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Elevated trace elements in sediments and seagrasses at CO2 seeps

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6	Elevated trace elements in sediments and seagrasses at CO2 seeps
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14	Abstract
15	Seagrasses often occur around shallow marine CO2 seeps, allowing assessment of trace
16	element accumulation. Here, we measured Cd, Cu, Hg, Ni, Pb and Zn levels at six CO2 seeps
17	and six reference sites in the Mediterranean. Some seep sediments had elevated metal
18	concentrations; an extreme example was Cd which was 43x more concentrated at a seep than
19	its reference. Three seeps had metal levels that were predicted to adversely affect marine biota,
20	namely Vulcano (for Hg), Ischia (for Cu) and Paleochori (for Cd and Ni). There were higher-
21	than-sediment levels of Zn and Ni in Posidonia oceanica and of Zn in Cymodocea nodosa,
22	particularly in roots. High levels of Cu were found in Ischia seep sediments, yet seagrass was
23	abundant, and the plants contained low levels of Cu. Differences in bioavailability and toxicity
24	of trace elements helps explain why seagrasses can be abundant at some CO ₂ seeps but not
25	others.
26	Highlights:
27	• Sandy CO ₂ seep sediments had higher concentration of trace elements than sandy
28	reference sites.
29	• Metals can be more toxic in areas affected by CO ₂ acidification, with adverse effects

- 30 on the sediment associated biota
- Seagrasses element accumulation at CO₂ seeps was highest in the roots

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

35 Introduction:

Around 30% of anthropogenic CO₂ emissions have dissolved into surface seawater 36 causing the pH to fall in a process known as 'ocean acidification' (Caldeira and Wickett, 2003). 37 Seawater acidification poses a threat to marine species and ecosystems, so one of the United 38 Nations Sustainable Development Goals is to "Minimize and address the impacts of ocean 39 40 acidification" (United Nations, 2015). Rising CO₂ levels are expected to reduce seascape complexity, alter trophic interactions (Nogueira et al., 2017; Milazzo et al., 2019) and reduce 41 biodiversity (Sunday et al., 2016; Agostini et al. 2018) causing impacts on a range of ecosystem 42 services (Lemasson et al., 2017). 43

44 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 45 46 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 47 toxicity depends on the chemical form. Arsenic, for example, is toxic in its metalloid form, Hg 48 49 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 50 ecosystems (Ivanina et al., 2015; Lewis et al., 2016) because lower seawater pH can increase 51 the bioavailability and toxicity of metals both in sediments (Roberts et al., 2013) and in the 52 water column (Millero et at., 2009). Lower pH can release metals to water column that were 53 previously bound to sediment (Atkinson et al., 2007). It can also alter the speciation of elements 54 such as Cu, Ni and Zn resulting in increased toxicity (Lacoue-Labarthe et al., 2009; 2012; Zeng 55 56 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 57 increase when seawater pH falls from 8.1 to 7.8, which is the change in surface seawater pH 58 59 that is underway this century (Byrne et al., 1998; Richards et al., 2011). The seawater free ion 60 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). 61

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 CO₂ seeps are little
understood.

Here, we investigated the levels of metals in sediments and seagrasses at acidified 71 volcanic seeps as well as reference sites. We chose seagrasses as they deliver important 72 ecosystem services in coastal habitats (Nordlind et al., 2016). They are also predicted to benefit 73 from rising CO₂ levels within their thermal limits (Koch et al., 2013; Brodie et al., 2014). 74 Seagrass habitats provide food and nurseries for fish, turtles and mammals (Whitfield et al., 75 2017), are important carbon sinks (Fourqurean et al., 2012). The seagrasses also sequester 76 contaminants such as excess nutrients (Constanza et al., 2014) and metals (Bonanno and 77 78 Orlando-Bonaca, 2017) and so are used as bioindicators (Catsiki and Panayotidis, 1993). The 79 plants take in trace elements via their roots, rhizomes or leaves and can translocate them 80 between these tissue compartments (Ralph et al., 2006). This introduces trace elements into the food web via grazing and decomposition (Lewis and Devereux, 2009). 81

82 Seagrass can be abundant at some shallow-water CO₂ seeps (Hall-Spencer et al., 2008; Russel et al., 2013) but are sparse or absent at other seeps (Vizzini et al., 2010, 2013). Studies 83 have shown upregulation of stress-related antioxidant genes in the seagrass Posidonia oceanica 84 at some CO_2 seeps (Lauritano et al., 2015) and work on the expression of genes involved in 85 photosynthesis and growth of another common Mediterranean seagrass, Cymodocea nodosa, 86 did not reveal beneficial effects of high CO₂ levels near a seep (Olivé et al., 2017). Under 87 88 laboratory CO₂ enrichment there was significantly increased expression of C. nodosa transcripts associated with photosynthesis (Ruocco et al., 2017). So, even though seagrasses 89 can be common at certain CO₂ seeps, toxins may cause stress and stunt their growth. 90

Laboratory studies have shown that, at elevated CO₂, 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 98 elements at volcanic seeps correlated with trace element accumulation in seagrass roots,99 rhizomes and leaves and whether seagrass are more tolerant of some metals than others.

100 Methods:

101 *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 CO₂ 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 CO₂ seeps were at 0-10 m depth with a tidal range of 0.30-0.50 m.

107 Vulcano, Italy

We sampled Levante Bay (38.4 N, 15.0 E) off Vulcano island (Fig. 1A). The underwater gas emissions are 97-98% CO₂ with 2.2% hydrogen sulfide (H₂S) at the seep site, decreasing to <0.005% H₂S 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 CO₂ seeps at 1 m depth.

113 Ischia, Italy

114 At Castello Aragonese, off Ischia ($40^{\circ}43'50.4"N$; $13^{\circ}57'48.2"E$), CO₂ bubbles up in 115 shallow water seeps (Fig. 1A). Here the gas is 90–95% CO₂, 3–6% N₂, 0.6–0.8% O₂, 0.2–0.8% 116 CH₄ and the seeps lack H₂S (Tedesco, 1996). Abundant *Posidonia oceanica* meadows were 117 sampled at 0.5m depth from the seep area and from a reference site (Fig. 2a).

118 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%CO₂, 2.99-6.23% N₂, 0.69-1.2% O₂
and 0.65-3% H₂S (Caramanna et al., 2010). Here *P. oceanica* was sampled at 5 m depth.

124 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% CO₂ with some CH₄ and H₂ (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).

132 *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% CO₂, 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.

139

140 *Water sampling*

Water samples (n=5) were collected at each CO₂ seeps and Reference station in 100 ml 141 142 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 143 144 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. 145 146 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 147 materials (CRM Batch 129, accuracy-98.7%, Dickson, 2013) for TA analysis. Temperature, 148 pH_{NBS} and TA data were used to calculate pCO_2 using CO₂SyS program following methods 149 given by Pierrot et al., (2006). Dissociation constants (K1 and K2) developed by Meherbach et 150 151 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 pCO_2 calculation. 152

153 Sediment & seagrass sampling

154 Sediment samples (n=5) were collected 1m apart from six CO₂ seeps and six Reference 155 stations by SCUBA diving. A 10-cm long and 2 cm diameter syringe with the tip cut off to was 156 used to collect the upper 5 cm of sand. The sediment samples were stored in plastic bags in 157 dark boxes and transferred to the laboratory. They were then dried at 40°C until a constant 158 weight was achieved and then analysed for grain size following dry sieving at Half Phi intervals 159 (Blott and Pye, 2001). After grain size analysis the fine and very fine sediment fractions (<180-160 63 μ m) 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.

168 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 (HNO₃) 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% HNO₃ (25 ml volume).

For dried seagrass (leaves, rhizomes and roots), 0.25g of sample was added to 6mL of HNO₃ 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 HNO₃ (69% w/w) were Ultrapure type (Ultrapure, Fischer Chemicals, USA). All glassware was soaked overnight in 10% HNO₃
and washed with distilled water and oven dried before use.

187 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.

195 The SQG-Q was calculated as follows:

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

197 Where PEL-Q_i = contaminant/PEL. The PEL-Qi represents the probable effect level quotient 198 (PEL-Q) of the i contaminant and n represents the total number of contaminants (trace metals). 199 Based on the SQG-Q index, the sediments were divided into three categories as established by 200 MacDonald et al. (2000). SGQ-Q \leq 0.1- low potential for adverse biological effects; 0.1< SQG-201 Q<1- moderate potential for adverse biological effects; SQG-Q \geq 1- high potential for adverse 202 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:

206

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).

212 *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,

215 Paleochori and Vulcano for *C. nodosa*), compartments (sediment and leaves, rhizomes, roots) 216 and stations (CO₂ seeps, Reference). All data were first checked for normality and 217 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 218 219 posteriori comparison among factor levels. Pearson's correlation co-efficient was applied to identify correlation between trace element concentration in sediment and seagrass 220 221 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 222 applied. All statistical tests were conducted with a significance level of $\alpha = 0.05$ and data were 223 reported as mean \pm standard error (SE). 224

226 **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 230 231 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 (5.3-232 233 fold) and Zn (2.39-fold) at Panarea, Cd (42.6-fold) at Paleochori and Cu (8.9-fold) at Adamas 234 seep sediments, compared to reference stations. Mercury was only observed at Italian CO_2 235 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 236 237 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 238 Sediment Quality Guidelines Quotient was mainly 'Moderate', although it was in the 'Low' to 239 240 '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). 241

We were especially interested in results from Ischia as P. oceanica was abundant within 242 243 the main CO₂ seep area (Fig.2a). The sediment at this seep has the highest Cu (32-fold), Zn (2fold) and Pb (1.5-fold) concentrations than other two seep locations sampled for P. oceanica, 244 245 but the seagrass tissues had low levels of these metals (Fig.3). On the other hand, P. oceanica 246 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 247 248 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 249 250 leaves of P. oceanica and C. nodosa at all seep locations (Figs. 3 and 4). Exceptions were Cd 251 (8-fold) concentrations within the rhizomes, Zn (42-fold) and Cu (5-fold) within leaves of P. 252 oceanica and Cd (6-fold), Pb (4-fold) and Hg (3-fold) within leaves of C. nodosa (Figs. 3 and 4). 253

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 CO₂ seep stations, which were all sampled between May -July 2014.



287

- Fig. 2. a) *Posidonia oceanica* and b) *Cymodocea nodosa* meadows at CO₂ seeps off Ischia
 (Italy) and Paleochori (Greece).
- 291 Photo credits for a) Posidonia oceanica, and b) Cymodocea nodosa meadows at Italy and
- 292 Greece: Jason Hall Spencer, University of Plymouth, UK and Thanos Dailianis of Hellenic
- 293 Centre for Marine Research, Greece respectively.









Fig. 3. Element concentrations (mean \pm SE, n=5) of Cd, Cu, Hg, Ni, Pb and Zn in *Posidonia oceanica* plant compartments and sediments at reference and CO₂ seep sites off Italy and

296 *oceanica* plant compartments and sediments at reference and CO₂ seep sites off Italy and Greece. Different letters indicate significant differences between reference and CO₂ seep stations.

10 9 Sediment Roots n 8 Rhizomes Leaves 7 6 Cd (mg Kg⁻¹) 5 g g C bd m o p q о_р q 0 Reference Seeps Reference Seeps Reference Seeps Vulcano Adamas Paleochori 300 280 Sediment С 260 Roots 240 Rhizomes Leaves 220 Г 200 Cu (mg Kg⁻¹) 180 160 50 b k С a q g u n S 0 **Reference Seeps** Seeps **Reference Seeps** Reference Vulcano Adamas Paleochori

Fig.4. continued





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 CO₂ seeps off Italy and Greece.
 Different letters indicate significant differences between reference and CO₂ seep stations.

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	$8.19{\pm}0.06$	2596	428 ± 2.3
CO ₂ seep	35.7	17.8	7.78±0.05	7.78±0.05 2589	
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
Mathana					
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 1: Seawater salinity, temperature, total alkalinity, pH and pCO_2 values (mean \pm SE, n=5) at six Mediterranean CO₂ seeps and Reference stations between May-July 2014.

Table 2. Sediment Quality Guidelines-quotient (SQG-Q) of sediment calculated with Probable

Effects Level for CO₂ seeps and Reference stations off Greece and Italy. SQG-Q <0.1 (low effect). <0.1 SOG-Q>1 (moderate effect), SQG-Q>1 (adverse biological effects). Numbers in

313	effect), <0.1 SQG-Q>1 (moderate effect), SQG-Q>1 (adverse biological effects). Num
21/	hold indicate possible adverse effects of trace elements

314	bold indicate	possible ad	lverse effects	of trace e	elements.
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		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	106 Moderate		Adverse
Isellia	Cu Hσ	0.55	0.86	Moderate	Moderate
	Ph	0.01	0.00	Moderate	Moderate
	Zn	0.11	0.10	Moderate	Moderate
	211	0.12	0.10	Moderate	Wioderate
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
7 Kumus	Ni	0.21	0.21	Moderate	Moderate
	141	0.51	0.41	Moderate	Woderate
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

315

Table 3. Three-way ANOVA differences in trace element levels between Location: 3 levels (Methana (M), Panarea(P) and Ischia (V)), Stations:2
 variables (CO₂ 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	<u>1</u>		Sedimen	t vs Compa	rtment	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 Station Compt.	<0.001 <0.001 <0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cu	Location Station Compt.	<0.001 <0.001 <0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.314	<0.001	<0.001	<0.001
Ni	Location Station Compt.	<0.001 <0.001 <0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pb	Location Station Compt.	<0.001 <0.001 <0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.652
Zn	Location Station Compt.	<0.001 <0.001 <0.001	<0.001	<0.001	<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 (CO₂ 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-Si	dak p valu	ies							
			Location	1		Sediment	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	0 636	<0.001	<0.001							
Cu	Station	< 0.001	0.020	<0.001	<0.001							
	Compt	<0.001				<0.001	0.621	<0.001	<0.001	<0.001	<0.001	
	Compt.	<0.001				<0.001	0.021	<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 CO₂ 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	t-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	

332

Table 6. Bio-Sediment Accumulation Factor (BSAF) of trace metals in *P. oceanica* and *C.*

334	nodosa roots at CO ₂ se	eens (seens)	and Reference ((Ref.) s	stations off Italy	and Greek coast.
JJ -				ILCI./ B	stations on man	y and Oreek coust.

335 Sediment (Sd), Roots (Ro). Bold numbers indicate TF>1 value.

	Location	Ischia	1	Panar	ea	Metha	na
Seagrass	Elements	BSAF	F(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
		Vulca	no	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

336

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

339 Greece. Data collected from literature only included the pristine sites with seagrass meadows around Greece and Italy and seagrass meadows

340 within contaminated sites and sediment samples taken from ship-based cores were excluded. Samples of CO₂ seeps off Italy and Greek coast are

341 indicated in bold. Sediment (Sd), Leaves (L), Rhizomes (Rh), Roots (R).

Sample/Lo	Study site	Sample	Cd	Cu	Hg	Ni	Pb	Zn	References
cation									
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	L	1.2	9.6	-	7.6	-	57.5	Nicolaidou and Nott,
	Evvoikos Gulf								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	

343 Discussion

Shallow water CO₂ seeps have been used as natural analogues for future coastal 344 ecosystems as they can have areas of seabed where entire communities of marine organisms 345 346 are exposed to the shifts in carbonate chemistry that are expected due to continued anthropogenic CO₂ emissions (Hall-Spencer et al., 2008; Enochs et al., 2015; Connell et al., 347 2017). At such seeps, there are often elevated levels of trace elements and H₂S, so care is 348 needed when using them to assess the effects of ocean acidification (Barry et al., 2010; Vizzini 349 et al. 2010). This is done by mapping areas affected by volcanic fluid toxics and avoiding those 350 areas when assessing the effects of increased pCO_2 in seawater (Boatta et al. 2013; Agostini et 351 352 al. 2018). The six CO₂ 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 353 354 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 355 356 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 357 biota. So, careful selection of study sites is needed to avoid the combined effects of various 358 factors like trace metals and toxic gases while conducting ocean acidification research. 359

The trace element levels observed within CO₂ seep sediments were higher for 360 Cd and Cu, were similar for Hg and lower for Ni, Pb and Zn than mean element levels observed 361 around Mediterranean coast of Italy (Table 7). We think that this is because the sediments 362 studied were sandy and lacked clay particles (<63µm) which bind more trace elements in finer 363 sediments. Trace element levels observed at seep sediments off Vulcano, Italy, were in the 364 365 same range for Cd, 5-fold higher for Hg and lower for Cu (1.7-fold), Pb (6-fold) and Zn (2fold) from previously measurements by Vizzini et al. (2013). Levels of Hg and Pb measured at 366 367 Panarea CO₂ 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 368 with increased input of elements, whereas no such influx was observed during our sampling. 369 Trace element levels in seep sediments of the Greece coast were 3-fold (Cu), 2-fold (Pb) and 370 1.2-fold (Zn) lower than previously reported by Hodkinson et al., (1994), whereas Cd and Ni 371 are reported for the first time for this coast (Table7). These higher levels of elements could be 372 373 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 374 element levels within the CO₂ seep sediments of Italy and Greece coasts indicate the 375

heterogeneous patchiness in metal concentrations around seep systems, variation in influx of elements from CO₂ seeps and the variable biogeochemical factors (such as variation in pH and sediment grain size) that influences the metal availability at the CO₂ seeps. These variations of trace element levels in sediment between CO₂ 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 382 383 reference sites. Seagrass element accumulation is more element and seagrass tissue-specific rather than species-specific (Bonanno and Bonaca, 2017) resulting in seagrass compartments 384 385 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 386 387 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 388 389 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 390 where they store and sequester certain elements to avoid damage to photosynthetic apparatus. 391 392 This root accumulation of elements is then internally regulated for elements like Cd, Ni and Pb 393 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 394 out through the leaves which are then shed, as observed in *P. oceanica* (Di Leo et al., 2013; 395 Richir and Gobert, 2016) and in C. nodosa (Malea and Haritinoids, 1999; Bonanno and Di 396 Martino, 2016). This transfer of trace elements from roots to leaves of P. oceanica and C. 397 nodosa also promote the release of these elements into the food webs of coastal ecosystems or 398 the water column. On the other hand, storage and sequestration of metals in the below ground 399 400 tissues like roots also reduces metal burden of seagrasses as below ground tissues are 401 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 402 are toxic (Kabata-Pendias and Mukherjee, 2007), similar preferences has been observed for 403 accumulation of Zn over Pb in both P. oceanica (Sanchiz et al., 2001) and C. nodosa (Malea 404 and Haritonidis, 1999; Llagostera et al., 2011). However, seagrasses also tend to store toxic 405 elements like Hg and Pb in the vacuoles of cortical tissue of roots outside the endodermis or in 406 cell walls, thereby preventing the uptake of these elements into rhizomes and leaves (Windham 407 408 et al., 2001).

409 Significant positive correlation of trace elements between seagrass tissues and sediment suggest the bioindication potential of seagrass tissues for that trace element (Bonanno and 410 Borg, 2018). For instance, positive correlation was found in *P. oceanica* for Cd through 411 sediment-root pathway and for Zn and Ni through sediment-rhizome, which indicates that roots 412 of P. oceanica are potential bioindicators of Cd and rhizomes of Zn and Ni at CO₂ seeps off 413 Italy. In C. nodosa no positive correlation was found for any of the elements analysed, which 414 indicates their low potential for being bioindicators of trace metals and this also suggests why 415 P. oceanica is used as a bioindicator in most of trace metal accumulation studies in 416 417 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 418 between sediment -roots and Zn between sediment- rhizomes. Negative correlation suggests 419 that the preferable route for Cd transfer in P. oceanica (Lafabrie et al., 2007; Di Leo et al., 420 2013) and Zn in C. nodosa (Malea et al., 1999) is through water column rather than the 421 sediment-root pathways. Similarly, elements such as Pb with negative correlation in C. nodosa, 422 423 suggests Pb being toxic is not uptake or stored within the seagrass compartments (Sanchiz et al., 2001). 424

Bio-Sediment Accumulation Factor analysis between elements in sediment and in 425 seagrass roots indicate that the pathway of uptake/storage is not always the sediment-root, even 426 though higher element concentrations were observed in the sediments at CO₂ seeps. Even 427 though, in *P. oceanica* Cd and Ni, were found with BSAF>1 in roots at all three seep stations, 428 which suggests that accumulation of elements like Cd and Ni are made through the sediment-429 root pathway, for elements like Cu, Hg, Pb and Zn a mixed response (higher at reference and 430 lower at seep sites or vice versa) of BSAF>1 was found, which indicates that for these trace 431 elements both sediment-root and water-root pathways may be used. BSAF >1 value observed 432 433 for trace elements in *P. oceanica* at the CO₂ 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. 434 nodosa Cu was the only element with BSAF>1 in roots found at all three seep stations, whereas 435 other elements showed mixed response. Cu being an essential element is preferred for root 436 accumulation through sediment-root pathway, whereas other elements can use a mixed 437 accumulation from sediment-roots or water-roots or water-leaves pathway (Bonanno and Di 438 Martino, 2016). However, it was observed for both P. oceanica at Ischia and Panarea and C. 439 nodosa at Vulcano seeps, that Hg accumulation from sediment-roots pathway (BSAF>1) was 440

441 not higher than reference sites. This suggests Hg being toxic to the plant roots is not preferred
442 for accumulation in seagrass (Bonanno and Di Martino, 2016).

At CO₂ seeps the low pH can alter the metal speciation and favour the release of metals 443 444 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 445 determining its bioavailability. Low pH of seawater near the CO₂ seeps tends to release the 446 metals that are less strongly associated with sediments, increasing their potential bioavailability 447 (Riba et al., 2004). Thus, low pH can increase the concentration of certain dissolved metals, 448 which could affect the sediment-seagrass associated biota e.g., by increasing Cu, Cd and Zn 449 450 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 451 increase the availability of Cd, Cu, Ni, Pb and Zn in their free ion forms (Roberts et al., 2013). 452 Low pH combined with increased availability can influence and increase seagrass uptake of 453 trace elements (Yang and Ye, 2009) that can lead to higher accumulation and storage of trace 454 elements in seagrass roots and leaves (Bonanno and Bonaca, 2017). Higher accumulation can 455 lead to metal stress once threshold levels are reached and affect the seagrass physiological 456 457 processes (Olive 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 458 on metal toxicity have been conducted on Cymodocea serrulata (Prange and Dennison. 2000), 459 Halophila ovalis and H. spinulosa (Prange and Dennison, 2000; Ambo-Rappe et al., 2011). 460 Considering the observed results from these ex-situ metal toxicity studies, there is a possibility 461 462 that elements such as Cu and Pb at the CO₂ seeps may affect P. oceanica and C. nodosa 463 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. 464

465 **Conclusion:**

We observed that Greek and Italian marine CO₂ 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 CO₂ 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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