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Elevated trace elements in sediments and seagrasses at CO_2 seeps

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Abstract

Seagrasses often occur around shallow marine CO_2 seeps, allowing assessment of trace element accumulation. Here, we measured Cd, Cu, Hg, Ni, Pb and Zn levels at six CO_2 seeps and six reference sites in the Mediterranean. Some seep sediments had elevated metal concentrations; an extreme example was Cd which was 43x more concentrated at a seep than its reference. Three seeps had metal levels that were predicted to adversely affect marine biota, namely Vulcano (for Hg), Ischia (for Cu) and Paleochori (for Cd and Ni). There were higher-than-sediment levels of Zn and Ni in Posidonia oceanica and of Zn in Cymodocea nodosa, particularly in roots. High levels of Cu were found in Ischia seep sediments, yet seagrass was abundant, and the plants contained low levels of Cu. Differences in bioavailability and toxicity of trace elements helps explain why seagrasses can be abundant at some CO_2 seeps but not others.

Highlights:

- Sandy CO_2 seep sediments had higher concentration of trace elements than sandy reference sites.
- Metals can be more toxic in areas affected by CO_2 acidification, with adverse effects on the sediment associated biota
- Seagrasses element accumulation at CO_2 seeps was highest in the roots

Keywords: Bioaccumulation, bioavailability, ocean acidification, *Posidonia oceanica*, *Cymodocea nodosa*.

Introduction:

Around 30% of anthropogenic CO_2 emissions have dissolved into surface seawater causing the pH to fall in a process known as ‘ocean acidification’ (Caldeira and Wickett, 2003). Seawater acidification poses a threat to marine species and ecosystems, so one of the United Nations Sustainable Development Goals is to “Minimize and address the impacts of ocean acidification” (United Nations, 2015). Rising CO_2 levels are expected to reduce seascape complexity, alter trophic interactions (Nogueira et al., 2017; Milazzo et al., 2019) and reduce biodiversity (Sunday et al., 2016; Agostini et al. 2018) causing impacts on a range of ecosystem services (Lemasson et al., 2017).

Trace elements, as the term suggests, normally occur in very low concentrations. At low levels they are not toxic, and some are essential for cellular process that support life (Avelar et al., 2013). At higher concentrations, trace elements such as arsenic (As), copper (Cu), lead (Pb) and mercury (Hg) can be harmful to coastal biota (Stumm Morgan, 1995). Element toxicity depends on the chemical form. Arsenic, for example, is toxic in its metalloid form, Hg and Pb are toxic as free ions, and Cu is toxic when reduced to Cu (I) (Tchounwou et al., 2014). Ocean acidification is expected to exacerbate the harmful effects of metal pollution in coastal ecosystems (Ivanina et al., 2015; Lewis et al., 2016) because lower seawater pH can increase the bioavailability and toxicity of metals both in sediments (Roberts et al., 2013) and in the water column (Millero et at., 2009). Lower pH can release metals to water column that were previously bound to sediment (Atkinson et al., 2007). It can also alter the speciation of elements such as Cu, Ni and Zn resulting in increased toxicity (Lacoue-Labarthe et al., 2009; 2012; Zeng et al., 2015). However, levels of toxicity will depend on the rate of metal uptake by marine organisms (Batley et al., 2004). The uptake and availability of Cd, Co, Cu, Hg, Ni, Pb and Zn increase when seawater pH falls from 8.1 to 7.8, which is the change in surface seawater pH that is underway this century (Byrne et al., 1998; Richards et al., 2011). The seawater free ion concentration of Cu, for example, is expected to increase by 115% (Pascal et al., 2010; Richards et al., 2011) and Pb by 4.6% (Millero et al., 2009; Dong et al., 2016).

So far, tests on the risks posed by trace metals in ocean acidification conditions have been carried out in laboratory conditions (Besar et al., 2008; Richir & Gobert, 2013; Bravo et al., 2016), which over simplify the complex behaviour of these metals in the marine environment (Millero et al., 2009). Most submarine volcanic seeps have gradients in pH and trace elements, providing natural conditions to assess their uptake by marine biota (Renzi et al., 2011; Kadar et al., 2012; Vizzini et al., 2013). While relationships between organisms, environmental factors and trace elements have received much attention at deep-sea hydrothermal vents (Kadar et al., 2007; Cravo et al., 2007), those at coastal CO_2 seeps are little understood.

Here, we investigated the levels of metals in sediments and seagrasses at acidified volcanic seeps as well as reference sites. We chose seagrasses as they deliver important ecosystem services in coastal habitats (Nordlind et al., 2016). They are also predicted to benefit from rising CO_2 levels within their thermal limits (Koch et al., 2013; Brodie et al., 2014). Seagrass habitats provide food and nurseries for fish, turtles and mammals (Whitfield et al., 2017), are important carbon sinks (Fourqurean et al., 2012). The seagrasses also sequester contaminants such as excess nutrients (Constanza et al., 2014) and metals (Bonanno and Orlando-Bonaca, 2017) and so are used as bioindicators (Catsiki and Panayotidis, 1993). The plants take in trace elements via their roots, rhizomes or leaves and can translocate them between these tissue compartments (Ralph et al., 2006). This introduces trace elements into the food web via grazing and decomposition (Lewis and Devereux, 2009).

Seagrass can be abundant at some shallow-water CO_2 seeps (Hall-Spencer et al., 2008; Russel et al., 2013) but are sparse or absent at other seeps (Vizzini et al., 2010, 2013). Studies have shown upregulation of stress-related antioxidant genes in the seagrass *Posidonia oceanica* at some CO_2 seeps (Lauritano et al., 2015) and work on the expression of genes involved in photosynthesis and growth of another common Mediterranean seagrass, *Cymodocea nodosa,* did not reveal beneficial effects of high CO_2 levels near a seep (Olivé et al., 2017). Under laboratory CO_2 enrichment there was significantly increased expression of *C. nodosa* transcripts associated with photosynthesis (Ruocco et al., 2017). So, even though seagrasses can be common at certain CO_2 seeps, toxins may cause stress and stunt their growth.

Laboratory studies have shown that, at elevated CO_2, Cu, Pb and Zn are toxic to the seagrasses *Zostera capricorni* (Ambo-Rappe et al., 2007) and *Halophila ovalis* (Ambo-Rappe et al., 2011). Many volcanic seeps around Greece and Italy have elevated levels of metals and are colonised by seagrass (Vizzini et al., 2010; Apostolaki et al., 2014) yet little is known about the accumulation of these metals in seagrass. Here, we expand on work undertaken by Vizzini et al., (2013) to quantify the concentrations of trace elements in sediments and seagrass at multiple seep sites around the Mediterranean. Our aim was to find out whether levels of trace elements at volcanic seeps correlated with trace element accumulation in seagrass roots, rhizomes and leaves and whether seagrass are more tolerant of some metals than others.

Methods:

Study sites

We surveyed six locations, all of which had seagrasses (*Posidonia oceanica* or *Cymodocea nodosa*) growing on sand in the naturally high salinity and high alkalinity waters of the Mediterranean Sea (Table 1). At each site, a high CO_2 station and a reference station were sampled between May - July 2014. The annual temperature range was around 18-22°C for all six locations and the CO_2 seeps were at 0-10 m depth with a tidal range of 0.30-0.50 m.

Vulcano, Italy

We sampled Levante Bay (38.4 N, 15.0 E) off Vulcano island (Fig. 1A). The underwater gas emissions are 97-98% CO_2 with 2.2% hydrogen sulfide (H_2S) at the seep site, decreasing to <0.005% H_2S towards the north-eastern part of the bay (Capaccioni et al., 2001; Boatta et al., 2013; Milazzo et al., 2014). *Cymodocea nodosa* was absent near the main vents so we, collected it on the periphery of the CO_2 seeps at 1 m depth.

Ischia, Italy

At Castello Aragonese, off Ischia (40°43'50.4"N; 13°57'48.2"E), CO_2 bubbles up in shallow water seeps (Fig. 1A). Here the gas is 90–95% CO_2, 3–6% N_2, 0.6–0.8% O_2, 0.2–0.8% CH_4 and the seeps lack H_2S (Tedesco, 1996). Abundant *Posidonia oceanica* meadows were sampled at 0.5m depth from the seep area and from a reference site (Fig. 2a).

Panarea, Italy

Panarea island (38°38'12.2"N; 15°06'42.5"E) is part of the Aeolian Archipelago in the Southern Tyrrhenian Sea (Fig.1A). On the main island and on the surrounding seafloor, tectonic faults have many gas seeps (Gabianelli et al., 1990; Voltattorni et al., 2009). The underwater gas emissions around these seeps are 92-95%CO_2, 2.99-6.23% N_2, 0.69-1.2% O_2 and 0.65-3% H_2S (Caramanna et al., 2010). Here *P. oceanica* was sampled at 5 m depth.

Milos Islands, Greece

Adamas thermal springs (36.70 N, 24.46 E) and Paleochori Bay (36.67 N, 24.51 E) are situated on southwest and southeast part of Milos island respectively (Fig. 1B). Milos island has an extensive submarine venting area, from the intertidal to depths of more than 100 m

(Dando et al., 1999). The released gases are 92.5% CO_2 with some CH_4 and H_2 (Bayraktarov et al., 2013). The underwater gas seeps at Adamas thermal station and Paleochori Bay where *Cymodocea nodosa* meadows were studied are located at 2m and 4m depth, respectively (Fig. 2b).

Methana, Greece

The Methana peninsula (37.638428 N; 23.359730 E) is the westernmost volcanic system of the northern Aegean Volcanic Arc (Fig. 1B), derived from the subduction of the African tectonic plate beneath the Eurasian plate. We sampled the area described by Baggini et al. (2014) near Agios Nikolaos village on the NE part of the peninsula. The gases were 90% CO_2, with small amounts of nitrogen, carbon monoxide and methane (D'Alessandro *et al.*, 2008). Here we sampled *Posidonia oceanica* meadows at 8-10 m depth.

Water sampling

Water samples (n=5) were collected at each CO_2 seeps and Reference station in 100 ml Winkler bottles and were fixed with 20 µl mercuric chloride and stored in dark cool- boxes for transport to the laboratory for total alkalinity (TA) analysis. The pH_{NBS} (using pH meter, Titrino Methron, Thermo Scientific) and temperature of the water samples were measured in the field immediately after collection and then measured in the laboratory again during the TA analysis. In the laboratory 80 ml water samples were analysed for TA using a Lab Titrino analyser following methods given by Dickson et al., (2007). Sterilized sea water was used as reference materials (CRM Batch 129, accuracy-98.7%, Dickson, 2013) for TA analysis. Temperature, pH_{NBS} and TA data were used to calculate $p\mathrm{CO}_2$ using CO_2SyS program following methods given by Pierrot et al., (2006). Dissociation constants (K_1 and K_2) developed by Meherbach et al., (1973) and refitted by Dickson and Millero, (1987) and dissociated constant for boric acid (K_B) developed by Dickson et al., (2007) was used in $p\mathrm{CO}_2$ calculation.

Sediment & seagrass sampling

Sediment samples (n=5) were collected 1m apart from six CO_2 seeps and six Reference stations by SCUBA diving. A 10-cm long and 2 cm diameter syringe with the tip cut off to was used to collect the upper 5 cm of sand. The sediment samples were stored in plastic bags in dark boxes and transferred to the laboratory. They were then dried at 40°C until a constant weight was achieved and then analysed for grain size following dry sieving at Half Phi intervals (Blott and Pye, 2001). After grain size analysis the fine and very fine sediment fractions (<180-63 µm) were collected and stored in plastic bottles for trace metal analysis.

Samples (n=5, whole plants) of *Cymodocea nodosa* (from Vulcano, Adamas and Paleochori islands) and of *Posidonia oceanica* (from Ischia, Panarea and Methana) were collected by SCUBA diving at each station. The plants were rinsed well to remove sediment, scraped to remove epiphytes and leaf scales were removed from rhizomes (*P. oceanica*) by hand and with soft tooth-brush and then washed with distilled water, air-dried and stored in polybags until analyses. Seagrass leaves, roots and rhizomes were oven dried at 40°C and powdered in a mortar and stored until further analysis.

Analytical Methods

Total trace element (Cd, Cu, Hg, Ni, Pb and Zn) concentrations were determined using Aqua Regia Soluble Total method (Modified by Laboratory of the Government Chemist (LGC) UK from ISO11466). Dried sediment (0.25 g) was put into digestion tubes (Tecator type). Cold and concentrated acids in the order: 4.5 mL Hydrochloric acid (HCl): 1.5 mL Nitric acid (HNO_3) was added to the tubes. The digestion tubes were left to pre-digest, for one hour then heated for 2 hours at 95 - 100°C. After cooling, the digest was filtered quantitatively into a volumetric flask and diluted using 2% HNO_3 (25 ml volume).

For dried seagrass (leaves, rhizomes and roots), 0.25g of sample was added to 6mL of HNO_3 following the same procedure as metals and the volume was made up to 25mL. Similarly, blanks and standards (LGC Reference Materials, UK, recovery-95%) used for sediments (LCG6156) and plants (LGC7162) were prepared using the same method. Analysis of Cd, Cu, Hg, Ni, Pb and Zn was performed using an ICP-MS (Thermo Scientific, iCAP 7000 Series) and an ICP-AES (Thermo Scientific, X Series-2) in triplicate with analytical detection precision of 99.5%.

All acids were analytical grade. Normal precautions for metal analysis were observed throughout the analytical procedures. HCL (37%w/w) and HNO_3 (69% w/w) were Ultrapure type (Ultrapure, Fischer Chemicals, USA). All glassware was soaked overnight in 10% HNO_3 and washed with distilled water and oven dried before use.

Data Analysis

To assess the sediment quality of all six locations we used Sediment Quality Guidelines Quotient (SQG-Q, Long and MacDonald, 1998). Among the environmental quality indices in the literature, this was chosen for its simplicity, comparability and robustness as reported by Caeiro et al., (2005). The SQG-Q consists of two values: a threshold effects level (TEL) and a probable effect level (PEL) (MacDonald et al., 1996). The TEL represent concentrations below which adverse biological effects occur rarely, the PEL represent concentrations above which adverse biological effects occur frequently.

The SQG-Q was calculated as follows:

$$\text{SQG-Q} = (\Sigma^{n}{}_{i=1}\ \text{PEL-Q}_{i})/\ n$$

Where PEL-Q_i = contaminant/PEL. The PEL-Qi represents the probable effect level quotient (PEL-Q) of the i contaminant and n represents the total number of contaminants (trace metals). Based on the SQG-Q index, the sediments were divided into three categories as established by MacDonald et al. (2000). SGQ-Q $\leq$ 0.1- low potential for adverse biological effects; 0.1< SQG-Q<1- moderate potential for adverse biological effects; SQG-Q$\geq$1- high potential for adverse biological effects.

To assess bio-accumulation of elements from sediment, we calculated the Bio Sediment Accumulation Factor (BSAF), which is defined as the ratio between metal concentration in the plant and that in the sediment (Lau et al., 1998; Szefer et al., 1999), given by:

$$\text{BSAF} = \text{Mp}\ /\text{Ms}$$

Where Mp is the concentration of the element in the seagrass and Ms is the concentration of the element in the sediment (Fergusson. 1990). BSAF is a key factor in expressing the efficiency of seagrass species to absorb elements from sediments and concentrate specific element in its roots. Higher BSAF values (>1) indicate a greater capability of accumulation (EPA, 2007).

Statistics

A three-way ANOVA was used to test for significant differences in trace element concentration among locations (Ischia, Panarea and Methana for *P. oceanica* Adamas, Paleochori and Vulcano for *C. nodosa*), compartments (sediment and leaves, rhizomes, roots) and stations (CO_2 seeps, Reference). All data were first checked for normality and homogeneity of variances. When variances were not homogenous, data were ln(x+1) transformed. When there were significant effects, the Holm-Sidak test was performed for a posteriori comparison among factor levels. Pearson's correlation co-efficient was applied to identify correlation between trace element concentration in sediment and seagrass compartments, after testing for normality of distribution on raw or log transformed data. When normality was not achieved, non-parametric Spearman's rank correlation coefficient was applied. All statistical tests were conducted with a significance level of $\alpha = 0.05$ and data were reported as mean ± standard error (SE).

Results

Dissolved CO_2 concentrations were highest (and pH lowest) at each of the seeps; reference sites had normal CO_2 and pH. Salinity, temperature and total alkalinity were not affected by the seeps (Table 1).

Grain size analysis showed that 99% of the sediment particles sampled at all locations were sand. Most sediment trace element levels were significantly higher at seeps than at reference stations, except at Ischia (Figs 3 and 4). Large differences were found for Ni (5.3-fold) and Zn (2.39-fold) at Panarea, Cd (42.6-fold) at Paleochori and Cu (8.9-fold) at Adamas seep sediments, compared to reference stations. Mercury was only observed at Italian CO_2 seeps, with 1.4-fold higher levels in the seeps sediments at Vulcano that at Ischia and Panarea. Zinc sediment concentrations were similar at all locations but were 1.7-fold lower at Methana than at Ischia. However, Zn levels at the seeps of Panarea were 2.3-fold higher than at reference sites. The environmental quality of seep sediments for trace elements derived from the Sediment Quality Guidelines Quotient was mainly ‘Moderate’, although it was in the ‘Low’ to ‘Moderate’ range for reference stations. ‘Adverse’ biological effects were considered likely due to high levels of Hg at Vulcano, Cu at Ischia plus Ni and Cd at Paleochori (Table 2).

We were especially interested in results from Ischia as *P. oceanica* was abundant within the main CO_2 seep area (Fig.2a). The sediment at this seep has the highest Cu (32-fold), Zn (2-fold) and Pb (1.5-fold) concentrations than other two seep locations sampled for *P. oceanica*, but the seagrass tissues had low levels of these metals (Fig.3). On the other hand, *P. oceanica* at the Ischia seeps had higher concentration of Cd (19-fold), Zn (4-fold), Ni (3-fold) and Hg (1.2-fold) than the sediment (Fig.3). The concentrations of Ni at Paleochori, Pb at Vulcano and Zn at Adamas seeps were 18-fold, 4-fold and 3-fold higher in the sediment than in *C. nodosa* (Fig.4). Trace element levels were generally significantly higher in the roots than rhizomes and leaves of *P. oceanica* and *C. nodosa* at all seep locations (Figs. 3 and 4). Exceptions were Cd (8-fold) concentrations within the rhizomes, Zn (42-fold) and Cu (5-fold) within leaves of *P. oceanica* and Cd (6-fold), Pb (4-fold) and Hg (3-fold) within leaves of *C. nodosa* (Figs. 3 and 4).

Significant differences between the three sampling sites in the levels of trace elements in sediment and tissues were observed for *P. oceanica* (Table 3). Element concentrations measured in sediments and *P. oceanica* compartments differed significantly except for Cu (sediment-leaves) and Zn (sediment-roots), whereas within *P. oceanica* compartments all elements, except Pb (roots-leaves) has significant differences at all three sites. The accumulation of elements in *P. oceanica* plant parts did not show consistent common patterns for the three sampling sites. Hg and Cu were generally higher in roots and leaves than in rhizomes in all reference and seep sites. Zn was much higher in the leaves than in other plant parts at Ischia and Panarea, indicating leaf uptake. On the other hand, Cd was higher in the rhizomes of *P. oceanica* in reference and seep sites of Ischia and Panarea indicating mobility and storage in this plant part (Fig.3).

Significant variation was observed in trace element levels for *C. nodosa* between the three sites, except for Cu at Adamas vs Paleochori, Ni at Vulcano vs Adamas and Pb at Vulcano vs Paleochori (Table 4). Element levels measured in sediment and in *C. nodosa* compartments differed significantly, except for Cu (sediment vs rhizomes). The accumulation of elements in *C. nodosa* plant parts did not show highly consistent common patterns as in *P. oceanica* (Fig.4). However, Cu was always much higher in roots than other plant parts and Hg was higher in both roots and leaves than in rhizomes.

Correlation between trace element content in sediments and those recorded in *P. oceanica* roots and rhizomes were significant and positive for Zn and Ni in rhizomes at Ischia and Panarea seeps respectively, where in roots Cd was observed with positive correlation only at Panarea seeps (Table 5). Correlations of trace element content in sediment and those observed in roots and rhizomes of *C. nodosa* were significant and negative for Pb in both roots and rhizomes and for Zn only in rhizomes at Vulcano seeps (Table 5).

The Bio-Sediment Accumulation Factor indicated that in *P. oceanica* there was high root accumulation of Cd at all three sites and of Cu at Panarea and Methana. In *C. nodosa*, there was high accumulation of Cu in the roots at all three sites (Table 6).

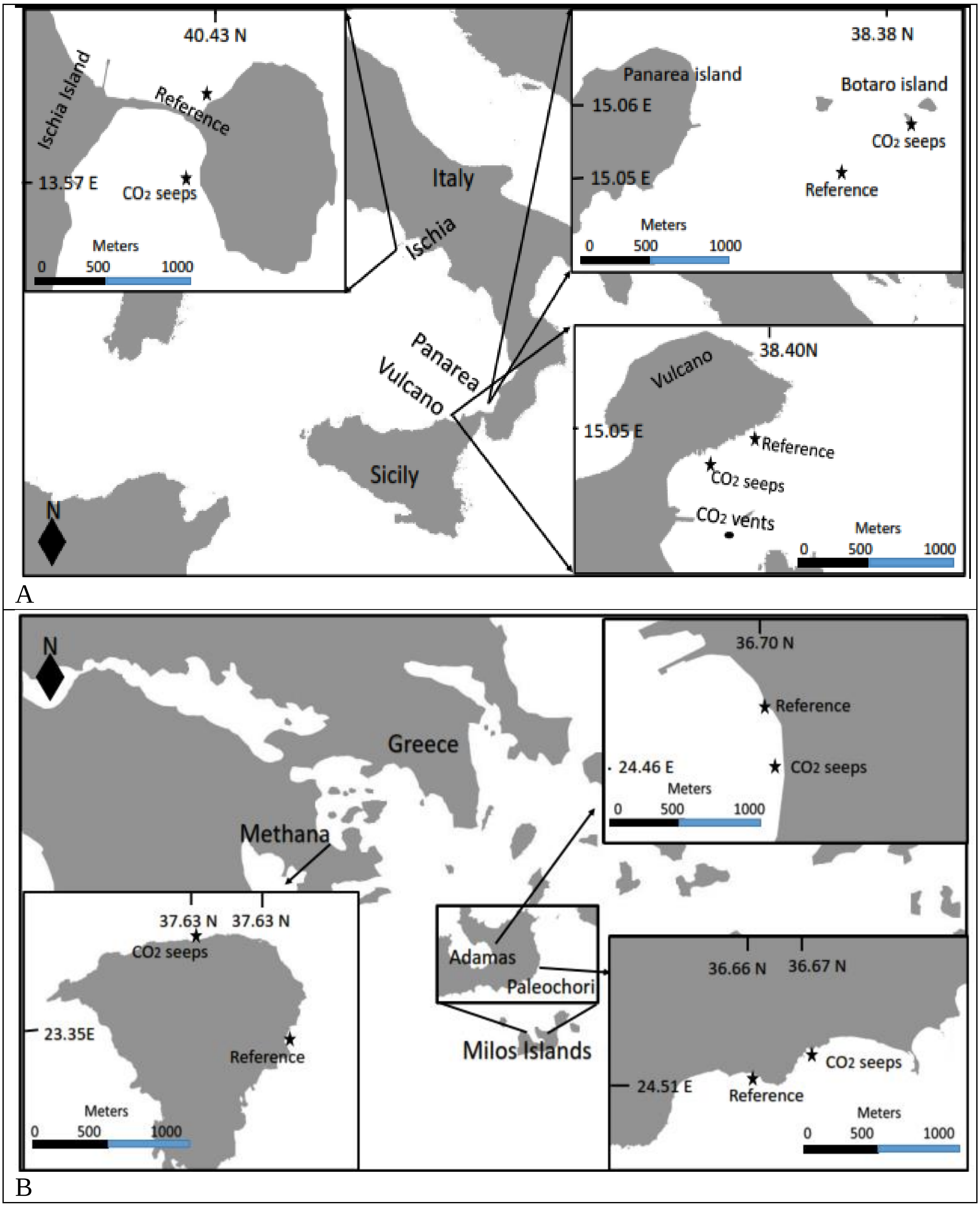
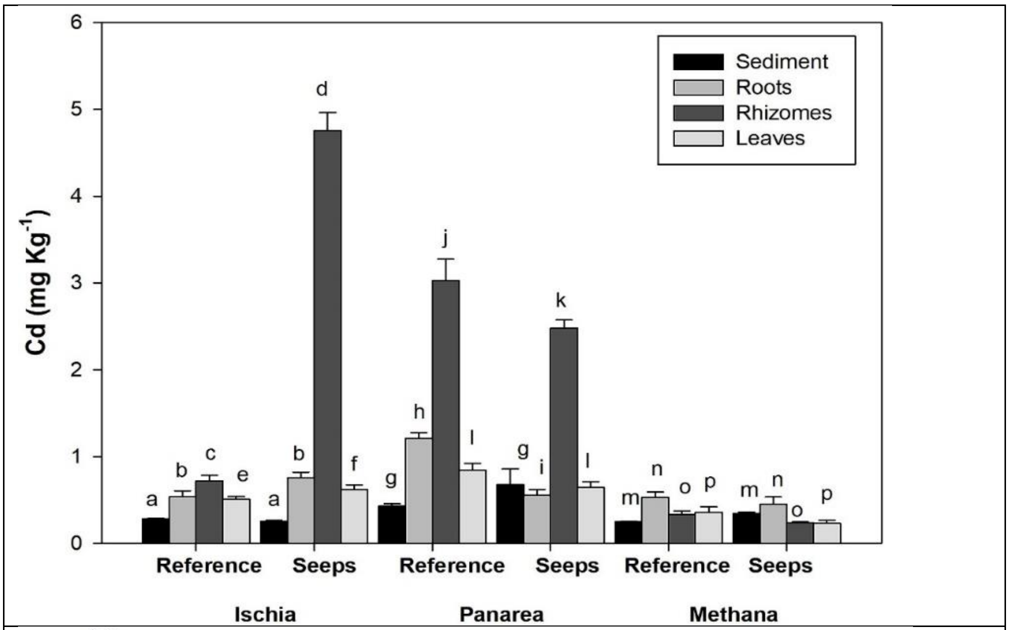
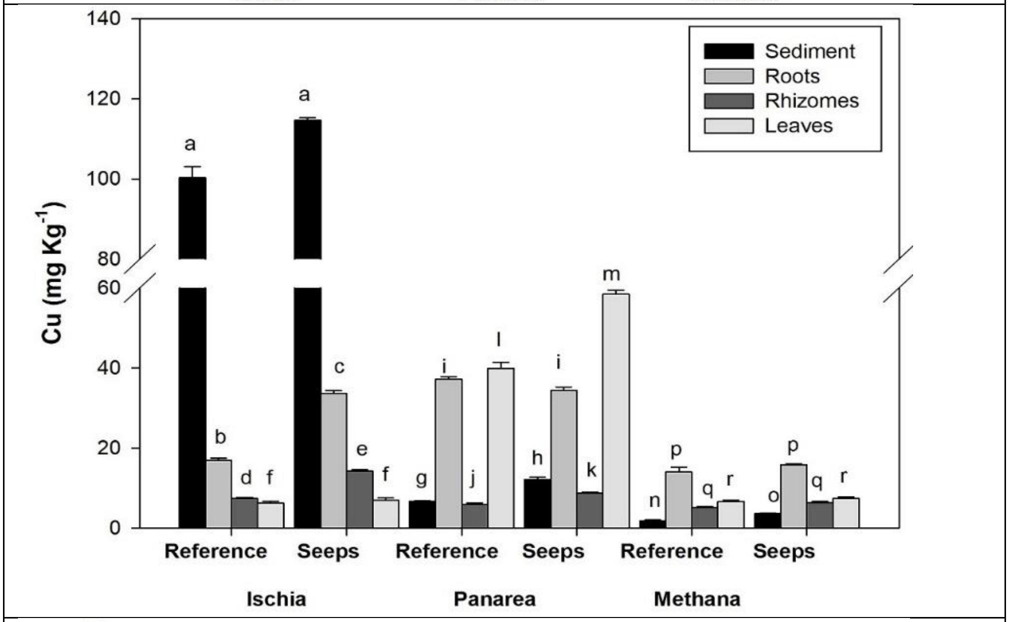


Fig.1. Study areas in a) Italy and b) Greece, showing reference and CO_2 seep stations, which were all sampled between May -July 2014.

Fig. 2. a) *Posidonia oceanica* and b) *Cymodocea nodosa* meadows at CO_2 seeps off Ischia (Italy) and Paleochori (Greece).

Photo credits for a) *Posidonia oceanica*, and b) *Cymodocea nodosa* meadows at Italy and Greece: Jason Hall Spencer, University of Plymouth, UK and Thanos Dailianis of Hellenic Centre for Marine Research, Greece respectively.


Cd (mg Kg^{-1})
0
1
2
3
4
5
6
Sediment
Roots
Rhizomes
Leaves
a b c e
a b d f
g h j l
g i k l
m n o p
m n o p
Reference
Seeps
Reference
Seeps
Reference
Seeps
Ischia
Panarea
Methana


Cu (mg Kg^{-1})
0
20
40
60
80
100
120
140
Sediment
Roots
Rhizomes
Leaves
a b d f
a c e f
g i j l
h i k m
n p q r
o p q r
Reference
Seeps
Reference
Seeps
Reference
Seeps
Ischia
Panarea
Methana

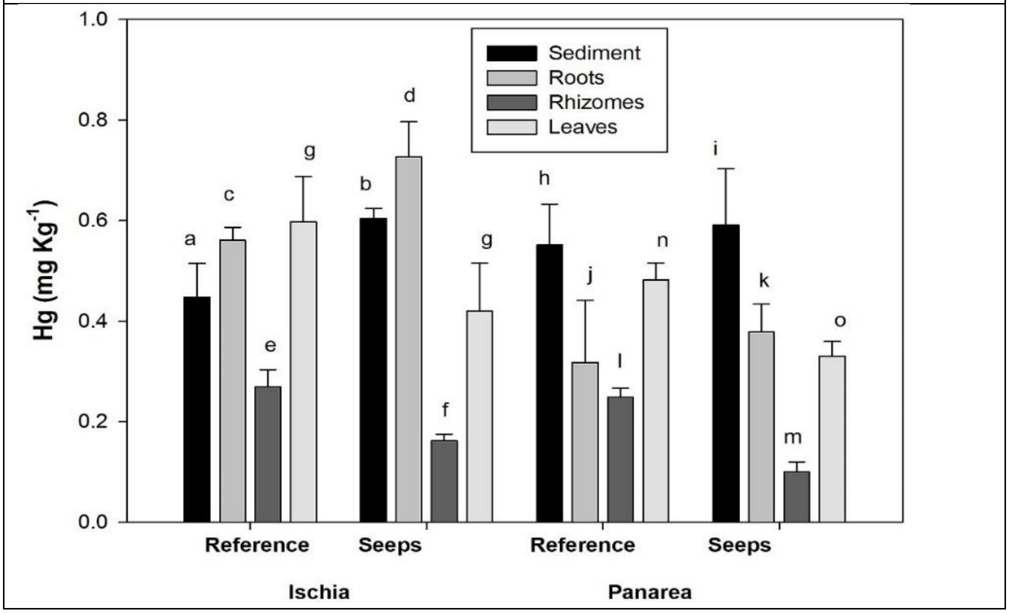
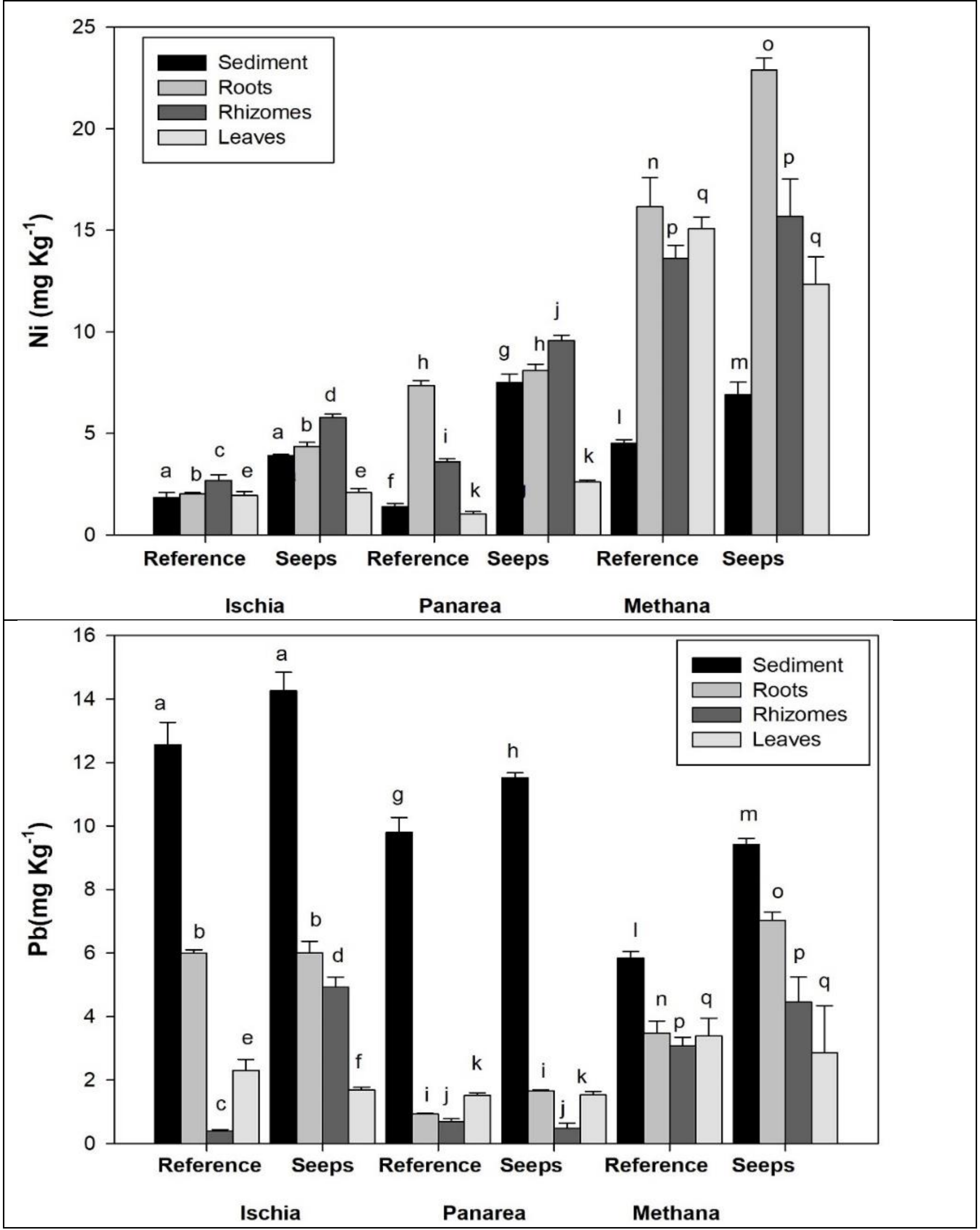

Hg (mg Kg^{-1})
0.0
0.2
0.4
0.6
0.8
1.0
Sediment
Roots
Rhizomes
Leaves
a c e g
b d f g
h j l n
i k m o
Reference
Seeps
Reference
Seeps
Ischia
Panarea

Fig.3. continued



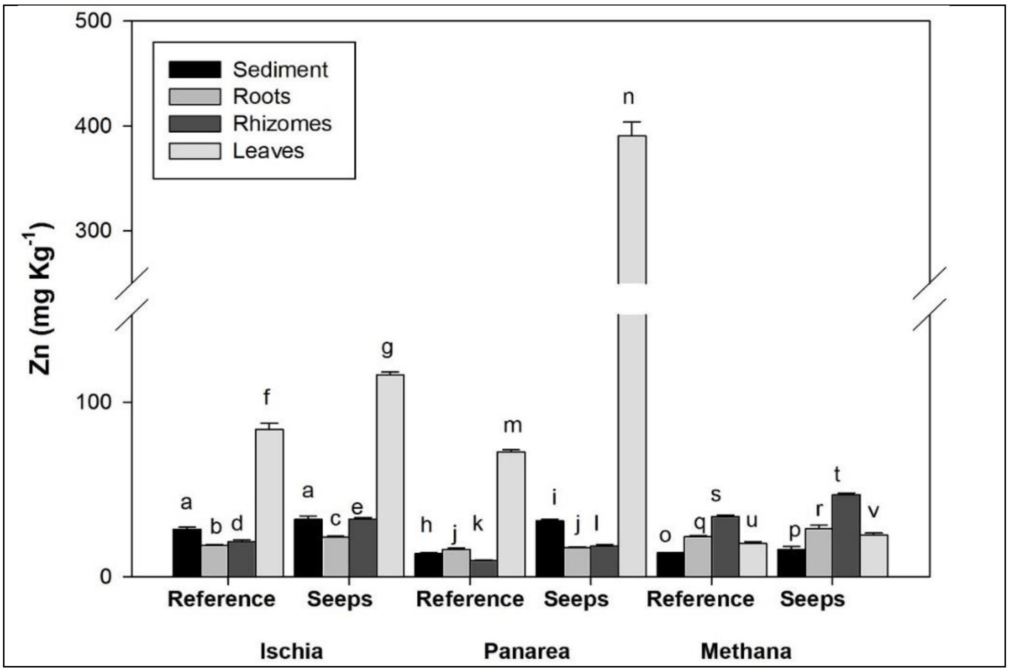


Fig. 3. Element concentrations (mean ± SE, n=5) of Cd, Cu, Hg, Ni, Pb and Zn in *Posidonia oceanica* plant compartments and sediments at reference and CO_2 seep sites off Italy and Greece. Different letters indicate significant differences between reference and CO_2 seep stations.

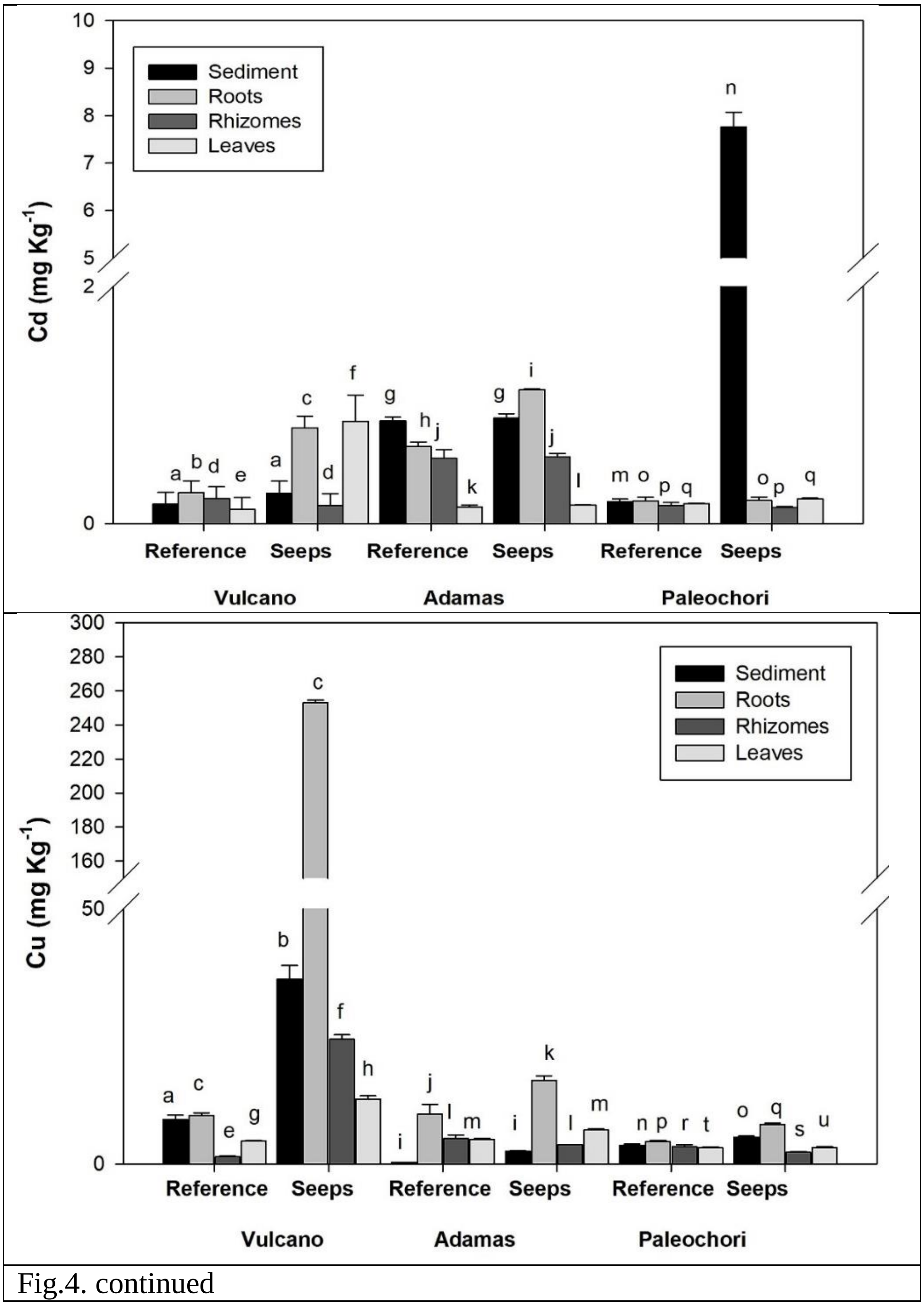
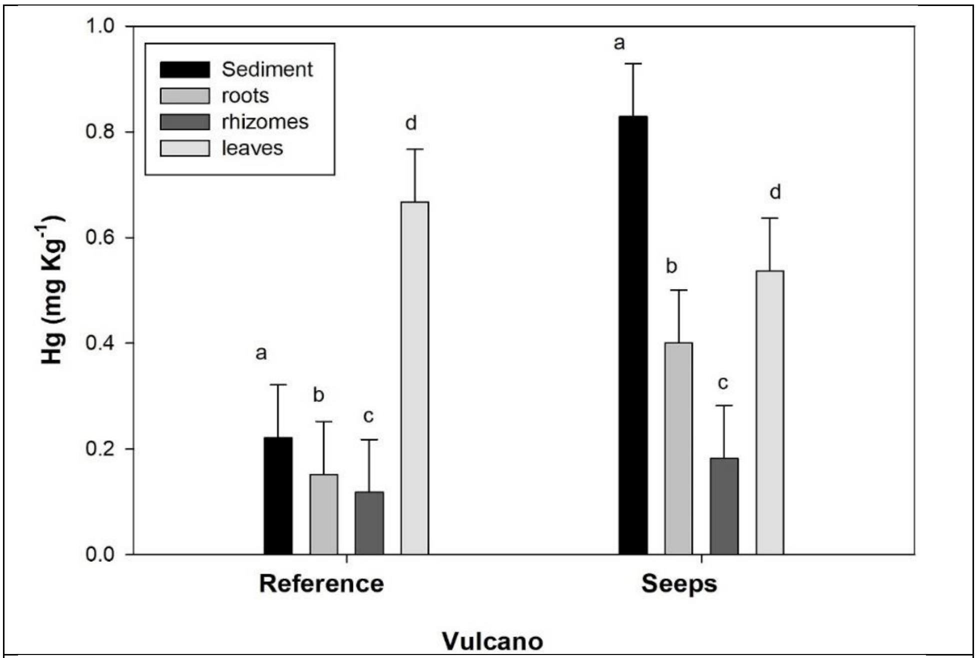
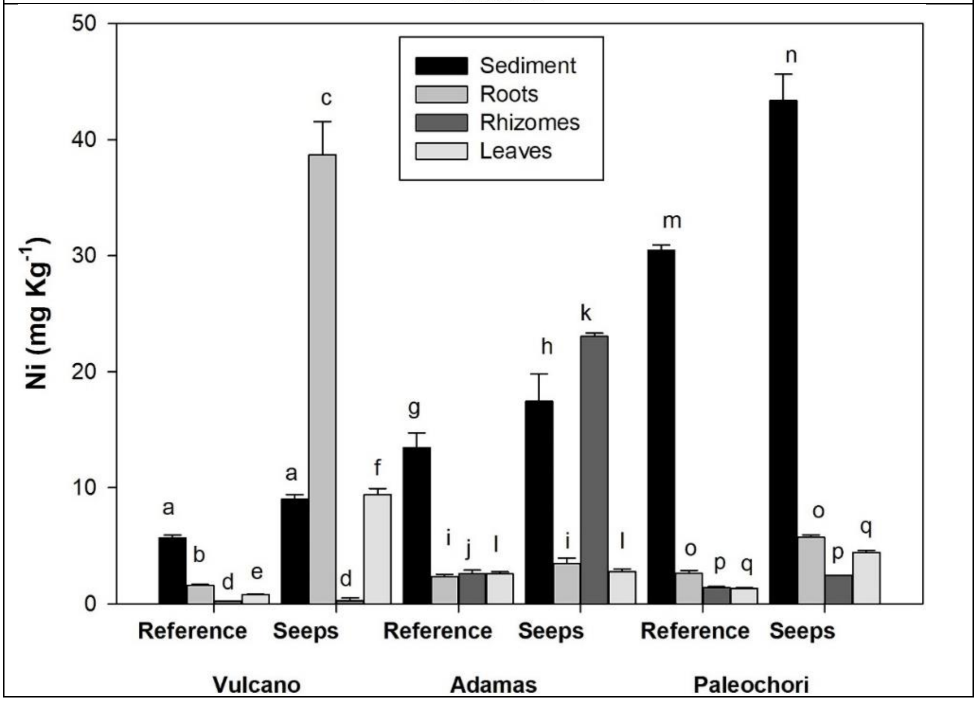


Fig.4. continued


Hg (mg Kg^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
Sediment
roots
rhizomes
leaves
a
b
c
d
a
b
c
d
Reference
Seeps
Vulcano


Ni (mg Kg^{-1})
50
40
30
20
10
0
Sediment
Roots
Rhizomes
Leaves
a
b
d
e
a
c
d
f
g
i
j
l
h
i
k
l
m
o
p
q
n
o
p
q
Reference
Seeps
Reference
Seeps
Reference
Seeps
Vulcano
Adamas
Paleochori

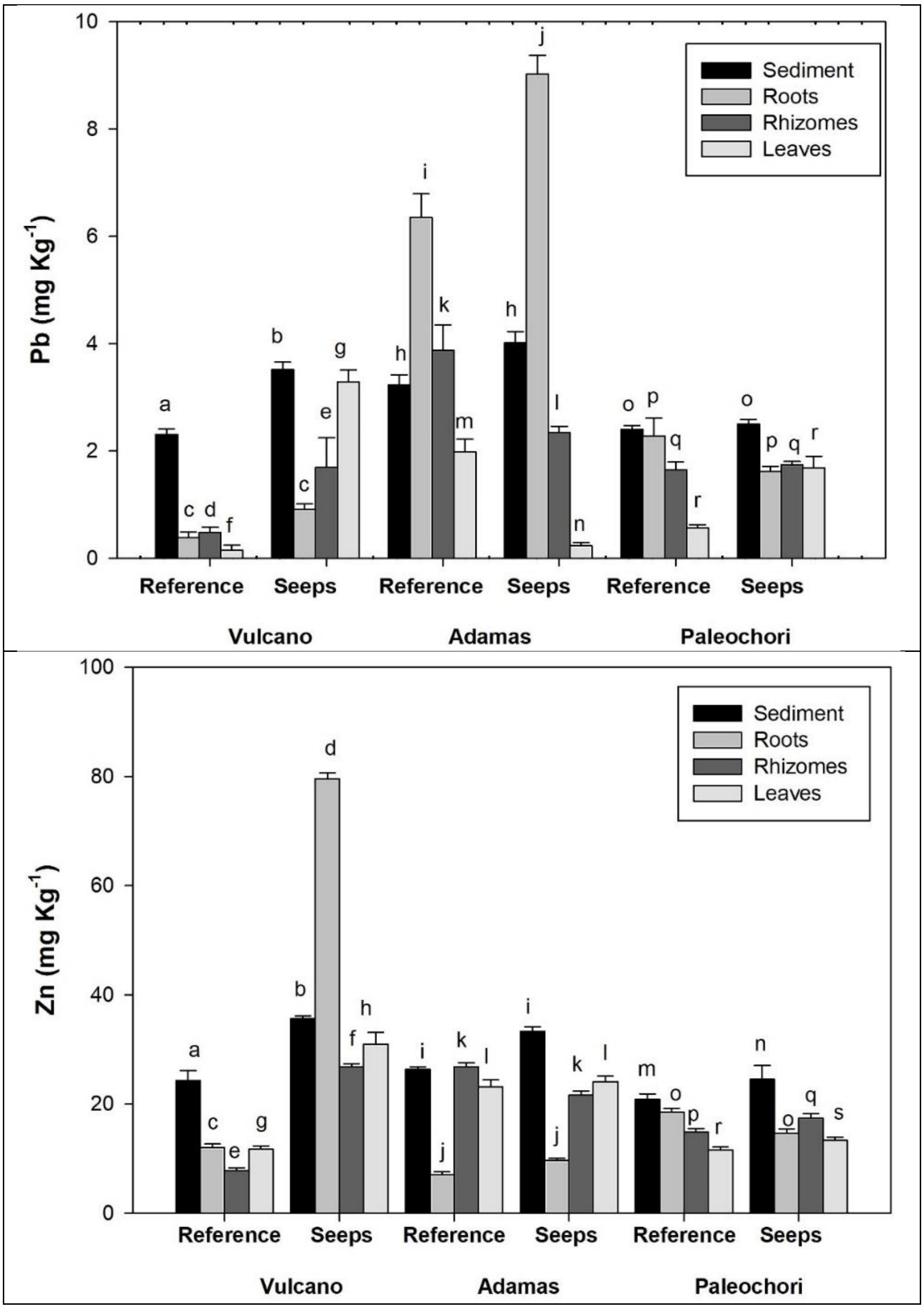


Fig. 4. Element concentration (mean ± SE, n=5) of Cd, Cu, Hg, Ni, Pb and Zn for *Cymodocea nodosa* in plant compartments and sediments at reference and CO_2 seeps off Italy and Greece. Different letters indicate significant differences between reference and CO_2 seep stations.

Table 1: Seawater salinity, temperature, total alkalinity, pH and pCO_2 values (mean ± SE, n=5) at six Mediterranean CO_2 seeps and Reference stations between May-July 2014.

Site	**Salinity (psu)**	**Temp.(°C)**	**pH_{NBS}**	**TA (µmol Kg SW^{-1})**	**pCO_2 (µatm)**
Vulcano					
Reference	35.8	21.6	8.17 ± 0.05	2439	427 ± 6.8
CO_2 seep	35.8	22.4	7.98 ± 0.08	2432	1928 ± 15.8
Ischia					
Reference	35.6	17.7	8.19± 0.06	2596	428± 2.3
CO_2 seep	35.7	17.8	7.78±0.05	2589	1653± 10.2
Panarea					
Reference	36.0	20.5	8.18±0.05	2507	420± 4.6
CO_2 seep	36.0	22.3	7.47±0.04	2500	3370± 2.3
Adamas					
Reference	36.7	22.6	8.2 ± 0.03	2715	405.5 ± 1.6
CO_2 seep	36.7	23.5	7.5 ± 0.04	2704	2457.9 ± 1.8
Paleochori					
Reference	36.0	22.6	8.2 ±0.01	2711	402.9 ± 1.1
CO_2 seep	36.0	22.8	7.9 ±0.01	2706	1884.3 ± 3.0
Methana					
Reference	36.8	22.8	8.2±0.01	2715	460 ± 6.9
CO_2 seep	36.8	23.0	7.8±0.02	2704	1980± 4.4

Table 2. Sediment Quality Guidelines-quotient (SQG-Q) of sediment calculated with Probable Effects Level for CO_2 seeps and Reference stations off Greece and Italy. SQG-Q <0.1 (low effect), <0.1 SQG-Q>1 (moderate effect), SQG-Q>1 (adverse biological effects). Numbers in bold indicate possible adverse effects of trace elements.

		SQG-Q		**Effects**	
Location	**Element**	**Reference**	**CO_2 seeps**	**Reference**	**CO_2 seeps**
Vulcano	Cu	0.08	0.33	Low	Moderate
	Hg	0.32	**1.18**	Moderate	**Adverse**
	Ni	0.13	0.21	Moderate	Moderate
	Zn	0.09	0.13	Low	Moderate
Ischia	Cu	0.93	**1.06**	Moderate	**Adverse**
	Hg	0.64	0.86	Moderate	Moderate
	Pb	0.11	0.13	Moderate	Moderate
	Zn	0.12	0.10	Moderate	Moderate
Panarea	Cd	0.10	0.16	Low	Moderate
	Cu	0.06	0.11	Low	Moderate
	Hg	0.79	0.84	Moderate	Moderate
	Ni	0.03	0.18	Low	Moderate
	Pb	0.09	0.57	Low	Moderate
	Zn	0.05	0.12	Low	Moderate
Adamas	Cd	0.21	0.21	Moderate	Moderate
	Ni	0.31	0.41	Moderate	Moderate
Paleochori	Cd	0.04	**1.84**	Low	**Adverse**
	Ni	0.71	**1.01**	Moderate	**Adverse**
Methana	Ni	0.11	0.16	Moderate	Moderate
	Pb	0.05	0.42	Low	Moderate

Table 3. Three-way ANOVA differences in trace element levels between Location: 3 levels (Methana (M), Panarea(P) and Ischia (V)), Stations:2 variables (CO$_2$ seeps, Reference)) and compartments :4 levels (Sediments (Sd), Rhizomes (Rh), Roots (R), Leaves (L)). Holm-Sidak significant test ($p<0.05$) is presented for locations, sediment and *P. oceanica* compartments. Numbers in bold indicate differences that were not significant.

			Holm-Sidak p values								
			Location			**Sediment vs Compartment**			**Compartments**		
Element	**Variation**	**p value**	**M vs P**	**M vs V**	**V vs P**	**Sd vs R**	**Sd vs Rh**	**Sd vs L**	**R vs Rh**	**Rh vs L**	**R vs L**
Cd	Location	<0.001	<0.001	<0.001	<0.001						
	Station	<0.001									
	Compt.	<0.001				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cu	Location	<0.001	<0.001	<0.001	<0.001						
	Station	<0.001									
	Compt.	<0.001				<0.001	<0.001	**0.314**	<0.001	<0.001	<0.001
Ni	Location	<0.001	<0.001	<0.001	<0.001						
	Station	<0.001									
	Compt.	<0.001				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pb	Location	<0.001	<0.001	<0.001	<0.001						
	Station	<0.001									
	Compt.	<0.001				<0.001	<0.001	<0.001	<0.001	<0.001	**0.652**
Zn	Location	<0.001	<0.001	<0.001	<0.001						
	Station	<0.001									
	Compt.	<0.001				**0.222**	<0.001	<0.001	<0.001	<0.001	<0.001

Table 4. Three-way ANOVA differences in Fe and trace element levels between Location: 3 levels (Adamas (A), Paleochori (P) and Vulcano (V)), Stations:2 variables (CO$_2$ seeps, reference) and compartments: 4 levels (Sediments (Sd), Rhizomes (Rh), Roots (R), Leaves (L). Holm-Sidak significant test ($p<0.05$) is presented for locations, sediment and *C. nodosa* compartments. Numbers in bold indicate differences that were not significant.

			Holm-Sidak p values **Location**			**Sediment vs Compartment**			**Compartments**		
Element	**Variation**	**p value**	**A vs P**	**A vs V**	**V vs P**	**Sd vs R**	**Sd vs Rh**	**Sd vs L**	**R vs Rh**	**Rh vs L**	**R vs L**
Cd	Location	<0.001	<0.001	<0.001	<0.001						
	Station	<0.001									
	Compt.	<0.001				<0.001	<0.001	<0.001	<0.001	**0.787**	<0.001
Cu	Location	<0.001	**0.626**	<0.001	<0.001						
	Station	<0.001									
	Compt.	<0.001				<0.001	**0.621**	<0.001	<0.001	<0.001	<0.001
Ni	Location	<0.001	<0.001	**0.853**	<0.001						
	Station	<0.001									
	Compt.	<0.001				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pb	Location	<0.001	<0.001	<0.001	**0.286**						
	Station	<0.001									
	Compt.	<0.001				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Zn	Location	<0.001	<0.001	<0.001	<0.001						
	Station	<0.001									
	Compt.	<0.001				<0.001	<0.001	<0.001	<0.001	**0.910**	<0.001

Table 5. Results of correlation analysis between trace elements content in sediments and seagrass (*P. oceanica* and *C. nodosa*) roots and rhizomes at high CO_2 sites off Italy and Greek coast. r is the correlation co-efficient and significance level ($p < 0.050$). Bold letters indicate significant correlation. Trace elements only with significant co-relation are presented.

Seagrass	**Location**	**Element**	**Sediment-roots**		**Sediment-rhizomes**	
			r	p value	r	p value
P. oceanica	Ischia	Zn	-0.234	0.704	0.870	**0.048**
	Panarea	Cd	0.841	**0.014**	-0.910	**0.032**
		Ni	-0.358	0.554	0.884	**0.046**
C. nodosa	Vulcano	Pb	-0.881	**0.048**	-0.889	**0.037**
		Zn	-0.795	0.108	-0.966	**0.007**

Table 6. Bio-Sediment Accumulation Factor (BSAF) of trace metals in *P. oceanica* and *C. nodosa* roots at CO_2 seeps (seeps) and Reference (Ref.) stations off Italy and Greek coast. Sediment (Sd), Roots (Ro). Bold numbers indicate TF>1 value.

	Location	**Ischia**		**Panarea**		**Methana**	
Seagrass	**Elements**	**BSAF(Ro/Sd)**		**BSAF(Ro/Sd)**		**BSAF(Ro/Sd)**	
		Ref.	**Ref.**	**Ref.**	**Seeps**	**Ref.**	**Seeps**
P. oceanica	Cd	**1.9**	**3.0**	0.08	**2.71**	**2.12**	**1.28**
	Cu	0.17	0.29	**5.15**	**3.10**	**8.21**	**4.49**
	Hg	0.47	0.43	**1.95**	0.13	-	-
	Ni	**1.12**	**1.11**	0.22	**1.10**	**3.63**	**3.42**
	Pb	**1.42**	**1.21**	0.75	0.74	0.61	0.75
	Zn	0.81	0.56	**1.12**	0.52	**1.70**	**1.87**
		Vulcano		**Adamas**		**Paleochori**	
C. nodosa	Cd	**1.71**	**2.23**	0.45	0.52	**1.03**	0.03
	Cu	**1.14**	**4.32**	**36.65**	**6.50**	**1.23**	**1.49**
	Hg	**1.97**	0.51	**-**	**-**	-	-
	Ni	0.28	**3.17**	**2.05**	**1.69**	0.09	0.13
	Pb	0.07	0.11	0.76	**1.28**	0.97	0.65
	Zn	0.50	**1.13**	**1.62**	**2.27**	0.90	0.62

Table 7. Mean range concentration (mg/Kg) of trace elements in sediment and *P. oceanica* and *C. nodosa* tissues off the coast of Italy and Greece. Data collected from literature only included the pristine sites with seagrass meadows around Greece and Italy and seagrass meadows within contaminated sites and sediment samples taken from ship-based cores were excluded. Samples of CO_2 seeps off Italy and Greek coast are indicated in bold. Sediment (Sd), Leaves (L), Rhizomes (Rh), Roots (R).

Sample/Location	**Study site**	**Sample**	**Cd**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**	**References**
Sediment									
Italy	Sicily	Sd	0.19-0.25	5.23-7.25	0.1-0.17	39.8-52.4	3.7-5.7	31.4-54.7	Bonanno and Raccuia, 2018
	Sicily	Sd	0.24	1.6	-	-	1.77	7.5	Campanella et al. 2001
	Sicily	Sd	0.15-0.30		0.18-0.6	-	4.31-7		Vizzini et al. 2013
	Ionian Sea	Sd	0.06	2.03		-	4.57	31.75	Cozza et al. 2013
	Taranto Gulf	Sd	0.12-0.17	8.0-22.3	0.1-1.79	-	14.2-29.1	35-68	Di Leo et al. 2013
	Vendicari, Sicily	Sd	0.3	0.20	-	3.23	2.2	10.5	Bonanno and Martino, 2017
	Vendicari, Sicily	Sd	0.15	3.04	-	5.4	6.22	11.4	Bonanno and Martino, 2016
	Panarea	**Sd**	**1**		**4.5**	**-**	**60**		**Renzi et al. 2011**
	Vulcano	**Sd**	**0.11-0.32**	**26.4-76.1**	**0.01-0.2**	**-**	**5.8-25.0**	**13.8-78.2**	**Vizzini et al., 2013**
	Vulcano	**Sd**	**0.23-0.31**	**29.41-44.80**	**0.74-1.09**	**8-9.87**	**3.2-3.97**	**34.2-36.7**	**This study**
	Ischia	**Sd**	**0.23-0.29**	**113-116**	**0.55-0.67**	**0.85-2.20**	**12.05-15.3**	**24.3-30.7**	**This study**
	Panarea	**Sd**	**0.18-0.98**	**6.01-6.91**	**0.38-0.95**	**0.99-1.39**	**11.1-14.8**	**11.9-15.7**	**This study**
Greece		Sd							
	Hellenic Volcanic arc	**Sd**	**-**	**18**	**-**	**-**	**20**	**43**	**Hodkinson et al. 1994**
	Adamas	**Sd**	**0.76-0.97**	**2.3-2.7**	**-**	**10-17**	**2.76-3.72**	**31.2-35.8**	**This Study**
	Paleochori	**Sd**	**0.14-0.28**	**4.8-5.6**	**-**	**35.2-48.1**	**2.27-2.74**	**14.7-28**	**This Study**
	Methana	**Sd**	**0.30-0.38**	**3.05-3.75**	**-**	**5.6-8.9**	**9-10**	**11.5-18.7**	**This Study**
P. oceanica									
Italy	Italy	L	0.4-1.76		0.01-0.04		0.4-1.96		Costantini et al. 1991

	Ischia	L	-	-	0.01-0.18	-	-	-	Pergent and Pergent-Martini. 1999
	North Sardinia	L	0.6-2	6-17	-	-	5.2-11.2	-	Baroli et al. 2001
		Rh	0.8-2.4	5.4-15.3	-	-	0.8-2.4	-	
	Ustica Island	L	3.6-7.5	19.8-53.2	-	-	1.1-5	142-260	Conti et al. 2007
		Rh	0.6-1.7	9.4-14.3	-	-	0.03-28	58	
	Linosa Island,	L	0.95-5.49	3.12-17.7	-	-	0.59-13.8	16.5-156	Conti et al.2010
	Tyrrhenian Sea	L	0.22-0.38	1.01-2.79	-	1.44-5.21	0.15-0.52	-	Bravo et al. 2016
	Sicily	L	1.45	10.5	-	9.5	2.1	55.7	Bonanno and Martino, 2017
		Rh	0.89	7.12	-	2.34	1.14	32.5	
		R	1.8	14.6	-	5.12	2.56	44.3	
	Ischia	**L**	**0.51-0.62**	**6.16-6.89**	**0.42-0.60**	**1.8-2.8**	**1.69-2.39**	**84.21-115**	**This study**
		Rh	**0.72-4.76**	**7.38-14.2**	**0.16-0.27**	**5.21-6.26**	**0.39-4.92**	**19.9-32.9**	
		Ro	**0.53-0.76**	**16.8-33.6**	**0.56-0.73**	**3.79-4.95**	**5.98-6.01**	**17.8-22.6**	
	Panarea	**L**	**0.64-0.84**	**39.8-58.3**	**0.33-0.48**	**0.82-1.47**	**1.51-1.53**	**71.4-390**	**This study**
		Rh	**2.48-3.03**	**5.92-8.6**	**0.10-0.25**	**3.1-3.97**	**0.48-0.70**	**9.37-17.6**	
		Ro	**0.55-1.21**	**37.2-34.3**	**0.32-0.38**	**6.9-7.9**	**0.93-1.65**	**15.6-16.7**	
Greece									
	Aegean Sea,	L	1.99	-	-	21.2	-	-	Catsiki and Bei. 1992
	Aegean Sea	L	-	0.44-45.8	-	21.1-60.9	-	-	Catsiki and Panayotidis. 1993
		Rh		0.41-58.6	-	3.34-46.2	-	-	
		R		0.25-36.1	-	3.34-46.2			
	Methana	**L**	**0.35-0.23**	**6.59-7.39**	**-**	**10.8-17.7**	**3.38-2.86**	**18.9-23.9**	**This study**
		Rh	**0.33-0.24**	**5.08-6.30**	**-**	**10.9-19.9**	**3.08-4.45**	**34.5-46.9**	
		Ro	**0.53-0.45**	**13.9-5.78**	**-**	**21.6-24.79**	**3.48-7.03**	**23.0-27.4**	
Italy									
C. nodosa	Sicily	L	0.39-3.82	-	0.36-0.7	-	3.32-33.42	-	Vizzini et al. 2013
	Sicily	L	0.55	3.9	-	5.57	1.85	43.4	Bonanno and Martino. 2016
		Rh	0.1	2.06	-	1.15	0.38	24.2	
		R	0.21	3.35	-	3.45	4.56	35.3	
	Vulcano	**L**	**0.45-1.61**	**-**	**-**	**-**	**2.86-8.26**	**-**	**Vizzini et al. 2013**

	Vulcano	**L**	**0.12-0.86**	**4.52-12.6**	**0.35-0.64**	**0.81-9.41**	**0.14-3.29**	**11.3-30.9**	**This study**
		Rh	**0.21-0.15**	**1.38-24.4**	**0.63-1.59**	**0.23-0.26**	**0.48-1.69**	**7.7-26.7**	
		Ro	**0.26-0.81**	**9.5-250.0**	**0.37-0.47**	**1.60-38.6**	**0.39-0.91**	**12.0-79.5**	
Greece									
	North Evvoikos Gulf	L	1.2	9.6	-	7.6	-	57.5	Nicolaidou and Nott, 1998
		Rh	2.1	7.7	-	1.2		23	
		R	2.1	12.8	-	5.2	-	22.92	
	Aegean Sea	L	-	2.1	-	2.8	-	-	Catsiki and Panayotidis, 1993
		Rh		0.19-11.1	-	1.4-8.95			
		R		1.11-75.4	-	3.4-50	-	-	
	Thessaloniki Gulf	L	-	-	-	2.33	-	-	Malea and Kevrekidis, 2013
		Rh	-	-	-	0.85	-	-	
		R				0.34-5.04			
	Milos	**L**	**0.14-0.15**	**4.74-6.63**	**-**	**2.56-2.76**	**0.23-1.98**	**23.1-24.2**	**This study**
		Rh	**0.55-0.56**	**4.95-3.43**	**-**	**2.61-23.0**	**2.34-3.88**	**21.6-26.8**	
		Ro	**0.65-1.13**	**9.78-16.3**	**-**	**2.33-3.49**	**6.35-9.02**	**7.02-9.72**	
	Paleochori	**L**	**0.17-0.21**	**3.15-3.21**	**-**	**1.33-4.4**	**0.57-1.69**	**11.5-13.3**	**This study**
		Rh	**0.15-0.14**	**3.43-2.29**	**-**	**1.40-2.42**	**1.65-1.74**	**14.8-17.3**	
		Ro	**0.19-0.19**	**4.35-7.71**	**-**	**2.62-5.75**	**2.28-1.62**	**14.4-18.5**	

Discussion

Shallow water CO_2 seeps have been used as natural analogues for future coastal ecosystems as they can have areas of seabed where entire communities of marine organisms are exposed to the shifts in carbonate chemistry that are expected due to continued anthropogenic CO_2 emissions (Hall-Spencer et al., 2008; Enochs et al., 2015; Connell et al., 2017). At such seeps, there are often elevated levels of trace elements and H_2S, so care is needed when using them to assess the effects of ocean acidification (Barry et al., 2010; Vizzini et al. 2010). This is done by mapping areas affected by volcanic fluid toxics and avoiding those areas when assessing the effects of increased $p\mathrm{CO_2}$ in seawater (Boatta et al. 2013; Agostini et al. 2018). The six CO_2 seeps that we surveyed showed sediments were enriched with Cd, Cu, Hg, Ni, Pb and Zn. This was expected since hydrothermal seep sediments often have high levels of metals (Aiuppa et al., 2000; Sternbeck et al., 2001) due to continuous input from the subsea floor into the sediments (Dando et al., 2000). The calculated Sediment Quality Guidelines Quotient (Long et al., 1998; MacDonald et al., 2000) suggests Hg (at Vulcano), Cu (at Ischia) plus Cd and Ni (at Paleochori) were at high enough levels to have adverse impacts on marine biota. So, careful selection of study sites is needed to avoid the combined effects of various factors like trace metals and toxic gases while conducting ocean acidification research.

The trace element levels observed within CO_2 seep sediments were higher for Cd and Cu, were similar for Hg and lower for Ni, Pb and Zn than mean element levels observed around Mediterranean coast of Italy (Table 7). We think that this is because the sediments studied were sandy and lacked clay particles (<63µm) which bind more trace elements in finer sediments. Trace element levels observed at seep sediments off Vulcano, Italy, were in the same range for Cd, 5-fold higher for Hg and lower for Cu (1.7-fold), Pb (6-fold) and Zn (2-fold) from previously measurements by Vizzini et al. (2013). Levels of Hg and Pb measured at Panarea CO_2 seeps were 5-fold and 4-fold lower from those reported by Renzi et al. (2011), probably because Renzi et al. (2011) sampling was made just after a massive outgassing event with increased input of elements, whereas no such influx was observed during our sampling. Trace element levels in seep sediments of the Greece coast were 3-fold (Cu), 2-fold (Pb) and 1.2-fold (Zn) lower than previously reported by Hodkinson et al., (1994), whereas Cd and Ni are reported for the first time for this coast (Table7). These higher levels of elements could be in part due to weathering and land run-off on-land which makes their way to these shallow volcanic seeps along with hydrothermal inputs (Hodkinson et al., 1994). The difference in element levels within the CO_2 seep sediments of Italy and Greece coasts indicate the heterogeneous patchiness in metal concentrations around seep systems, variation in influx of elements from CO_2 seeps and the variable biogeochemical factors (such as variation in pH and sediment grain size) that influences the metal availability at the CO_2 seeps. These variations of trace element levels in sediment between CO_2 seeps and pristine sites off Greek and Italy coast were also reflected in the plant accumulation of trace elements in roots, rhizomes and leaves (Table 7).

Element levels were higher in seagrass compartments at the seep sites compared to reference sites. Seagrass element accumulation is more element and seagrass tissue-specific rather than species-specific (Bonanno and Bonaca, 2017) resulting in seagrass compartments acting as metal accumulators of their surrounding environment, especially of heavy metals (Govers et al., 2014). In our analyses most elements in both seagrasses were more concentrated in roots than rhizomes which had more metals than the leaves, which is typical for *P. oceanica* and *C. nodosa* (Bonanno and Bonaca, 2017). Higher element accumulation in roots and leaves than rhizomes were also observed for *P. oceanica* and *C. nodosa* from pristine sites off Italy and Greek coast (Table 7). Root accumulation is common in both terrestrial and aquatic plants where they store and sequester certain elements to avoid damage to photosynthetic apparatus. This root accumulation of elements is then internally regulated for elements like Cd, Ni and Pb from roots to rhizomes to leaves suggesting that seagrasses have different tolerance mechanisms for dealing with trace elements that either accumulate in the roots or are moved out through the leaves which are then shed, as observed in *P. oceanica* (Di Leo et al., 2013; Richir and Gobert, 2016) and in *C. nodosa* (Malea and Haritinoids, 1999; Bonanno and Di Martino, 2016). This transfer of trace elements from roots to leaves of *P. oceanica* and *C. nodosa* also promote the release of these elements into the food webs of coastal ecosystems or the water column. On the other hand, storage and sequestration of metals in the below ground tissues like roots also reduces metal burden of seagrasses as below ground tissues are permanently buried (Windham et al., 2001). Seagrasses accumulate some elements, such as Cd and Ni, that are essential micronutrients (Sanz-Lazaro et al., 2012) rather than Hg or Pb that are toxic (Kabata-Pendias and Mukherjee, 2007), similar preferences has been observed for accumulation of Zn over Pb in both *P. oceanica* (Sanchiz et al., 2001) and *C. nodosa* (Malea and Haritonidis, 1999; Llagostera et al., 2011). However, seagrasses also tend to store toxic elements like Hg and Pb in the vacuoles of cortical tissue of roots outside the endodermis or in cell walls, thereby preventing the uptake of these elements into rhizomes and leaves (Windham et al., 2001).

Significant positive correlation of trace elements between seagrass tissues and sediment suggest the bioindication potential of seagrass tissues for that trace element (Bonanno and Borg, 2018). For instance, positive correlation was found in *P. oceanica* for Cd through sediment-root pathway and for Zn and Ni through sediment-rhizome, which indicates that roots of *P. oceanica* are potential bioindicators of Cd and rhizomes of Zn and Ni at CO_2 seeps off Italy. In *C. nodosa* no positive correlation was found for any of the elements analysed, which indicates their low potential for being bioindicators of trace metals and this also suggests why *P. oceanica* is used as a bioindicator in most of trace metal accumulation studies in Mediterranean Sea (Bonanno et al., 2017). In *P. oceanica* significant negative correlation was found for Cd in sediment-rhizomes and in *C. nodosa* negative correlation was found for Pb between sediment -roots and Zn between sediment- rhizomes. Negative correlation suggests that the preferable route for Cd transfer in *P. oceanica* (Lafabrie et al., 2007; Di Leo et al., 2013) and Zn in *C. nodosa* (Malea et al., 1999) is through water column rather than the sediment-root pathways. Similarly, elements such as Pb with negative correlation in *C. nodosa*, suggests Pb being toxic is not uptake or stored within the seagrass compartments (Sanchiz et al., 2001).

Bio-Sediment Accumulation Factor analysis between elements in sediment and in seagrass roots indicate that the pathway of uptake/storage is not always the sediment-root, even though higher element concentrations were observed in the sediments at CO_2 seeps. Even though, in *P. oceanica* Cd and Ni, were found with BSAF>1 in roots at all three seep stations, which suggests that accumulation of elements like Cd and Ni are made through the sediment-root pathway, for elements like Cu, Hg, Pb and Zn a mixed response (higher at reference and lower at seep sites or vice versa) of BSAF>1 was found, which indicates that for these trace elements both sediment-root and water-root pathways may be used. BSAF >1 value observed for trace elements in *P. oceanica* at the CO_2 seeps of Italy and Greek coast are within the range of BSAF values observed for *P. oceanica* in Mediterranean Sea (Bravo et al., 2016). In *C. nodosa* Cu was the only element with BSAF>1 in roots found at all three seep stations, whereas other elements showed mixed response. Cu being an essential element is preferred for root accumulation through sediment-root pathway, whereas other elements can use a mixed accumulation from sediment-roots or water-roots or water-leaves pathway (Bonanno and Di Martino, 2016). However, it was observed for both *P. oceanica* at Ischia and Panarea and *C. nodosa* at Vulcano seeps, that Hg accumulation from sediment-roots pathway (BSAF>1) was not higher than reference sites. This suggests Hg being toxic to the plant roots is not preferred for accumulation in seagrass (Bonanno and Di Martino, 2016).

At CO_2 seeps the low pH can alter the metal speciation and favour the release of metals from sediment (Simpson et al., 2004: Atkinson et al., 2007). The chemical form in which metals are present (e.g. whether they are bound to organic or inorganic compounds) is a key issue determining its bioavailability. Low pH of seawater near the CO_2 seeps tends to release the metals that are less strongly associated with sediments, increasing their potential bioavailability (Riba et al., 2004). Thus, low pH can increase the concentration of certain dissolved metals, which could affect the sediment-seagrass associated biota e.g., by increasing Cu, Cd and Zn bio-availability, their accumulation and possible toxic effects (Basallote et al., 2014).

In our research, all the CO_2 seeps had low pH (7.4-7.9) conditions, which are known to increase the availability of Cd, Cu, Ni, Pb and Zn in their free ion forms (Roberts et al., 2013). Low pH combined with increased availability can influence and increase seagrass uptake of trace elements (Yang and Ye, 2009) that can lead to higher accumulation and storage of trace elements in seagrass roots and leaves (Bonanno and Bonaca, 2017). Higher accumulation can lead to metal stress once threshold levels are reached and affect the seagrass physiological processes (Olìve et al., 2017). However, it is difficult to measure toxic effects of metals on seagrass in *in-situ* conditions due to variable environmental settings, but a few *ex-situ* studies on metal toxicity have been conducted on *Cymodocea serrulata* (Prange and Dennison. 2000), *Halophila ovalis* and *H. spinulosa* (Prange and Dennison, 2000; Ambo-Rappe et al., 2011). Considering the observed results from these ex-situ metal toxicity studies, there is a possibility that elements such as Cu and Pb at the CO_2 seeps may affect *P. oceanica* and *C. nodosa* photosynthesis as well as root and leaf structures (Prange and Dennison. 2000; Ambo -Rapee et al., 2011). This may be why seagrasses are abundant at some seeps but not at others.

Conclusion:

We observed that Greek and Italian marine CO_2 seeps had elevated levels of trace elements in sediments compared to reference sites, and that this can be used to investigate interactions between seawater pH, element bioavailability and element accumulation within marine organisms. Care is needed when using volcanic CO_2 seeps as analogues for the effects of ocean acidification as increased levels of trace elements can be harmful to marine biota. In some cases, such as Ischia, high levels of Cu in the sediment were not accumulated in seagrass. At other sites low pH increased the accumulation of trace metals in seagrass, such as with Zn off Vulcano, Panarea and Ischia. Our research shows that ocean acidification can affect the bioaccumulation of some trace elements, which is relevant to agencies responsible for monitoring the effects of contamination in the marine environment.

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