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ORIGINAL ARTICLE

Effect of exposure time on the bioaccumulation of Cd, Mg, Mn and Zn in *Cystoseira abies-marina* samples subject to shallow water hydrothermal activity in São Miguel (Azores)

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Conflicts of interest
All authors declare no conflicts of interest.

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Abstract

Shallow water hydrothermal vents can be compared to polluted places due to high concentrations of heavy metals, and are thus good models for bioaccumulation studies. The present study intended to estimate the time of exposure required for the accumulation of certain elements to stabilize in specimens of *Cystoseira abies-marina*, to be used as a reference in future work. *Cystoseira abies-marina* intertidal specimens were transplanted from Mosteiros (a non-hydrothermal and pristine site) to Ferraria (with hydrothermal activity) and left there. Transplanted samples were collected after 1, 2, 4 and 8 weeks and the concentrations of Cd, Mg, Mn and Zn were measured through flame atomic absorption spectrophotometry. Although further studies with increased periods of exposure are needed because the concentration of these elements never stabilized in the collected samples, there is strong evidence that increased time of exposure led to increased concentrations of Cd, Mg, Mn, but not Zn. These results are consistent with the assumption that *C. abies-marina* is bioaccumulating some of the heavy metals and can thus be a good indicator for polluted waters.

Problem

Increased coastal pollution causes change in ecosystems, namely due to species adaptation to stressing environmental conditions (Chapman & Bulleri 2003). It is important to study the phenomenon of pollution, such as increased nutrient and heavy- metal load, and its impact on living organisms, as bioaccumulation and biomagnification of such elements along trophic chains increase their toxicity in the aquatic environment over time (Gochfeld 2003; Kamala-Kannan *et al.* 2007).

Hydrothermal environments are extraordinary scenarios with high concentrations of metals such as Cd, Cu, Fe, Mg, Mn, Rb and Zn that arise from discharges of sulphides (Von Damm 1990), which makes them appropriate for the study of ecological impacts of those elements on communities that live there (Cosson & Vivier 1997; Ventox 2003).

Marine macroalgae are primary producers in coastal waters that accumulate such elements, which are further transferred along the trophic chain by herbivores and detritivores (Agadi *et al.* 1978). Many toxic pollutants are found only in trace amounts in the water, and often at elevated levels in sediments, thus risk assessments based only on data derived from water analyses may be misleading, and data from sediments may not be representative of pollutant concentrations in the overlying water column and cannot give information on patterns of contamination at higher levels of the food chain (Torres *et al.* 2008). As the concentration of metals in algae tissues is proportional to their diluted concentration in the environment, they are important bioindicators of the environmental exposure to those elements, thus useful as test organisms for marine pollution studies (Fletcher 1991). Brown algae tend to have a higher affinity for metal accumulation than green and red algae (Markham *et al.* 1980), mainly due to the polyphenolic substances and polysaccharides that constitute their cellular walls (Forsberg *et al.* 1988).

The present work focused on the bioaccumulation of cadmium, magnesium, manganese and zinc in *Cystoseira abies-marina* in a site with shallow water hydrothermal activity as a natural pollution source. It constitutes a preliminary approach to the use of this species as a tool to monitor water quality in the Azores.

Study Area

The present study was conducted at São Miguel island (Azores), consisting of transplanting *Cystoseira abies-marina* specimens from a site with no hydrothermal activity to a small enclosed bay with shallow water hydrothermal activity (Mosteiros and Ferraria, respectively; Fig. 1).

Material and Methods

Specimens were collected with a chisel from Mosteiros, transported to Ferraria and placed within the hydrothermally active basin of Ferraria. *Cystoseira abies-marina* specimens were entangled in a 20 cm × 10 cm square of plastic-coated wire mesh (1 cm × 1 cm), and subsequently screwed to the rock (Fig. 2). Specimens were collected for analysis at t = 0 and transplanted specimens were collected after being exposed for 1 week, 2 weeks, 4 weeks and 8 weeks, and brought to the laboratory. They were then cleaned of epiphytes with a soft nylon brush, dried to constant weight in a drying oven at 70 °C, and ground with a mortar and pestle. Subsequently, samples were subject to acid digestion: (i) 3 ml of HNO_3 (65%) was added to approximately 0.5 g of ground sample and kept 43 h at room temperature; (ii) subsequently these were diluted 10 times and transferred to a heating plate at 100 °C for 4 h; (iii) then 1 ml of H_2O_2 (30%) was added and the samples kept on the heating plate at 100 °C for one extra hour. Samples were then ultrasonicated (2 min at 50 Hz.) and spun at 3220 g for 10 min. The levels of Cd, Mg, Mn and Zn in the liquid fraction were quantified with flame atomic absorption spectrometry. EU-certified reference material (BCR-279 – *Ulva lactuca*; reference values for Cd and Zn) was used to validate the metal extraction process, and blank samples were used to validate sample treatment procedures. ANOVA procedures were used to test differences in metal levels of samples subject to different hydrothermal activity exposure times.

Results

Blank samples did not indicate contamination for any of the analysed elements. Metal extraction efficiency from reference material was greater for Zn (96.8%) than for Cd (60.9%); there were no certified values for Mg and Mn (Table 1).

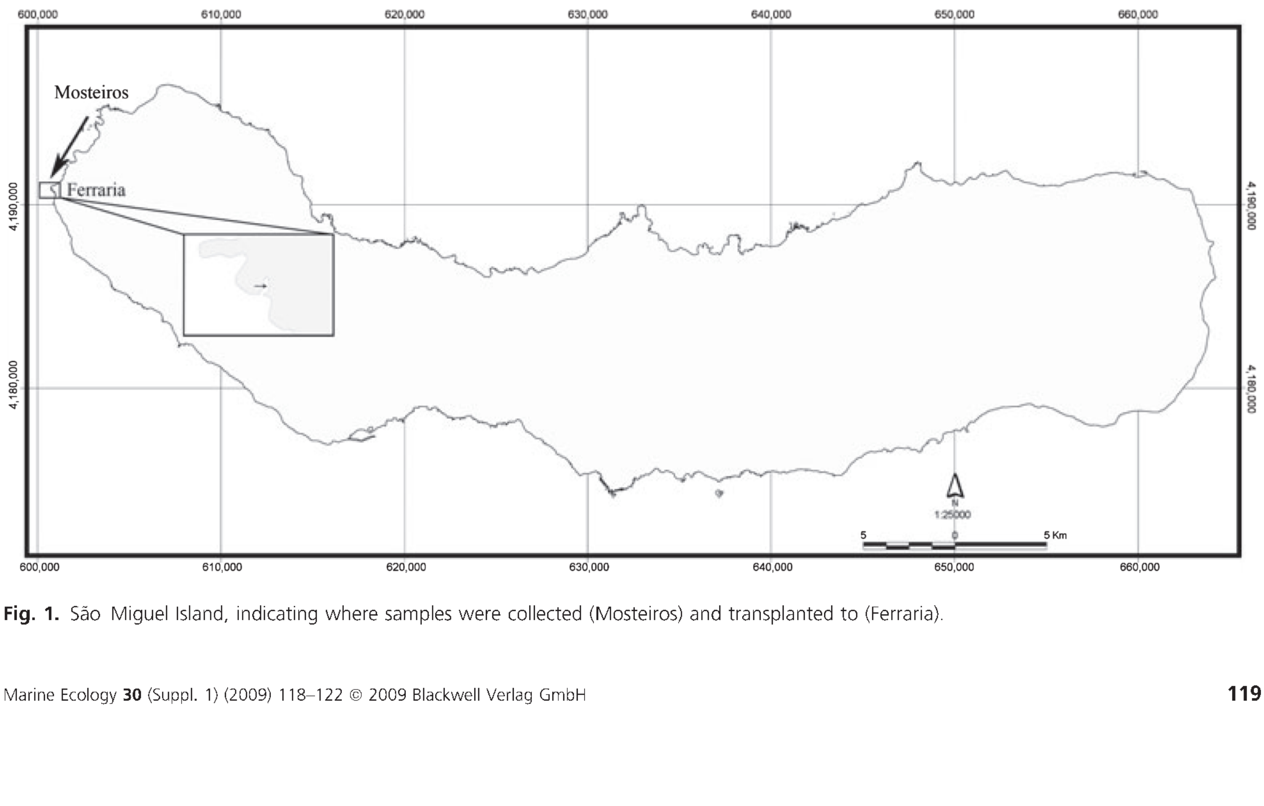

Fig. 1. São Miguel Island, indicating where samples were collected (Mosteiros) and transplanted to (Ferraria).

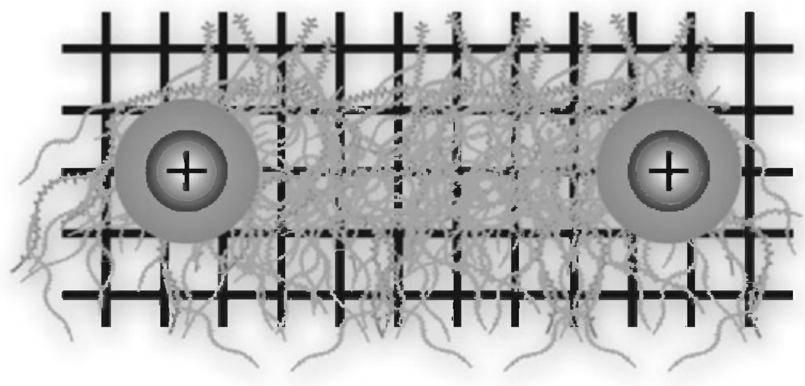

Fig. 2. Schematic representation of the transplantation method.

Table 1. Levels of Cd, Mg, Mn and Zn in *Cystoseira abies-marina* samples exposed to shallow water hydrothermal activity for 1 week, 2 weeks, 4 weeks and 8 weeks.

	Cd $mg \cdot kg^{-1}$ dry weight	Mg $mg \cdot kg^{-1}$ dry weight	Mn $mg \cdot kg^{-1}$ dry weight	Zn $mg \cdot kg^{-1}$ dry weight
0 weeks	1.014	666.639	4.054	25.655
	1.052	781.874	3.859	25.510
	0.552	712.627	3.567	31.172
1 week	0.000	1016.697	41.101	28.899
	0.000	1060.525	21.100	216.468
	0.000	870.383	25.285	29.113
2 weeks	0.195	1080.098	43.220	26.146
	0.087	962.674	50.868	26.997
	0.000	1016.551	29.684	35.140
4 weeks	0.412	936.294	257.647	20.529
	1.197	7826.590	179.356	21.057
	1.495	901.910	174.086	17.442
8 weeks	1.196	1094.616	1830.508	23.629
	1.811	8842.144	2164.510	26.691
	1.691	8649.610	2690.373	42.194
reference	0.330	12,584.830	1606.083	59.692
material	0.112	15,195.579	1716.373	42.038
(Cd = 0.274;	0.058	15,102.768	1632.669	47.320
Zn = 51.3)				
blank	0.000	0.000	0.000	0.004
	0.000	0.005	0.000	0.014
	0.000	0.003	0.000	0.009

Except for Zn, the concentration of all the elements showed an increase after the samples had been exposed to hydrothermal activity for 8 weeks relative to the time they were collected at the non-hydrothermal site (Table 1). However, for shorter periods these values varied for each element, and differences were significant only for Cd and Mn (Fig. 3). Compared to the initial Cd concentrations, there was first a reduction and then an increase to levels above the initial ones (Fig. 3a), whereas Mn concentrations started increasing significantly only after being exposed for 4–8 weeks (Fig. 3c). Although not significant, Mg concentrations also started increasing in a greater proportion after being exposed for 4 weeks (Fig. 3b).

Discussion

Cadmium levels are generally low and usually require accurate detection methods, ideally graphite furnace AAS. Flame AAS might not be accurate enough for detecting low Cd levels and thus reveal low extraction efficiency. Further assays using graphite furnace AAS are planned to confirm this theory, and if this has not been the issue, than alternative digestion protocols need to be tested to optimize results. Zn is generally present in much higher amounts and thus flame AAS is appropriate for its detection, as evidenced by the extraction efficiency obtained for the reference material.

The only work found on heavy metal accumulation in *Cystoseira abies-marina* (Lozano *et al.* 2003) reports Cd accumulation values similar to those of the present study associated with polluted sites in the Canary Islands. Other studies on heavy metal accumulation in different *Cystoseira* species focus on many elements, including Cd, Mg, Mn and Zn (Caliceti *et al.* 2002; Al-Masri *et al.* 2003). However, it is difficult to compare between species that might have different physiological response to heavy metal availability in the water. Given the objective of finding a tool to monitor water quality in the Azores, it seems more appropriate to study the usefulness of *C. abies-marina* in reflecting heavy metal concentration of surrounding waters rather than comparing its accumulation capacity throughout some geographical range, or with other related species.

Specimens collected in the non-hydrothermal site presented cadmium levels below what Lozano *et al.* (2003) report as polluted (<1 ppm). Polluted levels (1–2 ppm) were reached only after 4 weeks in the hydrothermal site. The initial decline in Cd levels is possibly related to the

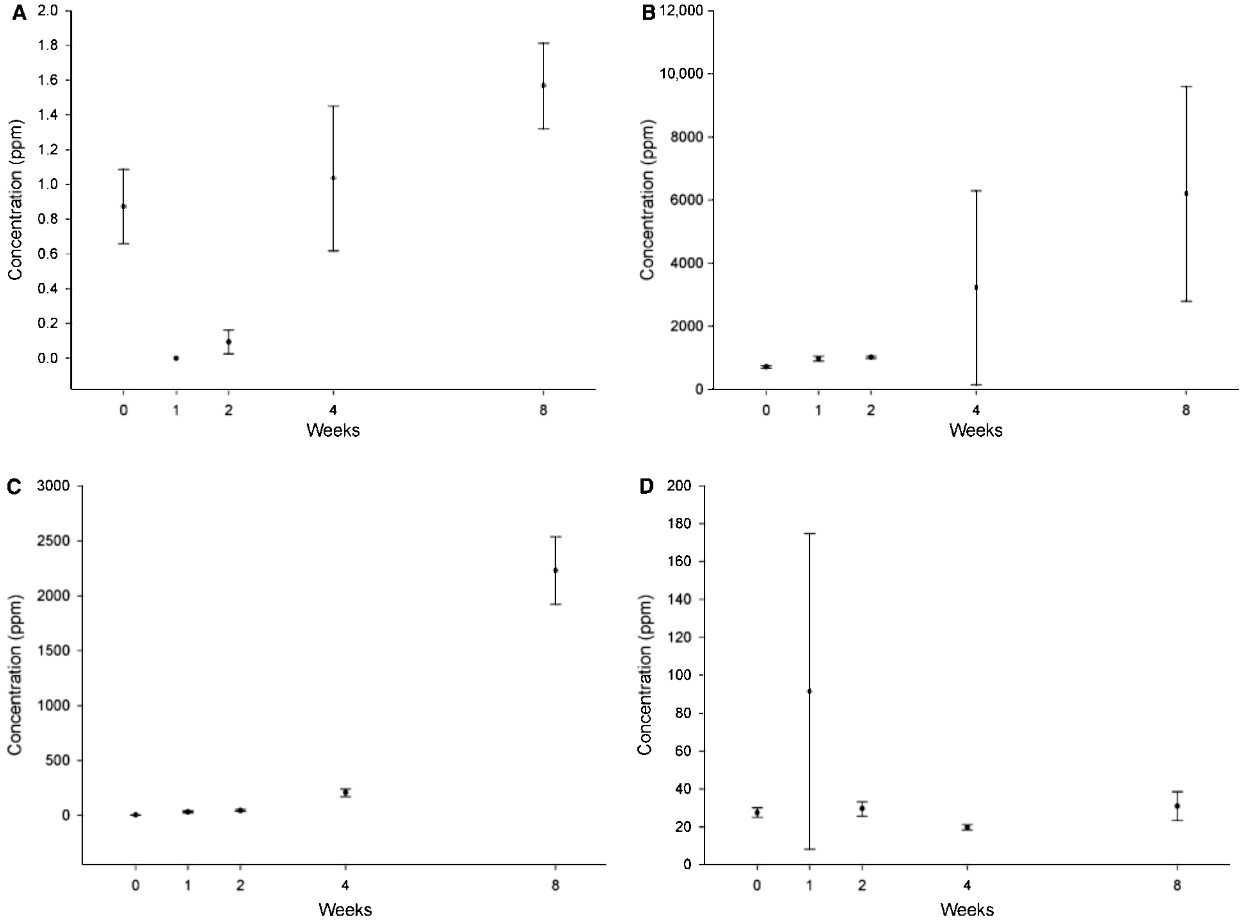

Fig. 3. Average concentration of Cd (a; anova P = 0.006), Mg (b; anova P = 0.1293), Mn (c; anova P = 0.0000) and Zn (d; anova P = 0.4160) in samples of *Cystoseira abies-marina* exposed to hydrothermal activity for 1 week, 2 weeks, 4 weeks and 8 weeks.

stressing new environment (on average, temperatures 6–8 °C higher and pH 1–1.5 lower than those of the open ocean in the Azores). Such conditions can induce a physiological response like the release of polyphenolic molecules (phlorotannins) that are strong chelators to heavy metals in solution (Toth & Pavia 2000; Topcuoglu *et al.* 2003; Stengel 2006). The gradual increase of Cd levels in the second week and take off after the fourth week indicate a possible physiological adaptation to the new conditions and the gradual synthesis of new polyphenols. Eight weeks of exposure were not enough for the levels of cadmium to stabilize, which probably indicates high concentrations of this element in the surrounding water.

The initial period of unresponsiveness in the accumulation of Mg and Mn is likely to be also related to the release of polyphenols under stressing conditions. Synthesis of new polyphenolic molecules is likely to take time, and thus the reduced absorption and/or adsorption capacity in an initial period after being exposed to the hydrothermal activity. Unresponsiveness of Zn accumulation suggest that there are no differences in Zn concentrations in hydrothermal and non-hydrothermal waters, which contradicts previous work conducted in the Azores in similar environments (Zaldibar *et al.* 2006; Amaral *et al.* 2007, 2008; Cunha *et al.* 2008). Zn is structurally present in many enzymes and is thus involved in physiological processes. Consequently, a delay in its accumulation might be related to the interference of adverse environmental conditions (increased temperature and acidity) with enzymatic activity, which might take longer, if ever, to respond.

Conclusions

Cystoseira abies-marina proved to accumulate heavy metals differently in hydrothermal and non-hydrothermal sites. This means that: (i) *Cystoseira abies-marina* can be used to monitor heavy metal levels in the water; and (ii) shallow water hydrothermally active sites can be used as models for natural pollution studies. However, further studies are needed to assess the maximum metal accumulation capacity of this species and the time required to reach it, and also relate it to heavy metal concentration in the surrounding water.

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