for effective decision-making and environmental resource management in coastal marine ecosystems.

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References

Bargagli, R., 1998. Trace Elements in Terrestrial Plants. An Ecophysiological Approach to Biomonitoring and Biorecovery. Springer, Berlin (324 pp.).

Barwick, M., Maher, W., 2003. Biotransference and biomagnification of selenium, copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie estuary, NSW, Australia. Mar. Environ. Res. 56, 471–502.

Borum, J., Greve, T.M., 2004. The four European seagrass species. In: Borum, J., Duarte, C.M., Krause-Jensen, D., Greve, T.M. (Eds.), European Seagrasses: An Introduction to Monitoring and Management. The M&MS Project, pp. 1–7.

Boudouresque, C.F., Bernard, G., Pergent, G., Shili, A., Verlaque, M., 2009. Regression of Mediterranean sea grasses caused by natural processes and anthropogenic disturbances and stress: a critical review. Bot. Mar. 52, 395–418.

Caçador, I., Vale, C., Catarino, F., 2000. Seasonal variation of Zn, Pb, Cu and Cd concentrations in the root-sediment system of *Spartina maritima* and *Halimione portulacoides* from Tagus estuary salt marshes. Mar. Environ. Res. 49, 279–290.
Cardwell, A.J., Hawker, D.W., Greenway, M., 2002. Metal accumulation in aquatic macrophytes from Southeast Queensland, Australia. Chemosphere 48, 653–663.
Carter, R.J., Eriksson, R.S., 1992. Investigation into the use of *Zostera muelleri* (Irmisch ex Aschers) as a sentinel accumulator for copper. Sci. Total Environ. 125, 185–192.
Catsiki, V.A., Panayotidis, P., 1993. Copper, chromium, and nickel in tissues of the Mediterranean seagrasses *Posidonia oceanica* and *Cymodocea nodosa* from Greek coastal areas. Chemosphere 26, 963–978.
Chaphekar, S.B., 1991. An overview on bio-indicators. J. Environ. Biol. 12, 163–168.
Conti, M.E., Cecchetti, G., 2003. A biomonitoring study: Trace metals in algae and molluscs from Tyrrhenian coastal areas. Environ. Res. 93, 99–112.
Demirezen, D., Aksoy, A., 2006. Common hydrophytes as bioindicators of iron and manganese pollutions. Ecol. Indic. 6, 388–393.
Deng, H., Yea, Z.H., Wong, M.H., 2004. Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China. Environ. Pollut. 132, 29–40.
Duarte, C.M., Sand-Jensen, K., 1990. Seagrass colonization: biomass development and shoot demography in *Cymodocea nodosa* patches. Mar. Ecol. Prog. Ser. 67, 97–103.
EPA, 2007. Framework for Metal Risk Assessment. U.S Environmental Protection Agency. Office of the Science Advisor, Washington D.C.
Faganelli, J., Vriser, B., Leskorsek, H., Cermelj, B., Planink, R., 1997. The impact of highway pollution on the coastal sea. Water Pollution IV: Modeling, Measuring and Prediction. Elsevier Applied Science, New York, NY, USA, pp. 161–173.
Ferrat, L., Pergent-Martini, C., Roméo, M., 2003. Assessment of the use of biomarkers in aquatic plants for the evaluation of environmental quality: application to seagrasses. Aquat. Toxicol. 65, 187–204.
Fitzgerald, E.J., Caffrey, J.M., Nesaratnam, S.T., McLoughlin, P., 2003. Copper and lead concentrations in salt marsh plants on the Suir Estuary, Ireland. Environ. Pollut. 123, 67–74.
Fränzle, O., 2006. Complex bioindication and environmental stress assessment. Ecol. Indic. 6, 114–136.
Fritioff, A., Greger, M., 2006. Uptake and distribution of Zn, Cu, Cd and Pb in an aquatic plant *Potamogeton natans*. Chemosphere 63, 220–227.
Fritioff, A., Kautsky, L., Greger, M., 2005. Influence of temperature and salinity on heavy metal uptake by submerged plants. Environ. Pollut. 133, 265–274.
G.U.R.I. (Gazzetta Ufficiale Della Repubblica Italiana), 2011. Decreto Ministero dell'ambiente e della tutela del territorio e del mare 8 Novembre 2010, N. 260. Regolamento Recante I Criteri Tecnici per La Classificazione Dello Stato Dei Corpi Idrici Superficiali, per La Modifica Delle Norme Tecniche Del Decreto Legislativo 3 Aprile 2006, N. 152, Recante Norme in Materia Ambientale, Predisposto Ai Sensi dell'articolo 75, Comma 3, Del Medesimo Decreto Legislativo. Supplemento N. 31 Alla Gazzetta Ufficiale 7 Febbraio 2011 N. 30.
Green, E.P., Short, F.T., 2003. World Atlas of Seagrasses. Prepared by the UNEP World Conservation Monitoring Centre and University of California Press, Berkeley, USA.
Greger, M., 2004. Metal availability, uptake, transport and accumulation in plants. In: Prasad, M.N.V. (Ed.), Heavy Metal Stress in Plants – From Biomolecules to Ecosystems, second ed. Springer-Verlag, Heidelberg, Berlin, Germany, pp. 1–27.
Guilizzoni, P., 1991. The role of heavy metals and toxic materials in the physiological ecology of submersed macrophytes. Aquat. Bot. 41, 87–109.
Halpern, B.S., Walbridge, S., Selkoe, K.A., et al., 2008. A Global map of human impact on marine ecosystems. Science 319, 948–952.
Harguinteguy, C.A., Cofré, M.N., Fernández-Cirelli, A., Pignata, M.L., 2016. The macrophytes *Potamogeton pusillus* L. and *Myriophyllum aquaticum* (Vell.) Verdc. as potential bioindicators of a river contaminated by heavy metals. Microchem. J. 124, 228–234.
Hemminga, M.A., Duarte, C.M., 2000. Seagrass Ecology. Cambridge University Press, Cambridge.
Jackson, L.J., 1998. Paradigms of metal accumulation in rooted aquatic vascular plants. Sci. Total Environ. 219, 223–231.
Jones, K.C., 1985. Gold, silver and other elements in aquatic bryophytes from a mineralized area of North Wales, UK. J. Geochem. Explor. 24, 237–246.
Kabata-Pendias, A., Pendias, H., 2001. Trace Elements in Soils and Plants. CRC Press, London.
Kaldy, J.E., 2006. Carbon, nitrogen, phosphorus and heavy metal budgets: How large is the eelgrass (*Zostera marina* L.) sink in a temperate estuary? Mar. Pollut. Bull. 52, 332–356.
Kapaj, S., Peterson, H., Liber, K., Bhattacharya, P., 2006. Human health effects from chronic arsenic poisoning – a review. J. Environ. Sci. Health A 41, 2399–2428.
Lewis, M.A., Devereux, R., 2009. Nonnutrient anthropogenic chemicals in seagrass ecosystems: fate and effects. Environ. Toxicol. Chem. 28 (3), 644–661.
Llagostera, I., Pérez, M., Romero, J., 2011. Trace metal content in the seagrass *Cymodocea nodosa*: differential accumulation in plant organs. Aquat. Bot. 95, 124–128.
Malea, P., Haritonidis, S., 1999. *Cymodocea nodosa* (Ucria) Aschers. as a bioindicator of metals in Thermaikos Gulf Greece, during monthly samplings. Bot. Mar. 42, 419–430.
Malea, P., Kevrekidis, T., 2013. Trace element (Al, As, B, Ba, Cr, Mo, Ni, Se, Sr, Tl, U and V) distribution and seasonality in compartments of the seagrass *Cymodocea nodosa*. Sci. Total Environ. 463-464, 611–623.
Marín-Guirao, L., Marín, A., Lloret, J., Martínez, E., García, A.J., 2005. Effects of mining wastes on a seagrass ecosystem: metal accumulation and bioavailability, seagrass dynamics and associated community structure. Mar. Environ. Res. 60, 317–367.
Martínez-Crego, B., Vergés, A., Alcoverro, T., Romero, J., 2008. Selection of multiple seagrass indicators for environmental biomonitoring. Mar. Ecol. Prog. Ser. 361, 93–109.
Matthews, D.J., Moran, B.M., McCabe, P.F., Otte, M.L., 2004. Zinc tolerance, uptake, accumulation and distribution in plants and protoplasts of five European populations of the wetland grass *Glyceria fluitans*. Aquat. Bot. 80, 39–52.
Mazzella, L., Scipione, M.B., Gambi, M.C., Buia, M.C., Lorenti, M., Zuppo, V., Cancemi, G., 1993. The Mediterranean seagrasses *Posidonia oceanica* and *Cymodocea nodosa*. A comparative review. 1st International Conference Mediterranean Coastal Environment, MEDCOAST'93, pp. 103–116 (Ankara, Turkey).
Nagajyoti, P.C., Lee, K.D., Sreekanth, T.V.M., 2010. Heavy metals, occurrence and toxicity for plants: a review. Environ. Chem. Lett. 8, 199–216.
Nicolaidou, A., Nott, J.A., 1998. Metals in sediment, seagrass and gastropods near a nickel smelter in Greece: possible interactions. Marine Pollut. Bulletin 36 (5), 360–365.
Orlando-Bonaca, M., France, J., Mavric, B., Grego, M., Lipej, L., Flander-Putrle, V., Sisko, M., Falace, A., 2015. A new index (MediSkew) for the assessment of the *Cymodocea nodosa* (Ucria) Ascherson meadow's status. Mar. Environ. Res. 110, 132–141.
Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck Jr., K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystems. Bioscience 56, 987–996.
OSPAR Commission, 2010. Background Document for *Cymodocea* Meadows. OSPAR Commission, London, p. 30 (487/2010).
Pergent-Martini, C., Pergent, G., 2000. Marine phanerogams as a tool in the evaluation of marine trace-metal contamination: an example from the Mediterranean. Int. J. Environ. Pollut. 13, 126–147.
Prange, J.A., Dennison, W.C., 2000. Physiological responses of five seagrass species to trace metals. Mar. Pollut. Bull. 41, 327–336.
Prasad, M.N.V., 2004. Heavy Metal Stress in Plants: From Biomolecules to Ecosystems. Springer Verlag.
Rainbow, P.S., 1995. Biomonitoring of heavy metal availability in the marine environment. Mar. Pollut. Bull. 31, 183–192.
Ralph, P.J., Burchett, M.D., 1998. Photosynthetic responses of *Halophila ovalis* to heavy metals stress. Environ. Pollut. 103, 91–101.
Ralph, P.J., Tomasko, D., Moore, K., Seddon, S., Macinnis-Ng, C., 2006. Human impacts on seagrasses: eutrophication, sedimentation, and contamination, chapter 24. In: Larkum, A.W., Orth, R.J., Duarte, C.M. (Eds.), Seagrasses: Biology, Ecology, and Concervation. Springer, Dordecht, The Netherlands.
Reboreda, R., Caçador, I., 2007. Halophyte vegetation influences in salt marsh retention capacity for heavy metals. Environ. Pollut. 146, 147–154.
Reizopoulou, S., Nicolaidou, A., 2004. Benthic diversity of coastal brackish-water lagoons in western Greece. Aquat. Conserv. Mar. Freshwat. Ecosyst. 14 (S1), S93–S102.
Roberts, D.A., Johnston, E.L., Poore, A.G.B., 2008. Contamination of marine biogenic habitats and effects upon associated epifauna. Mar. Pollut. Bull. 56, 1057–1065.
Romeo, M., Gnassia-Barelli, M., Juhel, T., Meinesz, A., 1995. Memorization of heavy metals by scales of the seagrass *Posidonia oceanica*, collected in the NW Mediterranean. Mar. Ecol. Prog. Ser. 120, 211–218.
Romero, J., Lee, K.-S., Pérez, M., Mateo, M.A., Alcoverro, T., 2006. Nutrient dynamics in seagrass ecosystems. In: Lrakum, A.W.D., Orth, R.J., Duarte, C. (Eds.), Seagrasses: Biology, Ecology and Conservation. Springer.
Sanchiz, C., García-Carrascosa, A.M., Pastor, A., 2000. Heavy metal contents in soft-bottom marine macrophytes and sediments along the Mediterranean coast of Spain. Mar. Ecol. 21 (1), 1–16.
Schlacher-Hoenlinger, M.A., Schlacher, T.A., 1998. Accumulation, contamination and seasonal variability of trace metals in the coastal zone—patterns in a seagrass meadow from the Mediterranean. Mar. Biol. 131, 401–410.
Sharma, P., Dubey, R.S., 2005. Lead toxicity in plants. Braz. J. Plant Physiol. 17, 35–52.
Siedlecka, A., Tukendorf, A., Skórzyńska-Polit, E., Maksymiec, W., Wójcik, M., Baszyński, T., Krupa, Z., 2001. Angiosperms (Asteraceae, *Convolvulaceae*, *Fabaceae* and *Poaceae*; other than *Brassicaceae*). In: Prasad, M.N.V. (Ed.), Metals in the Environment. Analysis by Biodiversity. Marcel Dekker, Inc., New York, pp. 171–217.
Sousa, A.I., Caçador, I., Lillebø, A.I., Pardal, M.A., 2008. Heavy metal accumulation in *Halimione portulacoides*: intra- and extra-cellular metal binding sites. Chemosphere 70, 850–857.
Stoltz, E., Greger, M., 2002. Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. Environ. Exp. Bot. 47, 271–280.
Vardanyan, L.G., Ingole, B.S., 2006. Studies on heavy metal accumulation in aquatic macrophytes from Sevan (Armenia) and Carambolim (India) lake systems. Environ. Int. 32, 208–218.
Wasserman, J.-C., Dumon, J.-C., Latouche, C., 1992. Bilan de 18 éléments-traces et de 7 éléments majeurs dans un environnement peuplé par de Zostères (*Zostera noltii*). Vie Milieu 42, 15–20.
Williams, T.P., Bubb, J.M., Lester, J.N., 1994. The occurrence and distribution of trace metals in halophytes. Chemosphere 28, 1189–1199.
Willis, J.M., Gambrell, R.P., Hester, M.W., 2010. Growth response and tissue accumulation trends of herbaceous wetland plants species exposed to elevated aqueous mercury levels. Int. J. Phytoremediation 12, 586–598.
Žáková, Z., Kočková, E., 1999. Biomonitoring and assessment of heavy metal contamination of streams and reservoirs in the Dyje/Thaya river basin, Czech Republic. Water Sci. Technol. 39, 225–232.