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1 **A first report of rare earth elements in Northwestern Mediterranean seaweeds**

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10

11 **Abstract**

12 The concentrations of rare earth elements (REE) were determined by ICP-MS in dominant seaweed  
13 species, collected from three locations of the northwestern Mediterranean Sea. This is the first study  
14 to define levels and patterns of REE in macro algae from these coastal areas.

15 Rare elements are becoming emerging inorganic contaminants in marine ecosystems, due to their  
16 worldwide increasing applications in industry, technology, medicine and agriculture.

17 Significant inter-site and interspecies differences were registered, with higher levels of REE in  
18 brown and green macro algae than in red seaweeds. Levels of light REE were also observed to be  
19 greater compared to heavy REE in all samples.

20 One of the investigated locations (Bergeggi, SV) had higher REE and  $\Sigma$ REE concentrations,  
21 probably due to its proximity to an important commercial and touristic harbor, while the other two  
22 sites were less affected by anthropogenic contaminations, and showed comparable REE patterns  
23 and lower concentrations.

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25 **Capsule:** rare earth elements in seaweeds

26

27 **Keywords:** REE, seaweeds, Mediterranean Sea, pollution tracers.

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## 1. Introduction

Rare earth elements (REE) are a group of chemical elements including yttrium (Y), scandium (Sc) and lanthanides (from lanthanum to lutetium). Despite their name, REE are not that rare in the natural environment, being the fifteenth most abundant component of the earth's crust (USEPA, 2012). REE are further subdivided into light REE (LREE), including lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd) and samarium (Sm); and heavy REE (HREE), including gadolinium (Gd), europium (Eu), terbium (Tb), dysprosium (Dy), thulium (Tm), ytterbium (Yb), holmium (Ho), erbium (Er), lutetium (Lu) and yttrium (Y) (Anastopoulos et al., 2016). REE mainly enter into oceans through atmospheric fallout (De Baar et al., 1983) and fluvial inputs (Frost et al., 1986), and have been frequently investigated as natural tracers of biogeochemical processes (Oliveri et al., 2010). As the distribution patterns of REE in the water column are already known, it is possible to utilize these patterns for tracing water masses or to identify pollution sources in seawater (Censi et al., 2004). In fact, in the last decade, the worldwide use of REE in industrial applications (electronics, nuclear energy, metallurgy, medicine, computer manufacturing) and in some countries (such as China) for use in fertilizer and feed additives, has increased levels of REE in water environments (Mashitah et al., 2012; Hermann et al., 2016). Thus, REE can be considered as emerging contaminants and pose a potential risk for marine and freshwater ecosystems. The Mediterranean Sea is a semi-enclosed sea; concentrations of trace elements and REE in this basin are higher than those registered in other nutrient-depleted surficial waters (Greaves et al., 1994; Strady et al., 2015). Numerous investigations regarding patterns of dissolved and particulate REE have been performed in this basin (e.g. Censi et al., 2004; Martinez-Boti et al., 2009; Tranchida et al., 2011; Roussiez et al., 2013; Ayache et al., 2016); conversely, occurrence and distribution of REE in marine biota have scarcely been investigated. To our knowledge, there are only two studies that have analyzed the distribution of REE in plankton from the Mediterranean Sea

56 (Strady et al., 2015; Battuello et al., 2017); examining REE in marine organisms is of great  
57 importance because of their increasing levels in seawater environments and, consequently, in the  
58 marine food chain.

59 Of the marine organisms that can be utilized as bioindicators of trace elements and REE in marine  
60 environments, seaweeds have several advantages as they are widespread, easy to collect and have a  
61 considerable ability to take-up trace elements in solution and concentrate them. Moreover, as they  
62 are at the base of the marine food chain, macro algae are essential in the transfer of trace elements  
63 to higher trophic levels.

64 We determined REE concentrations and distributions in seaweeds from three different sites located  
65 in Northwestern Mediterranean coastal areas. These sites have different environmental protection in  
66 the Ligurian and Northern Tyrrhenian Sea. The macro algae species collected for this study were  
67 the most abundant and widespread in all three sampling sites and were represented by the three  
68 phylum Chlorophyta (green algae), Ochrophyta (brown algae) and Rodophyta.

69 Macro algae species from these three locations were the subject of a previous investigation that  
70 focused on essential and nonessential trace elements, in the perspective of identifying the species  
71 potentially suitable for human and animal nutrition, as well to identify any potential risks for  
72 consumers due to the presence of toxic metals such as lead, cadmium and mercury in seaweeds of  
73 Mediterranean origin (Squadrone et al., under review).

74 In this study, we aimed to measure, for the first time, the concentrations of REE in marine  
75 Mediterranean seaweeds, identifying patterns and fractionations of REE, and verifying the potential  
76 use of REE as pollution tracers in the studied area.

77

## 78 **2. Materials and methods**

### 79 *2.1. Sampling area*

80 All three sampling locations were situated in the northwestern Mediterranean Sea (Figure 1).

81 The first sampling site was located in Bergeggi (SV), a Marine Protected Area of the Ligurian Sea  
82 (44°14'26. 94"N, 8°26'50. 98"E, General Reserve named B zone.) Here, human activities are  
83 restricted and regulated by the Italian law. Close to this site is located the industrial and commercial  
84 harbor of Vado Ligure (SV), characterized by high shipping traffic.

85 The second site was in the Island of Elba (Tyrrhenian Sea, 42°42'35. 17"N, 10°24'44. 97"E), five  
86 nautical miles off the Tuscan coast. Elba is the most populated island of the Tuscan archipelago,  
87 especially in summer.

88 The third sampling site was located in the little Capraia Island, (43°4'26. 90"N, 9°49'39.63"E, in the  
89 National Park of the Tuscan Archipelago, PNAT), another Marine Protected Area of the Ligurian  
90 Sea, about 30 nautical miles off Tuscan coast. The island has few inhabitants and no industrial  
91 activities.

92 Seaweed samples were collected in summer 2016. After collection, the macro algae were washed on  
93 board with seawater and then stored in refrigerated conditions. The specimens were transported to  
94 the laboratory and examined under the stereomicroscope, after being cut into thin sections, in order  
95 to identify the macro algae species. Before analyzing the seaweeds for REE content in the chemical  
96 laboratory, samples were rinsed with tap water, followed by a rinse with distilled water, then freeze-  
97 dried and homogenized to obtain a fine powder. Approximately 1-1.5 g of each sample were  
98 utilized for quantitation of REEs.

## 99 *2.2 Determination of REE*

100 Samples mineralization was performed using a microwave digestion lab station (Ethos 1, Milestone,  
101 Shelton, CT, USA), equipped with a 10 positions rotor for high pressures polytetrafluoroethylene  
102 (PTFE) digestion tubes.

103 All digestion tubes were cleaned with concentrated acid, rinsed with ultrapure water and dried at  
104 room temperature under a chemical hood. Disposable polypropylene tubes were used to storage  
105 mineralized samples. Freeze-dried samples (1.0-1.5 g) were directly weighed into PTFE digestion

106 tubes. 7 mL of HNO<sub>3</sub> (70% v/v) and 1.5 mL of H<sub>2</sub>O<sub>2</sub> (30% v/v) were then added before the  
107 microwave digestion process, programmed as follows: heating to 130°C in 8 min, hold for 2 min,  
108 heating to 200°C in 8 min, hold for 5 min; cooling for 30 min. Digested samples were then  
109 quantitatively transferred to 50 mL polypropylene tubes and gravimetrically diluted to a final  
110 weight of 50 g with ultrapure water.

111 REE determination was performed by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS  
112 Xseries II, Thermo Scientific, Bremen, Germany) equipped with a multi-vial auto sampler (ASX  
113 520, CETAC Technologies, Omaha, NE, USA). Instrument was tuned daily before each analytical  
114 trial. Certified Reference Materials (REE-1 from the National Institute of Standard and  
115 Technology), blank reagents and standard solutions were processed during each analytical session  
116 to verify performances of the methods. The limit of quantification (LOQ) was 0.010 mg Kg<sup>-1</sup>.

117 Results were expressed in mg Kg<sup>-1</sup> dry weight as the mean for each site with standard deviation; the  
118 sum of REE ( $\Sigma$ REE), of light REE (LREE) and of heavy REE (HREE) are also shown (Table 1).

### 119 *2.3 Statistical analysis*

120 The one-way analysis of variance (ANOVA) was employed to compare the average contents of the  
121 sum of REE ( $\Sigma$ REE) in macro algae between the three sampling sites and between the macro algae  
122 species in the same site (Table 2). The unpaired t-test was used to compare the average contents of  
123  $\Sigma$ REE in macro algae between sites 2 and 3. Results were considered statistically significant at p  
124 values of < 0.05. Graph Pad Statistics Software Version 6.0 (GraphPad Software, Inc., USA) was  
125 used for statistical evaluations.

## 126 **3. Results and Discussion**

127 REE are typical lithophile elements, with scarce presence in biological tissues. Seaweeds  
128 mostly develop in marine environments in contact with sediments and suspended particulate from  
129 geologic origin is present in their environment. Therefore, fine geological particulate, containing  
130 REE, are incorporated in plants tissues in different amounts.

131 The concentrations of REE in marine macro algae from the three collection sites of the  
132 Northwestern Mediterranean Sea are shown in Table 1 ( $\text{mg Kg}^{-1}$  dry weight). REE and  $\Sigma\text{REE}$  are  
133 also graphically presented for the three sites (Figure 2) and for the analyzed seaweeds (Figures 3  
134 and 4), to facilitate comparison.

135 A high variability in REE concentrations between the three sampling sites and between species was  
136 recorded; the REE concentrations, however, consistently followed the same trend, and  
137 concentrations of LREE were always higher than HREE.

### 138 3.1 Inter-site variability

139 In Figures 2a and 2b, mean REE levels in the three sampling sites are graphically represented. As  
140 shown, the specific area of collection greatly affected REE concentrations. In fact, macro algae  
141 from site 1 (Bergeggi, SV) had the highest concentrations for all the analyzed REE (Table 1). In site  
142 1, the highest REE values were found in the Ocrophyta *Halopteris filicina* (Ce  $8.8 \text{ mg Kg}^{-1}$ , La  $4.3$   
143  $\text{mg Kg}^{-1}$ , Nd  $4.1 \text{ mg Kg}^{-1}$ , Y  $3.4 \text{ mg Kg}^{-1}$ , Sc  $1.5 \text{ mg Kg}^{-1}$ , Pr  $1.1 \text{ mg Kg}^{-1}$ , Sm and Gd  $0.87 \text{ mg Kg}^{-1}$ ,  
144  $\text{Dy } 0.64 \text{ mg Kg}^{-1}$ , Er  $0.32 \text{ mg Kg}^{-1}$ , Yb  $0.27 \text{ mg Kg}^{-1}$ , Tb and Ho  $0.22 \text{ mg Kg}^{-1}$ , Eu  $0.17 \text{ mg Kg}^{-1}$ ,  
145 Tm  $0.80 \text{ mg Kg}^{-1}$ , Lu  $0.78 \text{ mg Kg}^{-1}$ ) and the lowest concentrations were found in the Rodhopyta  
146 *Ganonema farinosum* (Ce  $4.8 \text{ mg Kg}^{-1}$ , La  $2.5 \text{ mg Kg}^{-1}$ , Nd  $2.2 \text{ mg Kg}^{-1}$ , Y  $2.0 \text{ mg Kg}^{-1}$ , Sc  $0.84 \text{ mg}$   
147  $\text{Kg}^{-1}$ , Pr  $0.55 \text{ mg Kg}^{-1}$ , Sm and Gd  $0.46 \text{ mg Kg}^{-1}$ , Dy  $0.35 \text{ mg Kg}^{-1}$ , Er  $0.19 \text{ mg Kg}^{-1}$ , Yb  $0.15 \text{ mg}$   
148  $\text{Kg}^{-1}$ , Eu  $0.10 \text{ mg Kg}^{-1}$ , Tb and Ho  $0.080 \text{ mg Kg}^{-1}$ , Tm and Lu  $0.030 \text{ mg Kg}^{-1}$ ).

149 Despite being located in a marine protected area, site 1 seems to be greatly affected by being  
150 situated close to an important industrial and touristic harbor.

151 In site 2 (Elba Island, LI), we detected the highest levels of all elements in the Clorophyta *Flabellia*  
152 *petiolata* (Ce  $3.5 \text{ mg Kg}^{-1}$ , Y  $2.3 \text{ mg Kg}^{-1}$ , La  $2.2 \text{ mg Kg}^{-1}$ , Nd  $1.9 \text{ mg Kg}^{-1}$ , Sc  $0.66 \text{ mg Kg}^{-1}$ , Pr  
153  $0.47 \text{ mg Kg}^{-1}$ , Gd  $0.45 \text{ mg Kg}^{-1}$ , Sm  $0.40 \text{ mg Kg}^{-1}$ , Dy  $0.34 \text{ mg Kg}^{-1}$ , Er  $0.18 \text{ mg Kg}^{-1}$ , Yb  $0.15 \text{ mg}$   
154  $\text{Kg}^{-1}$ , Eu and Ho  $0.10 \text{ mg Kg}^{-1}$ , Tb  $0.091 \text{ mg Kg}^{-1}$ , Tm  $0.040$  and Lu  $0.033 \text{ mg Kg}^{-1}$ ).

155 In site 3 (Capraia Island, LI), the highest REE concentrations were found in the Ocrophyta  
156 *Halopteris scoparia* (Ce  $6.2 \text{ mg Kg}^{-1}$ , La and Nd  $2.7 \text{ mg Kg}^{-1}$ , Y  $1.9 \text{ mg Kg}^{-1}$ , Sc  $0.87 \text{ mg Kg}^{-1}$ , Pr

157 0.65 mg Kg<sup>-1</sup>, Sm and Gd 0.51 mg Kg<sup>-1</sup>, Dy 0.34 mg Kg<sup>-1</sup>, Er 0.17 mg Kg<sup>-1</sup>, Yb 0.13 mg Kg<sup>-1</sup>, Eu  
158 0.10 mg Kg<sup>-1</sup>, Ho 0.073 mg Kg<sup>-1</sup>, Tb 0.070 mg Kg<sup>-1</sup>, Tm 0.025 mg Kg<sup>-1</sup>, Lu 0.022 mg Kg<sup>-1</sup>) and the  
159 lowest levels were found in the Rodhopyta *Dudresnaya verticillata*.

160 Sites 2 and 3 seem to be less affected by anthropogenic contamination, and REE mean values were  
161 half of those registered in site 1.

162 In Table 2, the comparison between the ΣREE by one-way ANOVA showed a highly significant  
163 difference in concentrations between the three locations (p < 0.0001). However, the comparison  
164 between only sites 2 and 3 using the unpaired t-test resulted in a non-significant difference (p >  
165 0.05), highlighting that site 1 showed very different levels of REE in seaweeds, while between sites  
166 2 and 3, ΣREE values did not differ significantly.

### 167 3.2 Interspecies variability

168 The total levels of REE (ΣREE) are shown for each analyzed species in the three examined  
169 locations (Figure 3).

170 In site 1, Bergeggi (SV), the highest values were found overall, especially in green and brown  
171 macro algae, while in sites 2 and 3, REE values were lower; however, there was a high interspecies  
172 variability in the same sampling site. In fact, in site 1 (Figure 3), the total REE content was in the  
173 following decreasing order *Halopteris filicina* (ΣREE 27 mg Kg<sup>-1</sup> d.w.) > *Flabellia petiolata* >  
174 *Padina pavonica* > *Codium bursa* > *Ganonema farinosum* (ΣREE 15 mg Kg<sup>-1</sup> d.w.); in site 2, the  
175 order was *Flabellia petiolata* (ΣREE 15 mg Kg<sup>-1</sup> dry weight (d.w.) > *Dictyota dichotoma* > *Codium*  
176 *bursa* = *Padina pavonica* > *Peyssonnelia squamaria* > *Laurencia obtusa* > *Caulerpa racemosa* >  
177 *Halopteris filicina* (ΣREE 2.1 mg Kg<sup>-1</sup> d.w.); and finally, in site 3, the order was *Halopteris*  
178 *scoparia* (ΣREE 17 mg Kg<sup>-1</sup> d.w.) > *Padina pavonica* > *Halimeda tuna* > *Peyssonnelia squamaria*  
179 > *Cystoseira spp* > *Flabellia petiolata* > *Codium bursa* > *Dudresnaya verticillata* (ΣREE 2.3 mg  
180 Kg<sup>-1</sup> d.w.). A comparison between the three species that were collected in all three locations (*F.*  
181 *petiolata*, *C. bursa*, *P. pavonica*) is shown in Figure 4. The REE pattern was similar, but

182 concentrations differed in the same species in the two green macro algae *F. petiolata* and *C. bursa*  
183 from the three sites; the brown macro alga *P. pavonica* showed almost the same levels of REE in  
184 sites 2 and 3, but a higher value in site 1.

185 As shown in Table 2, the one-way interspecies comparison revealed highly significant differences  
186 between the different macro algae species for each site. This finding is in line with the scarce  
187 literature regarding REE in macro algae. In fact, other authors have underlined the interspecies  
188 variability in REE levels. Fu and coauthors (2000) suggested that REE patterns are division-  
189 dependent; while Sakamoto and coauthors (2008) indicated that the mechanism of uptake could be  
190 different and related to seaweed morphology, even if the accumulation of REE in seaweed is still  
191 not elucidated.

### 192 3.3 Chondrite-normalized REE pattern (Leedey Oklahoma chondrite)

193 To define a normalized REE pattern (Figure 5), we utilized the normalized values reported  
194 by Masuda (1975) for the Leedey chondrite, which is considered the most primitive chondrite  
195 (Sakamoto et al., 2008). Chondrite meteorites are, in fact, used as a reference for the normalization  
196 of REE, as they are thought to be similar to the original composition of the Earth's crust (Masuda et  
197 al., 1973). Moreover, during this meteorite formation, lanthanide fractionation did not occur (Song  
198 et al., 2006; Antonina et al., 2013), therefore, if fractionation between REE in seaweeds occurred,  
199 the comparison with the chondrite pattern could disclose this phenomenon.

200 In our study, the REE patterns were comparable in the three sites (Figure 5), even if, as previously  
201 indicated, site 1 (Bergeggi, SV) showed higher overall REE concentrations than the other two  
202 locations. The REE Ce and Eu have additional valences compared to the other lanthanides, and  
203 when Ce and/or Eu concentrations are enriched or depleted compared to the levels recorded in  
204 chondrite, this phenomenon is defined as a Ce or Eu (positive or negative) anomaly. In seaweeds  
205 from the three sites, a Eu negative anomaly was observed (Figure 5), while the REE ratios (Ce/La,  
206 Gd/Yb, La/Yb) were almost the same in the three different stations (mean values 1.8, 3.3 and 17,  
207 respectively).

208 The Eu anomaly is thought to be strictly dependent on lithology (Moller et al., 2004) and  
209 enrichment or depletion was explained by the Eu capacity to be mostly incorporated into  
210 plagioclase minerals.

211 Despite the very different sites of origin, the chondrite-normalized pattern profile of Mediterranean  
212 seaweeds appeared to be very similar to the pattern found by Mashitah and co-authors (2012) in  
213 brown seaweeds from Malaysian coasts.

214 The REE patterns normalized by chondrite (Figure 5) are typical of geological materials  
215 such as sediments, confirming that REE measurements are compatible with a geological material  
216 incorporated in macro algae tissues in different amounts. Moreover, the patterns are similar among  
217 sampling sites suggesting that correspond to sediments of similar origin.

#### 218 3.4 Comparison with REE in biota

219 In a previous study, we analyzed Ce and La concentrations in marine zooplankton from the  
220 Northwestern Mediterranean Sea (Battuello et al., 2017). We observed that concentrations for both  
221 these elements decreased from herbivorous to carnivorous copepods, and were in the average range  
222 of 0.50 - 1.86 mg Kg<sup>-1</sup> for Ce and 0.28 - 0.88 mg Kg<sup>-1</sup> d.w. for La (lowest values in carnivores).

223 In this investigation, Ce and La in seaweeds were in the medium range of 7.2 - 2.5 mg Kg<sup>-1</sup> and 3.7  
224 - 1.3 mg Kg<sup>-1</sup> d.w., respectively, showing the higher ability of REE to accumulate in seaweeds  
225 compared to zooplankton. As far as we know, we cannot compare these results with other seaweeds  
226 from the Mediterranean Sea, but a few studies have been performed in other parts of the world. For  
227 example, Hou and Yan (1998) analyzed La levels in Chinese coast seaweeds, finding the highest  
228 values reported in seaweeds to date, 10.14 mg Kg<sup>-1</sup> d.w. in green macro algae and 6.73 mg Kg<sup>-1</sup> d.w.  
229 in red macro algae, while the highest value we found for lanthanum was 4.3 mg Kg<sup>-1</sup> in *H. filicina*  
230 and *P. pavonica* from site 1.

231 Masitah (2012) analyzed REE concentrations in *P. pavonica* (Malaysian coast), and found ΣREE  
232 values ranging from 62 to 8.4 mg Kg<sup>-1</sup>, higher concentrations than in the Mediterranean area, where

233 the range we registered was 22 - 7.9 mg Kg<sup>-1</sup> (Table 1, Figure 2). REE in *Padina sp.* from the  
234 Malaysia areas decreased in the following order:  
235 Ce>Nd>La>Pr>Gd>Sm>Dy>Er>Yb>Eu=Tb>Ho>Tm>Lu.

236 In our study, in site 1 (Bergeggi, SV), the order of REE was:  
237 Ce>La>Nd>Y>Pr>Gd=Sm>Sc>Dy>Er>Yb>Eu>Tb>Ho>Tm>Lu, while in sites 2 and 3, the order  
238 was the same, namely Ce>Y>La>Nd>Sc>Pr>Gd>Sm>Dy>Er>Yb>Eu>Ho>Tb>Tm>Lu,  
239 demonstrating that *P. pavonica* samples in these two sites have the same geological “fingerprint”.  
240 Moreover, it was evident that these findings reflected a different pattern of REE in seaweeds, not  
241 only between the two different marine areas (Malaysia and Mediterranean), but also between the  
242 Mediterranean stations that we investigated. Sakamoto and co-authors (2008) investigated REE  
243 patterns in seaweed species collected in the Pacific Ocean (Japan). They found that seaweeds  
244 accumulated REE at levels 10<sup>3</sup> times higher than concentrations detected in seawater, and that the  
245 accumulation factor was higher for the heavy REE.

246 In order to estimate the bio concentration factor (BCF), which is defined as the accumulation of a  
247 chemical from water in an organism (Landis et al., 2011), we used the REE concentrations  
248 measured in surface waters of the Mediterranean Sea (Censi et al., 2004), ranging from 0.00013 µg  
249 L<sup>-1</sup> (Yb) to 0.0029 µg L<sup>-1</sup> (La). In fact, as REE partitioning is controlled by complexation and  
250 binding constants on an ocean-wide basis (Sholkovitz et al., 1994; Strady et al., 2015), we decided  
251 to apply them on a regional basin-wide basis, with the approach already utilized in the study by  
252 Strady and co-authors (2015).

253 BCF was usually expressed as the ratio of the concentration of the chemical in the organism and in  
254 water; our findings are shown in Figure 6.

255 The BCFs were expressed in the following decreasing order:  
256 Ce>Yb>Pr>La>Nd>Sm>Eu>Tb>Gd>Dt>Ho>Tm>Lu>Er, and with the exception of Yb, the bio  
257 concentration factors were higher in LREEs than in HREEs.

258 In a previous investigation in the studied area (Squadrone et al., under review), we found that  
259 different macro algae accumulated trace elements from seawater to different extents, and that brown  
260 and green macro algae had higher values than red macro algae. In fact, in seaweeds from site 1,  
261 important concentrations of iron, aluminum, manganese, copper, nickel and chromium were  
262 registered.

263 In this study, the same site (Bergeggi, SV) showed the highest levels of REE.

264 We suggest that REE concentrations in macro algae from sites 2 and 3, being non-contaminated  
265 areas, could constitute a baseline record for these elements in Mediterranean seaweeds, due to their  
266 characteristic elemental profile. In the presence of anthropogenic sources, such as in site 1, this  
267 unique profile was altered, and we can, therefore, suggest the use of REEs as pollution tracers.

268

#### 269 **4. Conclusions**

270 REE, due to their unique chemical properties have become crucially important in many industrial  
271 applications, and the global demand is still increasing. Following the consequent release of REE in  
272 terrestrial and aquatic environments, due to the extraction process and production of several  
273 industrial components, REE can be considered new emerging inorganic contaminants, for which the  
274 potential risks for human health and ecosystems have still not been investigated. Seaweeds have  
275 been shown to constitute a useful tool for biomonitoring REE, as they can concentrate REE at  
276 higher levels than in seawater. This study constitutes the first investigation of REE in seaweeds  
277 from the Northwestern Mediterranean Sea, providing the first record, which can be utilized for  
278 future comparisons.

279

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283 **References**

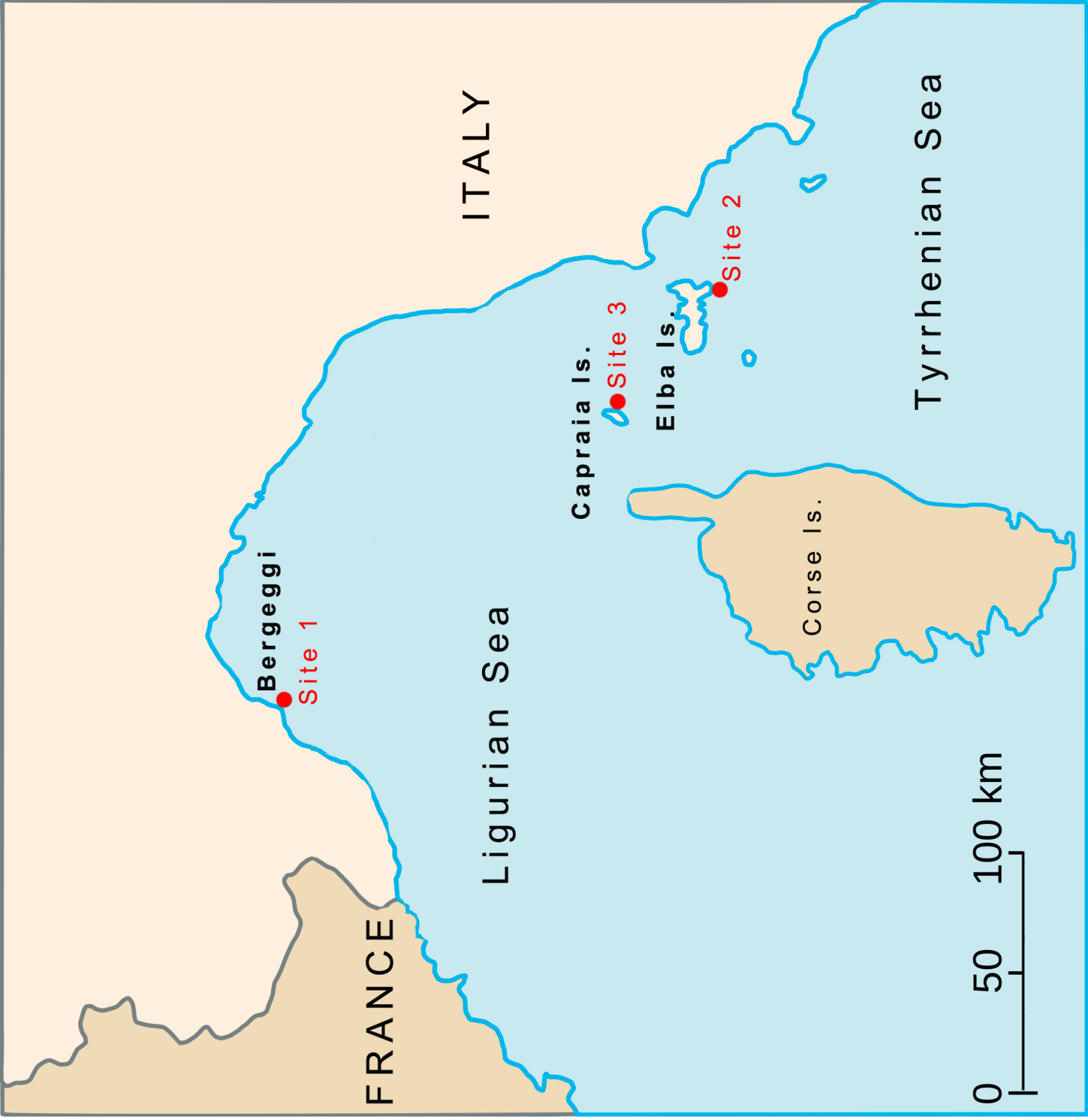
- 284 Anastopoulos I, Bhatnagar A, Lima EC, 2016. Adsorption of rare earth metals: A review of recent  
285 literature. *Journal of Molecular Liquids* 221: 954–962
- 286 Antonina AN, Shazili NAM, Kamaruzzaman B Y, Ong M C, Rosnan Y, Sharifah FN, 2015.  
287 Geochemistry of the Rare Earth Elements (REE) Distribution in Terengganu Coastal Waters: A  
288 Study Case from Redang Island Marine Sediment. *Open Journal of Marine Science* 3: 154-159
- 289 Ayache M, Dutay J, Arsouze T, Révillon S, Beuvier J, Jeandel C, 2016. High-resolution  
290 neodymium characterization along the Mediterranean margins and modelling of <sup>143</sup>Nd distribution in  
291 the Mediterranean basins. *Biogeosciences* 13: 5259–5276
- 292 Battuello M, Mussat Sartor R, Brizio P, Nurra N, Pessani D, Abete MC, Squadrone S, 2017. The  
293 influence of feeding strategies on trace element bioaccumulation in copepods (Calanoida).  
294 *Ecological Indicators* 74, 311-320
- 295 Battuello M, Brizio P, Mussat Sartor R, Nurra N, Pessani D, Abete MC, Squadrone S, 2016.  
296 Zooplankton from a North Western Mediterranean Area as a model of metal transfer in a marine  
297 environment. *Ecological Indicators* 66, 440-451
- 298 Censi P, Incarbona A, Oliveri E, Bonomo S, Tranchida G, 2010. Yttrium and REE signature  
299 recognized in Central Mediterranean Sea (ODP Site 963) during the MIS 6–MIS 5 transition.  
300 *Palaeogeogr. Palaeoclimatol. Palaeoecol.* doi:10.1016/j.palaeo.2010.03.045.
- 301 Censi P, Mazzola S, Sprovieri M, Bonanno A, Patti B, Punturo R., Spoto SE, Saiano F, Alonzo G,  
302 2004. Rare earth elements distribution in seawater and suspended particulate of the central  
303 Mediterranean Sea. *Chemistry and Ecology* 20(5): 323–343
- 304 De Baar HJW, Brewer PG, Bacon MD 1985. Anomalies in rare-earth distributions in seawater: Gd  
305 and Tb. *Geochimica et Cosmochimica Acta* 49: 1961–1969
- 306 DeForest DK, Brix KV, Adams WJ, 2007. Assessing metal bioaccumulation in aquatic  
307 environments: the inverse relationship between bioaccumulation factors, trophic transfer factors and  
308 exposure concentration. *Aquatic Toxicology* 84:236–246 DOI 10.1016/j.aquatox.2007.02.022.
- 309 Frost CD, O’Nions RK, Goldstein SL, 1986. Mass balance for Nd in the Mediterranean Sea.  
310 *Chemical Geology* 55: 45–50
- 311 Fu FF, Akagi T, Yabuki S, Iwaki M, Ogura N, 2000. Distribution of rare earth elements in seaweed:  
312 implication of two different sources of rare earth elements and silicon in seaweed. *Journal of*  
313 *Phycology* 36: 62–70.
- 314 Greaves MJ, Statham PJ, Elderfield H, 1994. Rare earth element mobilization from marine  
315 atmospheric dust into seawater. *Marine Chemistry* 46: 255–260
- 316 Hermann H, Nolde J, Berger S, Heise S, 2016. Aquatic ecotoxicity of lanthanum – A review and an  
317 attempt to derive water and sediment quality criteria. *Ecotoxicology and Environmental Safety*  
318 124:213–238

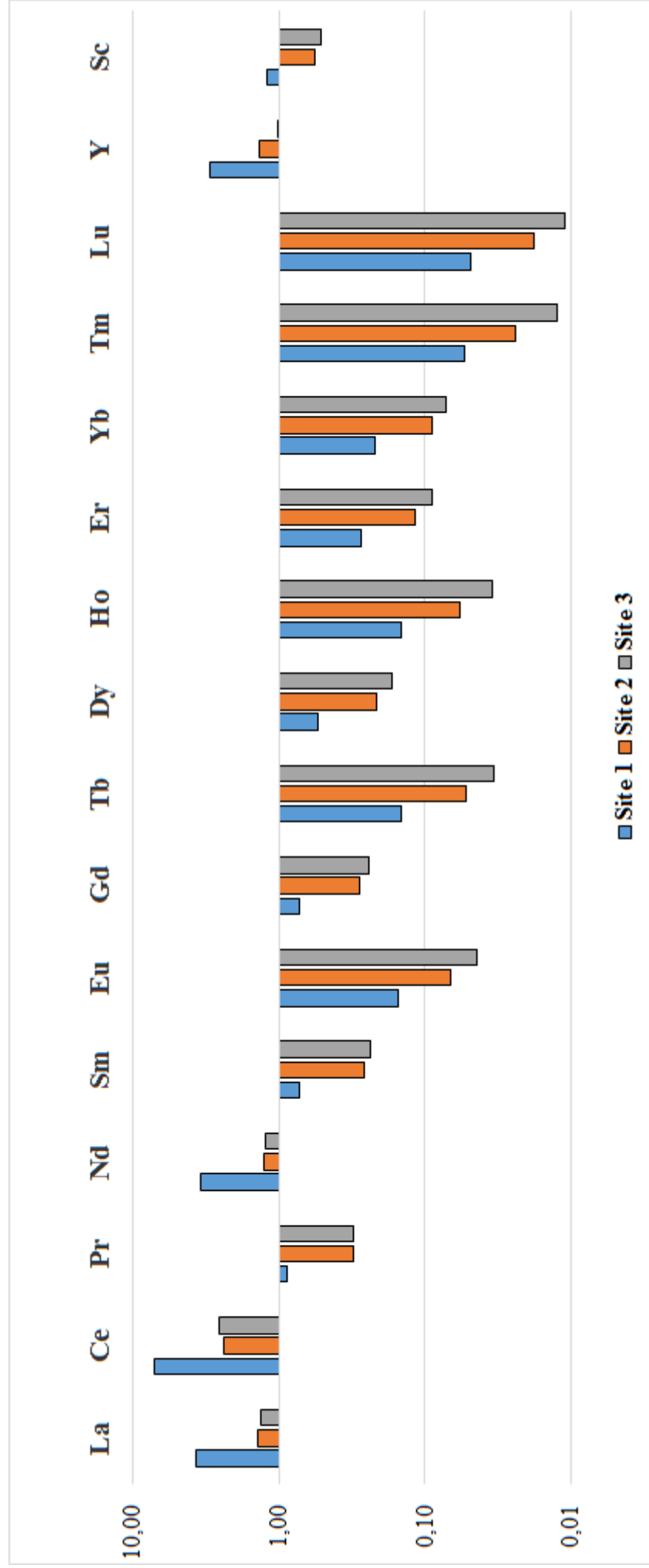
- 320 Hou X, Yan X, 1998. Study on the concentration and seasonal variation of inorganic elements in 35  
321 species of marine algae. *Science of the Total Environment* 222(3):141–156.  
322 [http://dx.doi.org/10.1016/S0048-9697\(98\)00299-X](http://dx.doi.org/10.1016/S0048-9697(98)00299-X).
- 323 Martinez-Bot MA, Vance D, Mortyn P., 2009. Nd/Ca ratios in plankton-towed and core top  
324 foraminifera: Confirmation of the water column acquisition of Nd. *Geochem. Geophys. Geosyst.*  
325 10.
- 326 Mashitah SM, Shazili NAM, Rashid MKA, 2012. Elemental concentrations in Brown Seaweed,  
327 *Padina* sp. along the east coast of Peninsular Malaysia, *Aquatic Ecosystem Health & Management*  
328 15(3): 267-278
- 329 Masuda A, 1975. Abundance of monoisotopic REE, consistent with Leedey chondrite values.  
330 *Geochemistry Journal* 9:183-184
- 331 Masuda A, Nakamura N, Tanaka T, 1973. Fine structure of mutually normalized rare-earth patterns  
332 of chondrites. *Geochimica et Cosmochimica Acta* 37: 239-248
- 333 Moldoveanu, G.A., Papangelakis, V.G. Recovery of rare earth elements adsorbed on clay minerals:  
334 I. Desorption mechanism. *Hydrometallurgy* 117 (2012) 71–78.
- 335 Oliveri E, Neri R, Bellanca A, Riding R, 2010. Carbonate stromatolites from a Messinian  
336 hypersaline setting in the Caltanissetta Basin, Sicily: petrographic evidence of microbial activity  
337 and related stable isotope and rare earth element signatures. *Sedimentology* 57: 142–161
- 338 Roussiez V, Aubert D, Heussner S, 2013. Continental sources of particles escaping the Gulf of Lion  
339 evidenced by these elements are becoming emerging inorganic contaminants in marine ecosystems,  
340 due to their worldwide increasing applications in industry, technology, medicine and agriculture.  
341 Rare earth elements: Flood vs. normal conditions. *Marine Chemistry* 153: 31–38
- 342 Sakamoto N, Kano N, Imaizumi H 2008. Determination of rare earth elements, thorium and  
343 uranium in seaweed samples on the coast in Niigata Prefecture by inductively coupled plasma mass  
344 spectrometry. *Applied Geochemistry* 23: 2955–2960
- 345 Song ZL, Liu CQ, Han GL, Wang ZL, Zhu ZZ, Yang , Y. 2006 Enrichment and Release of Rare  
346 Earth Elements during Weathering of Sedimentary Rocks in Wujiang Catchments, Southwest  
347 China, *Journal of Rare Earths* 24 (4):491-496
- 348 Squadrone S, Battuello M, Brizio P, Nurra N, Mussat Sartor R, Riva A, Staiti M, Benedetto A,  
349 Pessani D, Abete MC, 2017. Mediterranean seaweeds for feed and food: trace metals occurrence,  
350 *under review*.
- 351 Strady E, Kim I, Radakovitch O, Kim G, 2015. Rare earth element distributions and fractionation in  
352 plankton from the northwestern Mediterranean Sea. *Chemosphere* 119, 72–82.
- 353 Tranchida G, Oliveri E, Angelone M, Bellanca A, Censi P, D'Elia M, Neri R, Placenti F, Sprovieri  
354 M, Mazzola S, 2011. Distribution of rare earth elements in marine sediments from the Strait of

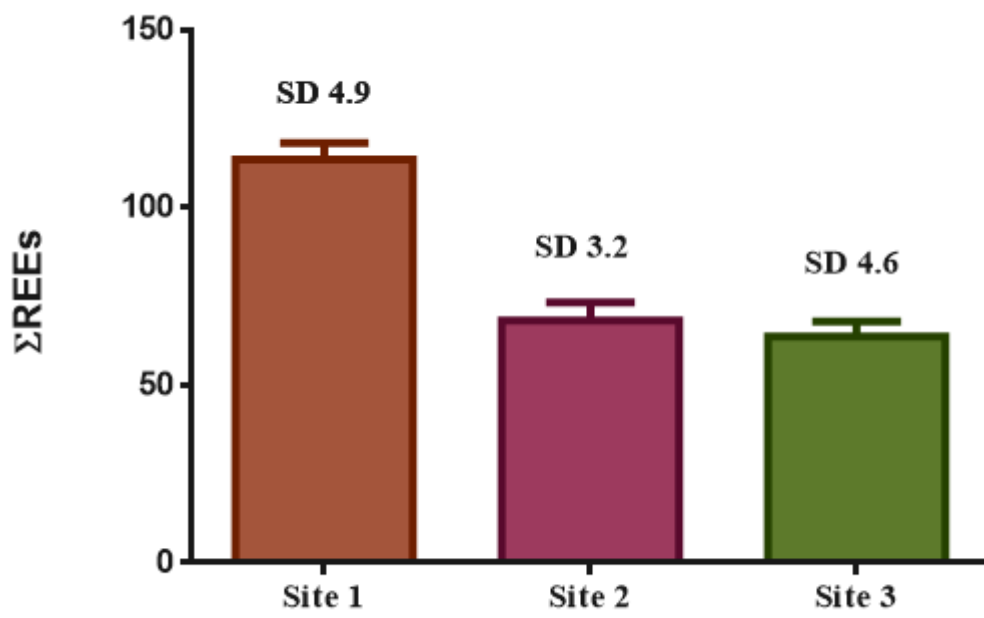
355 Sicily (western Mediterranean Sea): Evidence of phosphogypsum waste contamination Marine  
356 Pollution Bulletin 62: 182–191

357 USEPA 2012. Rare Earth Elements: A Review of Production, Processing, Recycling, and  
358 Associated Environmental Issues. United States Environmental Protection Agency, Cincinnati, OH,  
359 EPA/600/R-12/572.

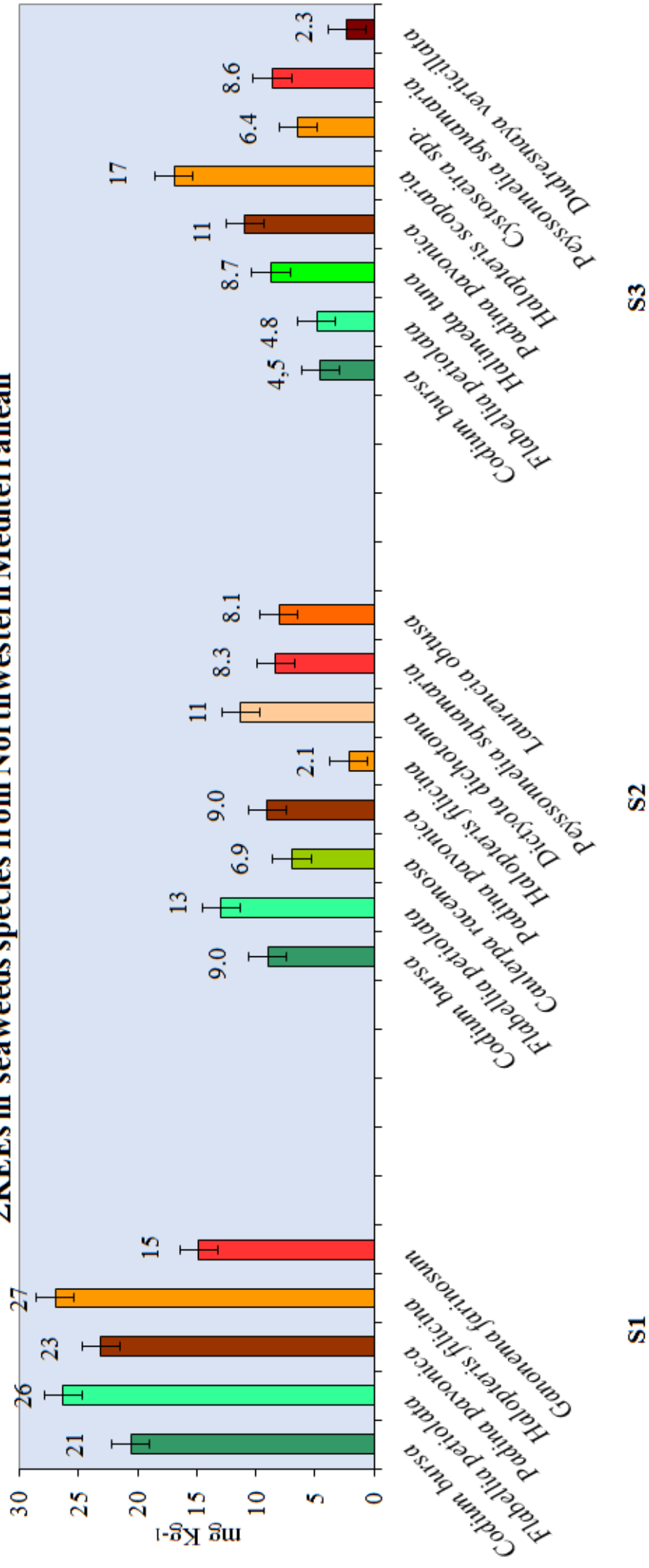
360

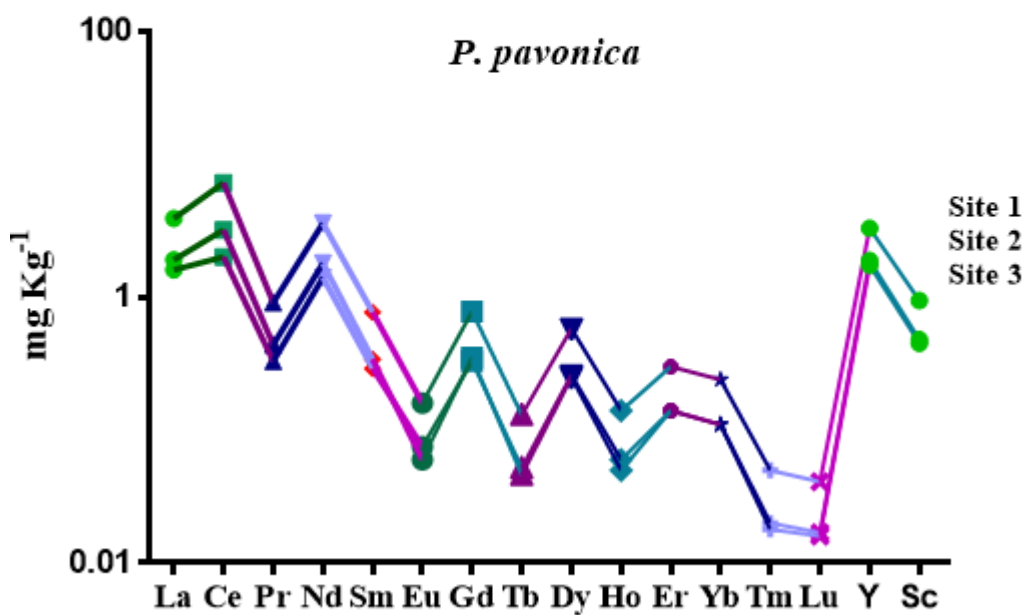
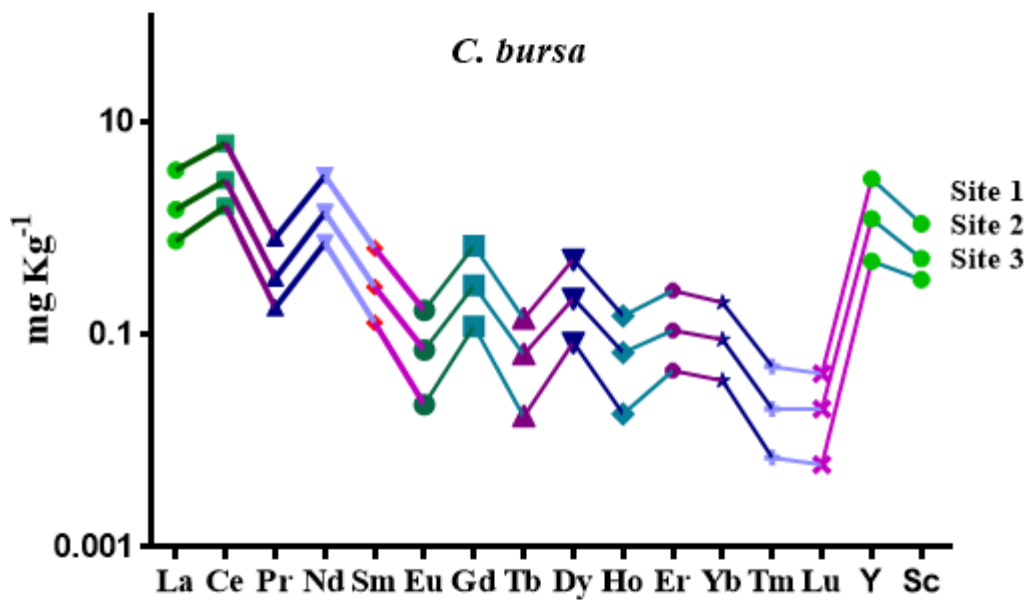
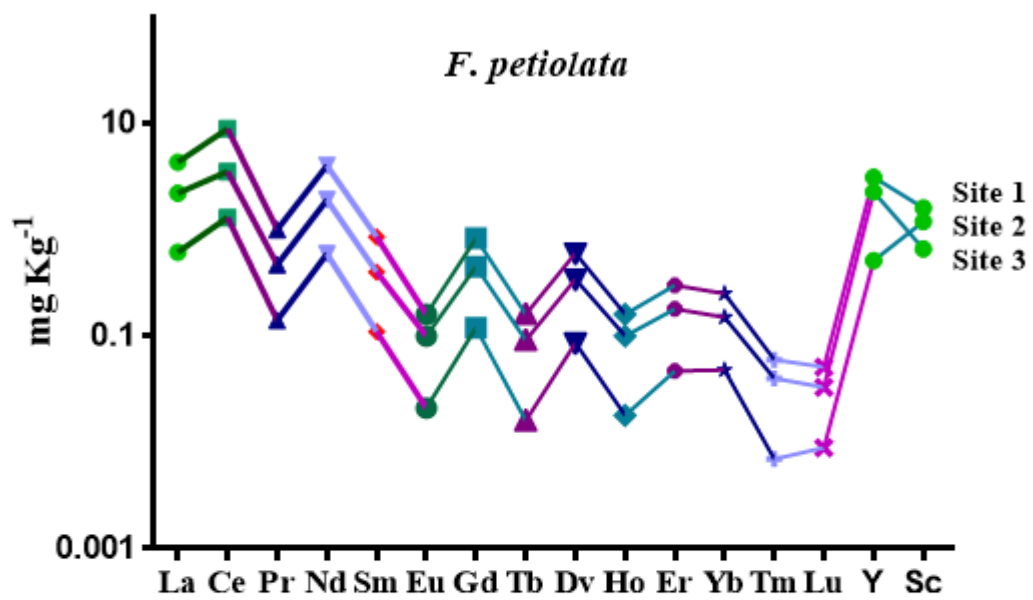


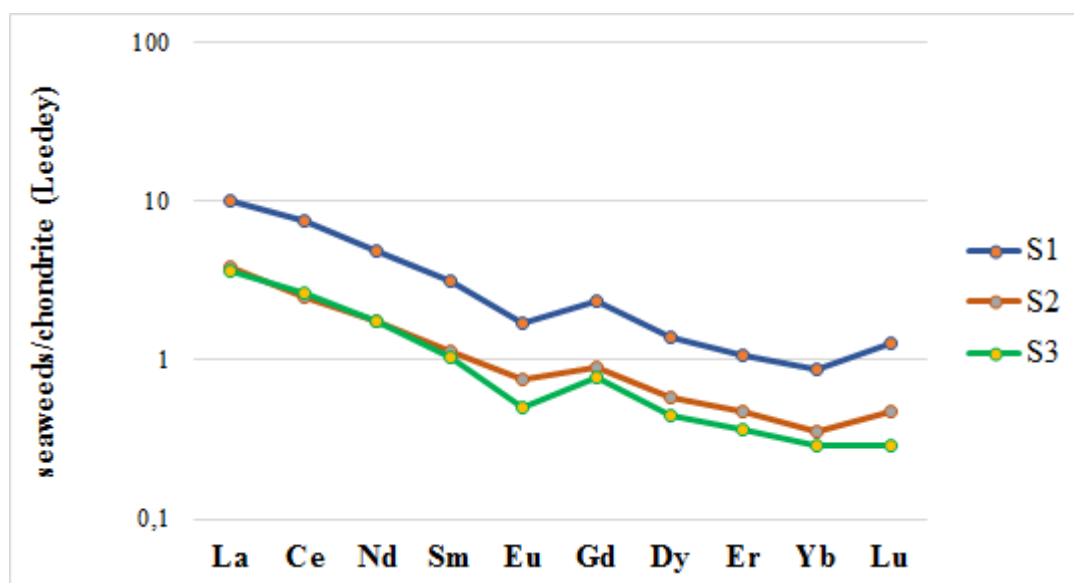


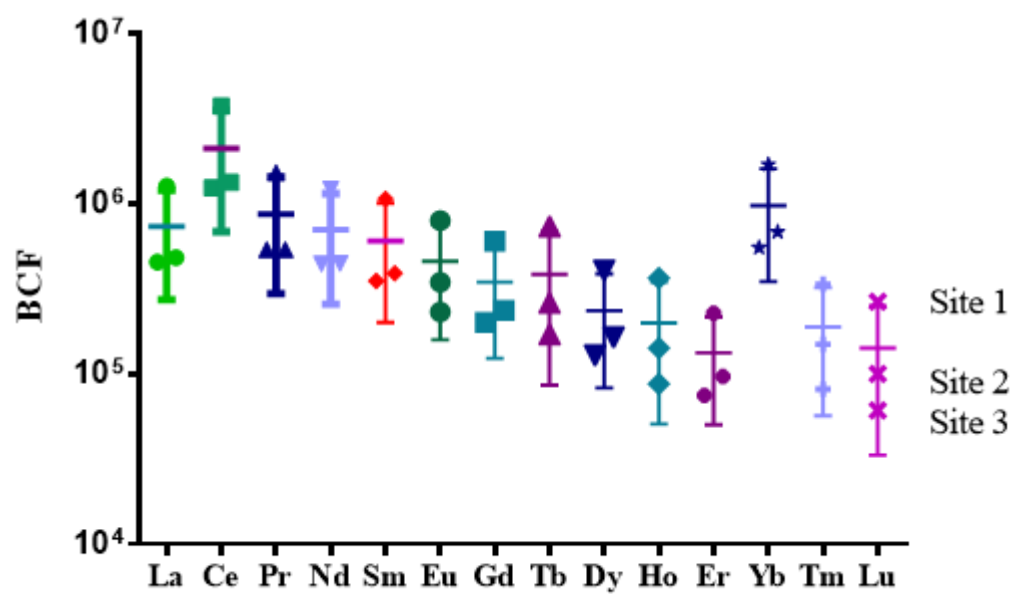


### ΣREEs in seaweeds species from Northwestern Mediterranean









**Concentrations of REE in macro algae from Mediterranean Sea (mg Kg<sup>-1</sup> d.w.)**

Site	Species	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Yb	Tm	Lu	Y	Sc	ΣREE	LREE	HREE
<b>S1</b>	<i>Codium bursa</i>	3.5	6.3	0.81	3.1	0.65	0.17	0.68	0.14	0.51	0.15	0.26	0.20	0.050	0.043	2.9	1.1	21	14	5.1
	<i>Flabellia petiolata</i>	4.3	8.8	1.0	4.0	0.85	0.16	0.83	0.16	0.60	0.16	0.30	0.25	0.060	0.051	3.1	1.6	26	19	5.6
	<i>Padina pavonica</i>	3.9	7.2	0.91	3.6	0.77	0.16	0.78	0.13	0.58	0.14	0.30	0.24	0.050	0.041	3.3	0.94	23	16	5.8
	<i>Halopteris filicina</i>	4.3	8.8	1.1	4.1	0.87	0.17	0.87	0.22	0.64	0.22	0.32	0.27	0.080	0.078	3.4	1.53	27	19	6.3
	<i>Ganonema farinosum</i>	2.5	4.8	0.55	2.2	0.46	0.10	0.47	0.08	0.35	0.08	0.19	0.15	0.030	0.030	2.0	0.84	15	10	35
	<b>mean</b>	<b>3.7</b>	<b>7.2</b>	<b>0.87</b>	<b>3.4</b>	<b>0.72</b>	<b>0.15</b>	<b>0.73</b>	<b>0.15</b>	<b>0.53</b>	<b>0.15</b>	<b>0.27</b>	<b>0.22</b>	<b>0.054</b>	<b>0.048</b>	<b>2.9</b>	<b>1.2</b>	<b>22</b>	<b>16</b>	<b>6.5</b>
	<b>SD</b>	0.77	1.72	0.21	0.77	0.17	0.03	0.16	0.051	0.11	0.049	0.051	0.048	0.018	0.018	0.55	0.36	4.9	3.6	1.1
<b>S2</b>		<b>La</b>	<b>Ce</b>	<b>Pr</b>	<b>Nd</b>	<b>Sm</b>	<b>Eu</b>	<b>Gd</b>	<b>Tb</b>	<b>Dy</b>	<b>Ho</b>	<b>Er</b>	<b>Yb</b>	<b>Tm</b>	<b>Lu</b>	<b>Y</b>	<b>Sc</b>	<b>ΣREE</b>	<b>LREE</b>	<b>HREE</b>
	<i>Codium bursa</i>	1.5	2.8	0.34	1.4	0.28	0.072	0.29	0.065	0.22	0.068	0.11	0.090	0.020	0.020	1.22	0.52	9.0	6.3	2.2
	<i>Flabellia petiolata</i>	2.2	3.5	0.47	1.9	0.40	0.10	0.45	0.091	0.34	0.10	0.18	0.15	0.040	0.033	2.26	0.66	13	8.5	3.8
	<i>Caulerpa racemosa</i>	1.1	1.8	0.24	1.0	0.22	0.050	0.24	0.040	0.20	0.050	0.11	0.080	0.020	0.020	1.30	0.50	6.9	4.3	2.1
	<i>Padina pavonica</i>	1.6	2.0	0.33	1.4	0.29	0.075	0.33	0.052	0.26	0.060	0.14	0.11	0.020	0.017	1.88	0.48	9.0	5.6	2.9
	<i>Halopteris filicina</i>	0.29	0.64	0.07	0.31	0.070	0.010	0.070	0.010	0.050	0.010	0.030	0.020	0.031	0.003	0.23	0.25	2.1	1.4	0.5
	<i>Dictyota dichotoma</i>	1.8	3.3	0.41	1.6	0.34	0.090	0.36	0.060	0.27	0.070	0.15	0.11	0.020	0.020	1.61	1.13	11	7.4	2.8
	<i>Peyssonnelia squamaria</i>	1.6	2.6	0.32	1.3	0.25	0.070	0.26	0.050	0.18	0.050	0.090	0.070	0.020	0.010	1.02	0.45	8.3	6.0	1.8
	<i>Laurencia obtusa</i>	1.3	2.3	0.28	1.2	0.25	0.060	0.27	0.050	0.21	0.050	0.11	0.080	0.020	0.020	1.33	0.61	8.1	5.2	2.2
	<b>mean</b>	<b>1.4</b>	<b>2.4</b>	<b>0.31</b>	<b>1.3</b>	<b>0.26</b>	<b>0.066</b>	<b>0.28</b>	<b>0.052</b>	<b>0.22</b>	<b>0.057</b>	<b>0.12</b>	<b>0.089</b>	<b>0.024</b>	<b>0.018</b>	<b>1.4</b>	<b>0.58</b>	<b>8.5</b>	<b>5.6</b>	<b>2.9</b>
<b>SD</b>	0.56	0.91	0.12	0.47	0.10	0.028	0.11	0.023	0.084	0.025	0.046	0.038	0.008	0.009	0.60	0.26	3.2	2.1	1.0	
<b>S3</b>		<b>La</b>	<b>Ce</b>	<b>Pr</b>	<b>Nd</b>	<b>Sm</b>	<b>Eu</b>	<b>Gd</b>	<b>Tb</b>	<b>Dy</b>	<b>Ho</b>	<b>Er</b>	<b>Yb</b>	<b>Tm</b>	<b>Lu</b>	<b>Y</b>	<b>Sc</b>	<b>ΣREE</b>	<b>LREE</b>	<b>HREE</b>
	<i>Codium bursa</i>	0.76	1.6	0.18	0.73	0.13	0.022	0.12	0.017	0.085	0.018	0.046	0.037	0.0070	0.006	0.49	0.33	4.5	3.4	0.9
	<i>Flabellia petiolata</i>	0.61	1.3	0.14	0.59	0.11	0.021	0.12	0.016	0.086	0.018	0.047	0.048	0.0070	0.0089	0.51	1.19	4.8	2.7	0.9
	<i>Halimeda tuna</i>	1.51	3.0	0.37	1.4	0.26	0.040	0.25	0.030	0.17	0.030	0.090	0.071	0.012	0.0090	1.04	0.42	8.7	6.5	1.7
	<i>Padina pavonica</i>	1.9	3.2	0.44	1.8	0.34	0.060	0.35	0.046	0.25	0.050	0.14	0.11	0.018	0.016	1.74	0.45	11	7.7	2.8
	<i>Halopteris scoparia</i>	2.73	6.2	0.65	2.7	0.51	0.10	0.51	0.070	0.34	0.073	0.17	0.13	0.025	0.022	1.87	0.871	17	13	3.3
	<i>Cystoseira spp.</i>	1.00	1.5	0.23	0.99	0.22	0.050	0.25	0.040	0.20	0.040	0.12	0.096	0.016	0.015	1.38	0.32	6.4	3.9	2.2
	<i>Peyssonnelia squamaria</i>	1.52	2.9	0.35	1.4	0.26	0.050	0.26	0.040	0.18	0.040	0.090	0.070	0.013	0.011	1.01	0.40	8.6	6.4	1.8
	<i>Dudresnaya verticillata</i>	0.52	0.80	0.090	0.35	0.060	0.010	0.070	0.010	0.040	0.010	0.020	0.013	0.0020	0.0020	0.20	0.10	2.3	1.8	0.4
	<b>mean</b>	<b>1.32</b>	<b>2.5</b>	<b>0.31</b>	<b>1.2</b>	<b>0.24</b>	<b>0.044</b>	<b>0.24</b>	<b>0.034</b>	<b>0.17</b>	<b>0.035</b>	<b>0.090</b>	<b>0.072</b>	<b>0.013</b>	<b>0.011</b>	<b>1.0</b>	<b>0.51</b>	<b>7.9</b>	<b>5.7</b>	<b>2.3</b>
<b>SD</b>	0.75	1.7	0.18	0.75	0.14	0.03	0.14	0.020	0.10	0.021	0.051	0.039	0.007	0.0062	0.61	0.35	4.6	3.5	1.0	



**Table 2 Statistical evaluation**

	<b>P value</b>	<b>P value</b>
<b>One-way ANOVA (3 SITES, ΣREE COMPARISON)</b>	P < 0.0001	****
<b>Unpaired t test (SITE 2 AND SITE 3 ΣREE COMPARISON)</b>	P = 0.0811 (P > 0.05)	NS
<b>One-way ANOVA (Site 1, ΣREE interspecies COMPARISON)</b>	P < 0.0001	****
<b>One-way ANOVA (Site 2, ΣREE interspecies COMPARISON)</b>	P < 0.0001	****
<b>One-way ANOVA (Site 3, ΣREE interspecies COMPARISON)</b>	P < 0.0001	****

\*\*\*\* Significant at the 0.01 probability level  
NS not statistically significant

**Figure 1**

Sampling sites, study area.

**Figure 2a**

Rare earth elements in macro algae from three Northwestern Mediterranean locations (mg Kg<sup>-1</sup> dry weight, log scale).

**Figure 2b**

Box-plot diagrams of  $\Sigma$ REE (mean  $\pm$  SD) in the three sampling locations (mg Kg<sup>-1</sup> dry weight).

**Figure 3**

Box-plot diagrams of  $\Sigma$ REE (mean  $\pm$  SEM) in the macro algae species (mg Kg<sup>-1</sup> dry weight) from the three examined locations.

**Figure 4**

REE distribution (mg Kg<sup>-1</sup> dry weight) in *Flabellia petiolata*, *Codium bursa* and *Padina pavonica* in the three sampling sites.

**Figure 5**

Chondrite (Leedeey, Oklahoma) normalized patterns of REE in Northwestern Mediterranean macro algae (log scale).

**Figure 6**

Bioconcentration factors in macro algae from the Northwestern Mediterranean Sea.