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Kassandra de Pao Mendonca, Bernard Angeletti, Aurélie Dufour, Carole Borchellini, Lars-Eric Heimbürger-Boavida, Emmanuelle Renard, Julien Issartel

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1

2 *The sponge **Oscarella lobularis** (Porifera, Homoscleromorpha)*
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4 *Mediterranean coastal ecosystems*

5

6 [Kassandra De Pao Mendonca](#)^{1,4}, [Bernard Angeletti](#)^{3,5}, [Aurélie Dufour](#)², [Carole Borchiellini](#)^{1°},
7 [Lars-Eric Heimbürger-Boavida](#)^{2°}, [Emmanuelle Renard](#)^{1,4,5°+}, [Julien Issartel](#)^{1,5°+}

8

9 ¹Aix Marseille Univ, Avignon Univ, CNRS, IRD, IMBE, Marseille, France

10 ²Aix Marseille Université, CNRS/INSU, Université de Toulon, IRD, Mediterranean Institute of
11 Oceanography (MIO), Marseille, France

12 ³Aix Marseille Univ, CNRS, IRD, INRAE, Coll France, CEREGE, Aix-en-Provence

13 ⁴Aix Marseille Univ, CNRS, IBDM, Marseille, France

14 ⁵Aix Marseille Univ, CNRS, FR 3098 ECCOREV, F-13545 Aix-en-Provence, France

15

16 ° co-PI

17 + corresponding authors :

18 emmanuelle.renard@imbe.fr ; +33 6 76 94 79 38 ; Mediterranean Institute of marine and terrestrial
19 Biodiversity and Ecology, 22 Chemin de la Batterie des Lions, 13007 Marseille, France

20 julien.issartel@imbe.fr ; +33 (0)4 13 94 93 51 ; Mediterranean Institute of marine and terrestrial
21 Biodiversity and Ecology, Europôle de l'Arbois, Bâtiment Villemin, 13545, Aix-en-Provence, France

22 Abstract

23 The biomonitoring of metallic contamination in marine ecosystems is often focused on animal
24 species of commercial interest and in lesser extent on non-model marine invertebrates. The
25 aim of the study was to compare the metal concentrations (Li, Al, Ti, Cr, Fe, Ni, Cu, Zn, As, Ag,
26 Cd, Hg, Pb) in seven marine sponges with a particular interest in the homoscleromorph sponge
27 *Oscarella lobularis* at different sites of the Bay of Marseille, France. Inter-species variabilities
28 suggest that the seven sponge species studied accumulate metals differently. In *O. lobularis*,
29 a multi-site analysis shows different bioaccumulation between the eight sampled populations.
30 This inter-site differences may reflect differences in the hydrodynamic features and in past
31 and present industrial activities. Because *Oscarella lobularis* shows a homogeneous metal
32 accumulation pattern in comparison with the other tested species, it appears to be suitable
33 for metal contamination biomonitoring in Mediterranean coastal waters, in particular of the
34 coralligenous communities.

35 Keywords: Sponge, bioconcentration factors, coralligenous, trace metals.

36 1.Introduction

37

38 The Mediterranean Sea is recognized as a major biodiversity hotspot, counting 7% of the
39 global marine species (Bianchi & Morri, 2000; European Environment Agency, 2006). During
40 the past century, this area has been exposed to intense anthropic pressure resulting from the
41 increasing population of the Mediterranean-bordering countries. Today, 450 million people
42 live in this area, without counting the important population increase occurring during summer
43 in a world leading tourist destination (Lanquar, 2011). As a consequence, agricultural,
44 domestic, commercial, and industrial land-based activities considerably intensified, increasing
45 contaminant inputs onto coastal marine environments via wastewaters, watersheds and
46 atmospheric inputs (Danovaro, 2003; Durrieu de Madron et al., 2011; Heimbürger et al., 2011;
47 Heimbürger-Boavida et al., 2010). Metal contamination, known to impact aquatic
48 communities (size, distribution and density, species richness, composition of community) is a
49 major concern (Nogales et al., 2011; Zhou et al., 2008; Zhu et al., 2020). Marseille is the most
50 important city of the French Mediterranean coast with a dense urbanization and important
51 industrial, port and tourism activities. Thus, there is a need to monitor and to map the metallic
52 contamination and to provide reliable bioindicators in order to accurately evaluate the
53 abundance of hazardous metals that could affect coastal marine communities of this area.

54

55 Evaluation of metal contamination on marine organisms and ecosystems is often focused on
56 species of commercial interest such as teleosts, decapods, bivalves and sea urchins, partly in
57 order to estimate the sanitary risks for consumers or the economic risks of the loss of
58 productivity for fisheries or farms (Liu et al., 2019; Mille et al., 2018; Penz et al., 2011;
59 Schuhmacher et al., 1994; Zhang et al., 2012). Today, the most widely used bioindicators in

60 the marine environment are bivalves (Aly et al., 2013; Azizi et al., 2018; Beyer et al., 2017;
61 Gentric, 2016; Lafabrie et al., 2007; Pérez et al., 2004). Considering the ongoing major
62 biodiversity crisis (Ceballos et al., 2015, 2017; Eisenhauer et al., 2019), it is timely to extend
63 our knowledge concerning the impact of anthropic activities on ecosystem contamination by
64 studying a larger set of marine animals (Chifflet et al., 2022). To this purpose, several studies
65 investigated metal accumulation in sponges, as being filter feeders and sessile organisms,
66 mainly in Demospongiae and Calcarea (Cebrian, 2007; Cebrian et al., 2003; Hansen et al., 1995;
67 Müller et al., 1998; Patel et al., 1985; Perez et al., 2005; Pérez et al., 2004). We propose to
68 evaluate for the first time the relevance of a Mediterranean sponge belonging to
69 Homoscleromorpha, *Oscarella lobularis* (Schmidt, 1862), as bioindicator for metal
70 contamination. Sponges (Porifera) are present in all marine habitats and gather about 9500
71 described species (de Voogd et al., 2022). In the Mediterranean Sea, sponges represent more
72 than 12% of marine fauna diversity (the same order as vertebrates) and are one of the main
73 organisms within benthic communities (Coll et al., 2010). The large amounts of water filtered
74 by sponges through their aquiferous system (Morganti et al., 2019; Weisz et al., 2008) yields
75 to the accumulation of metals present both in the dissolved and particulate phases (Caplat et
76 al., 2011; Orani et al., 2020; Rao et al., 2006). Several sponge species have already been used
77 as bioindicators of metal contamination (Keren et al., 2017; Khati et al., 2018; Mahaut et al.,
78 2013; Pan et al., 2011). However, only few studies explored if the accumulation of metals
79 depends on sponge species (Orani, Barats, Vassileva, et al., 2018; Pérez et al., 2004; Roveta et
80 al., 2020) thereby questioning the relevance of using only one species to monitor a large set
81 of environmental metal contaminants. The number of sponge species for which a convenient
82 set of biological knowledge and experimental tools are available is still limited, making it thus

83 far difficult to use sponges as model organisms for environmental assessment and
84 ecotoxicology.

85

86 In this context, *Oscarella lobularis* (Schmidt, 1862) provides several advantages: i) being
87 abundant in shallow rocky substrates of the north-western Mediterranean Sea, including at
88 the vicinity of the Marseille Metropole and the French Riviera (Ereskovsky et al., 2009; Renard
89 et al., 2021). *Oscarella lobularis* is part of the coralligenous biocenosis which represents a
90 biodiversity hotspot in Mediterranean ecosystems (Bertolino et al., 2013; Martin et al., 2014);
91 ii) the taxonomic identification of the species is clear (Gazave et al., 2012, 2013) and the life
92 cycle, development and morphoanatomical organization were deeply studied (Ereskovsky et
93 al., 2009, 2013; Fierro-Constain et al., 2017); iii) the high regeneration capability (Ereskovsky
94 et al., 2015) and the high number of buds obtained *in vitro* by asexual reproduction (Renard et
95 al., 2021; Rocher et al., 2020) provide useful replicates for experimental ecotoxicological
96 studies; iv) in addition, both genomic and transcriptomic resources (Belahbib et al., 2018;
97 Schenkelaars et al., 2015; Vernale et al., 2021) and molecular tools are available for this
98 species (Borchiellini et al., 2021; Fierro-Constain et al., 2017; Fierro-Constain et al., 2021;
99 Renard et al., 2021; Rocher et al., 2020). This is convenient and suitable to perform
100 ecotoxicological experiments at different complementary levels of the biological scale (from
101 morphological, to physiological, cellular, and molecular levels).

102 The aim of this study was to evaluate the suitability of *O. lobularis* as a biomonitor of metallic
103 contamination in the Mediterranean Sea. For this purpose, (i) the concentrations of thirteen
104 metals of environmental concern (Li, Al, Ti, Cr, Fe, Ni, Cu, Zn, As, Ag, Cd, Hg, Pb) were measured
105 in individuals sampled at seven locations of the Bay of Marseille, and (ii) the metal

106 concentrations in *O. lobularis* were compared with six other sponge species, commonly found
107 in sympatry to assess potential interspecific variations. The study sites are characterized by
108 contrasted anthropogenic activities and were used to assess possible links between metal
109 accumulation by these organisms to past and present anthropogenic activities.

110 2. Materials and Methods

111

112 2.1. Sampling strategy

113 According to the distribution of the homoscleromorph sponge *Oscarella lobularis* populations,
114 seven sites in the Bay of Marseille (Mejean, Frioul, Prado, Maire, Perez cave, Caramassaigne,
115 Riou) and one site out of the bay (Fourmigues) were selected (Figure 1).

116 **Figure 1**

117 At each site, 3-14 adult individuals and surface sediment were both collected manually by
118 scientific divers at the same depth (Supplementary Data Table 1). Because Maire Island is a
119 known study site in our laboratory for its large quantities and diversity of sponges species, 2
120 or 3 individuals of six other sponge species were sampled at this site for comparative
121 purposes: three Desmospongiae (*Aplysina cavernicola* (Vacelet, 1959), *Agelas oroides*
122 (Schmidt, 1864), *Axinella damicornis* (Esper, 1794)), one Calcarea (*Clathrina clathrus* (Schmidt,
123 1864)), one homoscleromorph of the Plakinidae family (*Corticium candelabrum* (Schmidt,
124 1862)) and one homoscleromorph of the Oscarellidae family, the sister species of *Oscarella*
125 *lobularis*, *Oscarella uberulate* (Schmidt, 1868). All samples were placed in situ in clean (never
126 used) plastic resealable-zip storage bags and brought back to the laboratory in a cool box
127 within 1 hour.

128

129 **2.2. Sample preparation and metal measurements**

130 Sponge epibionts were removed with ceramic forceps under a magnifier, organisms were
131 briefly rinsed with distilled water to limit salt crystals, dried on paper towel and then stored
132 at -20°C. For the sediments, after the residual sea water was removed, samples were stored
133 at -20°C. The frozen sponges and sediments were freeze-dried (Heto PowerDry PL3000 freeze
134 dryer, Thermo Scientific). Sediments were sieved to recover the fraction less than 2 mm and
135 sponges were homogenized in an agate mortar.

136 An Inductively Coupled Plasma Mass Spectrometry (ICP-MS Perkin Elmer NexION® 300X) was
137 used to quantify Li, Al, Ti, Cr, Fe, Ni, Cu, Zn, As, Ag, Cd, and Pb (Supplementary Data Table 2
138 and 3). Before measurements, 300 mg (dry weight) of each sponge sample were digested (1.5
139 mL HCl 37% + 1.5 mL HNO₃ 69% + 0.5 mL H₂O₂ 30%, quality TMA (Trace Metal Analysis)) and
140 50 mg (dry weight) of sediment were digested (1.5 mL HCl 37% + 1.5 mL HNO₃ 69% + 0.5 mL
141 HF 40%, quality TMA (Trace Metal Analysis)). The samples were then mineralized with a
142 microwave (UltraWave, Milestone) (20-130°C in 15min – 130°C during 10 min – 130°C to 210°C
143 in 10 min – 210°C during 10 min). Samples were diluted by a factor of 10-50 before analysis
144 by ICP-MS. Procedural blank and a standard sample (corn powder V463, INRAE) were analyzed
145 for each series of mineralization (Fehlauer et al., 2022).

146 Total Hg measurements were performed on aliquots of about 50 mg dry weight
147 (Supplementary Data Table 3) using atomic absorption spectrometry (AAS, Advanced Mercury
148 Analyzer-254, Altec) equipped with a low Hg optical cell. The method detection limit was 2 pg,
149 calculated as 3 times the standard deviation of the blank samples. The measured values were
150 always within ± 5% of the recommended values of the certified reference materials MESS-4
151 (marine sediment, National Council of Canada) and DORM-4 (fish protein, National Council of
152 Canada).

153 **2.3. Data analyses**

154 Principal Components Analysis (PCA) was performed to highlight the different trends in
155 sponge metal content between sites and species. Because the Shapiro-Wilk and the Bartlett
156 tests (performed in Rstudio) showed that the data do not fit the normality and
157 homoscedasticity criteria, the significance of metal variations was assessed using a non-
158 parametric Kruskal-Wallis analysis, followed by a Dunn post hoc test (p value < 0.05 considered
159 significant). All PCA and statistical analyses were performed using Rstudio open-source edition
160 package (<https://www.rstudio.com/>).

161 To calculate the bioconcentration factor (BCF), the metal concentrations found in sponge
162 tissues were compared to the metal concentrations found in sediments sampled at the same
163 sites (Pan et al., 2011).

164 3. Results

165

166 3.1. Difference in metal contents between Porifera species

167 3.1.1. Intraspecific variability.

168 Variability between different individuals of the same species is calculated from the relative
169 standard deviation (RSD) expressed in %. The highest mean variability is found for Cr (49.8%),
170 Pb (30.7%) and Ag (30.3%). *Oscarella uberulate* has the highest variability in Cr (RSD = 88.7%)
171 and Ag (RSD = 68.5%) while *Agelas oroides* has the highest RSD for Pb (60.4%) (Supplementary
172 Data Table 4). In contrast, the lowest variations are observed for As (mean RSD = 13.6%) and
173 Zn (mean RSD = 14.3%). The lowest variability for these two metals is found in *Corticium*
174 *candelabrum* with 1.4% for Zn and 3.3% for As.

175

176 3.1.2. Interspecific variability.

177 A principal component analysis (PCA) of metal concentrations in the seven marine sponge
178 species collected in the Maire site (for more information see Supplementary Data Table 5) was
179 performed (Figure 2). The first two axes of the PCA explain 60.2% of the total variability in the
180 data. Axis 1 of the PCA shows that the two demosponges, *Aplysina cavernicola* and *Agelas*
181 *oroides*, and the homoscleromorph *Corticium candelabrum*, display high concentrations of As
182 (211 µg/g, 200 µg/g, 387 µg/g respectively) and Hg (207 ng/g, 460 ng/g, 197 ng/g respectively)
183 comparatively to the other sponge species. *Axinella damicornis* has high concentrations of Cr
184 (5.92 µg/g), Cu (52.2 µg/g), and *Clathrina clathrus* (Calcarea) has the highest concentrations
185 of Ti (32.7 µg/g) and Li (2.67 µg/g). Moreover, Ni, Cd, Zn, representing the second axis, are

186 strongly concentrated in the homoscleromorph *Oscarella uberculata* (1564 $\mu\text{g/g}$, 48.5 $\mu\text{g/g}$,
187 4394 $\mu\text{g/g}$, respectively.

188 In contrast, *Oscarella lobularis*, is localized at the center of the PCA. These finding highlights
189 that in contrast to the other species sampled at the Maire site, *O. lobularis* displays a more
190 homogenous metal bioaccumulation profile than the other sponges studied here (Figure 2).

191 **Figure 2**

192 3.1.3. Bioconcentration factors in other marine sponges.

193 The bioconcentration factor (BCF) (Supplementary Data Table 6) determines the accumulation
194 capacity of organisms in their tissues (Pan et al., 2011).

195 The comparison of the bioconcentration factors between the seven sponges shows that the
196 bioaccumulated metals are often similar (e.g., Cu and As show $\text{BCF} > 1$ in all species; Ag and Cd
197 show $\text{BCF} > 1$ in all except one species, see Supplementary Data Table 6). In agreement with
198 PCA analysis (Figure 2), figure 3 shows that some metals are much more highly accumulated
199 in some species than in others (for example Ni and Cd in *Oscarella uberculata*).

200 **Figure 3**

201 **3.2. *O. lobularis*' metal concentrations in eight sites of Marseille**

202 3.2.1. Inter-site variations.

203 Metal concentrations measured in *Oscarella lobularis* at the different sites are presented in
204 figure 4. Al, Fe, Ni and Zn are the metals with the highest concentrations (Figure 4B) with
205 notable differences between sites. The Fourmigues individuals show the highest
206 concentrations of Zn (4920 $\mu\text{g/g dw}$), Al (834 $\mu\text{g/g dw}$), Ni (3277 $\mu\text{g/g dw}$) and Cd (120 $\mu\text{g/g}$
207 dw), 11 times higher for Zn than those sampled at Riou, in which the sampled specimens

208 present the lower Zn content. Sponges from the Prado reefs display the highest Fe body
209 content with 2460 µg/g (4 times higher than sponges from Fourmigues) and the Maire site
210 individuals show the highest As concentration (61.5 µg/g dw), 3 times higher than in the ones
211 from Fourmigues (Figure 4A). For the other metals, the variations between sites are slighter.

212 **Figure 4**

213 A PCA was performed to analyze inter-site variations. The first two axes of the PCA explain
214 60.7% of the total variability of the data (Figure 5). The PCA plot shows two distinct clusters:
215 an upper left one characterized by Cr, Fe, Cu, As, Hg, Pb and an upper right one influenced by
216 Li, Al, Ti, Ni, Zn, Ag, Cd. The first axis is mainly explained by the concentrations of Zn, Cd and
217 Ni in the tissues of *Oscarella lobularis*. In addition, body contents in Zn, Ni and Cd are
218 significantly higher in sponges from Fourmigues and in a lesser extent from Caramassaigne
219 and Frioul ($p < 0.05$) (Figure 5 and Supplementary Data Table 5). The second axis of the PCA is
220 mainly driven by the concentrations of Al, Ti, Fe, Hg and Pb. Sponges from the Prado reef site
221 are characterized by high concentrations of Fe, Hg and Pb (Figure 5). Concerning Fe, Prado's
222 reef and in a lesser extent Mejean populations display the significantly ($p < 0.05$) highest
223 concentrations (2460 ± 912 µg/g and 1740 ± 197 µg /g respectively) (Supplementary Data
224 Table 5). The Frioul site is at the center of the PCA plot, this means that individuals display
225 more homogeneous metal contents than those of the other sites. The population of *O.*
226 *lobularis* from the Perez cave, Caramassaigne and Riou sites, located at the bottom of the
227 biplot, show lower concentrations of metals than those of the other sites located in front of
228 the urban coast (Figure 5).

229 **Figure 5**

230 3.2.2. Bioconcentration factors in *Oscarella lobularis*.

231 As for other sponges, bioconcentration factor (BCF) for *Oscarella lobularis* was calculated
232 (Supplementary Data Table 6 for BCF).

233 BCFs are higher than 1 for Ag, As, Cd, Cu, Ni, Zn, and Hg, meaning that concentrations in
234 *Oscarella lobularis* are higher than those in the sediments whatever the sampling sites
235 (Supplementary Data Table 6). The highest BCF values are observed on 3 sites and for 3 metals
236 (Figure 6): Ni at Frioul, Caramassaigne and Fourmigues (BCF ranging from 522 to 850), Cd at
237 Caramassaigne (BCF = 584) and Zn at Fourmigues (BCF = 259). In contrast, Al, Cr, Fe, Li, Pb and
238 Ti, show bioconcentration factors lower than 1, meaning that the concentrations in *Oscarella*
239 *lobularis* tissues are lower than those measured in the surrounding sediment.

240 **Figure 6**

241 4. Discussion

242
243 Metals are naturally present in the environment in the form of geochemical backgrounds. The
244 geological formations of the Marseille basin are composed of many deposits of bauxite
245 (characterized by Al_2O_3 and iron oxides) formerly exploited, and many layers of *terra rossa*
246 (i.e., iron oxides). Even though these rocks naturally contain metals, because the concerned
247 area is characterized by the dominance of calcareous sedimentary rocks, the basicity of these
248 rocks makes metals hardly mobile and available (Ciesielski, 2007). Thereby natural origin of
249 metals in the sea is usually considered neglectable in this geographic area (Chandesris et al.,
250 2013; Tchounwou et al., 2012; Urien et al., 2017). Anthropic rock mining can nevertheless
251 increase ground leaching and bring natural metals to the sea by runoff, in addition to industrial
252 activities commonly considered to be responsible for most metal contaminations in the area.

253 The analysis of metals on the surface sediment in the bay of Marseille shows higher
254 concentrations for Ni, Cd, Cu, Zn (with a mean of 4.46 µg/g, 0.172 µg/g, 4.31 µg/g and 51.0
255 µg/g respectively) and lower concentrations for Cr and Pb (with a mean of 10.6 µg/g and 21.4
256 µg/g respectively) (Supplementary Data Table 5) compared to the sediments in the Gulf of
257 Lions (Roussiez et al., 2006). Moreover, in the Ligurian Sea, the concentrations are higher for
258 Cr, Cu, Li, Ni, Zn and are lower for Al, Fe, Hg compared to surface sediment in the bay
259 (Heimbürger et al., 2012). Concerning the bay of Toulon, the metal concentrations are much
260 higher than in the bay of Marseille, this is in agreement of a higher anthropic contamination
261 of this area (Tessier et al., 2011). However, the surface sediment represents the first
262 centimetres and thus supposedly the most recent particle deposits in the study area at a given
263 time (Martín et al., 2009; Tessier et al., 2011), making it a partial indicator of the quality of the
264 environment. In contrast, sponges, which are sessile organisms and active filter feeders,
265 represent a relevant proxy to monitor and evaluate metal contamination in addition to
266 sediment metal contents (Cebrian, 2007; Pan et al., 2011).

267 **4.1. Species-dependent and metal-dependent concentrations in sponge tissues.**

268 According to our results for a given site, metal concentrations are both sponge species-
269 dependent and metal-dependent. Among the different species sampled in our study,
270 *Oscarella lobularis* and *Agelas oroides* show the highest mean variabilities (26.8% and 33.1%
271 respectively), and concerning metals, As, Zn and Cu show the lowest mean variabilities (13.6%,
272 14.3% and 15.3% respectively) (Supplementary Data Table 4), like in the study by Orani *et al.*,
273 2018. Whatever the sponge species considered, Ag, As, Cd, Cu, Ni, Zn and Hg are present in
274 higher concentrations in sponges than in sediments (i.e., 2- to 37-fold higher than in sediment)
275 (Figure 3), as observed for other sponge species from the Mediterranean Sea (M. Araújo et al.,
276 2003; Cebrian, 2007; Orani, Barats, Vassileva, et al., 2018) or from the Pacific Ocean (Padovan

277 et al., 2012). This consensual finding could give an indication of the bioavailability in Ag, As,
278 Cd, Cu, Ni, Zn and Hg. High metal concentrations in tissues are generally considered to present
279 a risk for organisms health, but bioaccumulation of some metals within organism tissues can
280 sometimes be explained by their biological role. For example, Cu is involved in sponge
281 metabolism as well as various enzymatic processes (Tian et al., 2014) while As can play a
282 protective role against oxidative stress (Orani, Barats, Zitte, et al., 2018; Yamaoka et al., 2001).

283 Metal concentrations vary across species with well-defined signatures for each species (Figure
284 2; As and Hg for *C. candelabrum*; Cr, Ti, Li and Cu for *A. damicornis* and *C. clathrus*; Ni, Cd and
285 Zn for *O.tuberculata*). In contrast *Oscarella lobularis* displays a more homogeneous metal
286 accumulation pattern than the other sponge species studied. High inter-specific differences in
287 metal bioaccumulation were already observed in previous studies performed on different sets
288 of sponge species from different geographic areas (Batista et al., 2014; Genta-Jouve et al.,
289 2012; Patel et al., 1985). This finding may be partly explained by i) different filtration speed
290 and efficiency between sponge species (Cebrian, 2007; Morganti et al., 2019; Weisz et al.,
291 2008); ii) different molecular, cytological and histological features between species, as it had
292 been shown that different Demospongiae species accumulate metals in various structures like
293 collagen, skeleton fibers, pinacoderm (*i.e.*, external cell layer of sponge body) or even
294 mesohylar cells (*i.e.*, internal tissue of sponge) (Orani, Barats, Zitte, et al., 2018; Padovan et
295 al., 2012), by iii) different food preference (Maldonado, 2016; Perez et al., 2005), or by iv)
296 differences in associated microorganisms (Keren et al., 2017; Lee et al., 2009; Selvin et al.,
297 2009; Tian et al., 2014). In our case, the huge differences observed between *O.lobularis* and
298 *O.tuberculata* BCFs (Supplementary Data Table 6) cannot be explained neither by molecular,
299 cytological nor histological differences. Indeed, the high similarity between these sister
300 species has for long resulted in the absence of species discrimination (Boury-Esnault et al.,

1992; Gazave et al., 2013). Such differences between species of a same genus has also been observed in the genus *Mytilus* (Sussarellu et al., 2022). The microbial communities of *Oscarella lobularis* display a species-specific microbial diversity dominated by a Rhodobacteria (Gerçe et al., 2011; Gloeckner et al., 2013; Jourda et al., 2015) but further studies would be needed to decipher the relative contribution to metal accumulation of the sponge and its symbiotic microorganisms.

4.2. Intersite differences in *Oscarella lobularis* and potential links with the industrial past and present anthropic activities.

Although *Oscarella lobularis* accumulates metals more homogeneously than other sponge species, we note a variability of metal contents in tissues between the different sampling sites (Figure 5). These differences may be related to the past and present industrial activities in the bay of Marseille. The industrialization of Marseille increased in the middle of the 19th century with the creation of factories of several types (glass, petroleum, soda, tartaric acid, etc.). No obvious direct link has been made between metal contaminations in the bay and the factories, except concerning the industrial sites of Montredon (1873-1884), Escalette (1851-1924) and Goudes (1854-1877). Located in the east of Marseille, these sites were specialized in Ag and Pb production (Daumalin et al., 2016) (Figure 7). The metallic contamination in terrestrial and water compartments in the area of these former industries, located in front of the Maire site, was previously characterized (Lasalle, 2007), and high concentrations of Pb and As in soils (633 µg/g and 29.4 µg/g respectively), in marine sediments (99 to 1189 µg/g and 8.2 to 648 µg/g) and in marine organisms were highlighted. For example, in mussels, Pb and As are respectively up to 4 and 2 times higher in comparison with mussels from other sites of the Mediterranean sea (Andral et al., 2004; Santos-Echeandía et al., 2021), with highest Pb and As body amounts ranging from 4.5 to 5.3 µg/g dw and from 25.4 to 26.2 µg/g dw, respectively. Interestingly, *O.*

325 *lobularis* specimens sampled in the Maïre site, located in the vicinity of this former industrial
326 site, show the highest concentrations of Pb ($8.76 \pm 0.87 \mu\text{g/g dw}$) and As ($61.5 \pm 10.3 \mu\text{g/g dw}$)
327 measured among the different studied *O. lobularis* populations. Our results are therefore in
328 agreement with previous studies reporting that this area of the Bay of Marseille is still exposed
329 to a significant metallic contamination despite that these factories have been closed since a
330 century (Daumalin et al., 2016; Lasalle, 2007).

331 Marseille is the most important trade and cruise ship port of the France south littoral and has
332 the largest underground wastewater treatment plant in Europe in Calanques National Park
333 located at 5 km from the city. The rejected treated water, not free of metals, discharges Ag,
334 Cu, Pb, Cr, Hg and Fe at the Cortiou Cove during dry conditions (Millet et al., 2018; Oursel,
335 2013; Tumolo et al., 2020) and into the autonomous harbour ("*Port autonome*") of Marseille,
336 the old harbour ("*vieux port*") and at the level of the Prado site, without prior passage through
337 the treatment plant During rainy conditions. Finally, the small coastal rivers such as the
338 Huveaune, the Jarret and the Aygalades that run through the town, as well as irregular big
339 intrusion of the Rhône River (Frayssé et al., 2013), were already shown to collect and transport
340 contaminants toward the bay (i.e., Hg up to 62 ng/L in the Huveaune and Aygalades) (Jany &
341 Zebracki, 2012) (Figure 7). The hydrodynamics of the Gulf of Lion, with the southeast marine
342 winds and the North Current, could explain the spread of contaminants and their localization
343 on certain sites in the Bay of Marseille. In accordance with previous studies on sponges of the
344 Marseille region (Perez et al., 2005), our results indicate for example that the populations
345 present in the bay (i.e., Prado) have significant quantities of Fe. These large quantities of Fe
346 could be due to the use of ferric chloride as a coagulation agent for the physico-chemical
347 treatment of wastewater at the Cortiou plant and could be transported to the Prado reefs
348 with the North Current toward the bay (Figure 7). Fe isotope studies would confirm or refute

349 this hypothesis by specifically determining its source (natural or anthropogenic), as performed
350 in previous studies for other metals in the Mediterranean Sea (D. F. Araújo et al., 2019; Orani,
351 Barats, Vassileva, et al., 2018). Specimens sampled at the sites of Riou, Caramassaigne and
352 Perez Cave are all present in the bottom of the PCA, below the second axis, reflecting lower
353 metallic bioaccumulations in comparison to the other populations, that may be due to the
354 hydrodynamic characteristics of these sites (Figure 7) and their respective distance from the
355 urbanized area.

356 **Figure 7**

357 Sponges sampled in the Fourmigues site, located at the east of the Giens peninsula, are all
358 grouped in the right side of the PCA and are characterized by high concentrations of Zn, Ni
359 and Cd (Figure 5). The Toulon Bay, located at about 10 km from the Fourmigues sampling site,
360 is characterized by important industrial, touristic, and military nautical activities, and is
361 submitted to significant inputs of metals. In a previous study, high Zn concentrations with a
362 range of 24.3 – 1340 $\mu\text{g/g dw}$ were found in sediments in the large bay of Toulon (Tessier et
363 al., 2011). High Zn content in sediments could be due to the intensive use of antifouling paints
364 on boat hulls to prevent corrosion and fouling, which would be consistent with the intensive
365 nautical activity of this area (Jones & Turner, 2010). In addition, the bay of Toulon was also
366 reported to present high Cd concentration in seawater (between 0.007 and 0.14 $\mu\text{g/L}$) (Rossi
367 & Jamet, 2008) and, in the port of Porquerolles Island (located in the vicinity of Toulon), Zn,
368 Ni and Cd contamination in water, sediment and marine organisms were linked to the
369 maritime activities of the port (Augier, 1980). Therefore, our data at the Fourmigues site
370 suggest that at least part of the contamination from the bay of Toulon and from Porquerolles
371 Island may extend to the surrounding coastal waters. In particular, the North Current may be

372 responsible for the high concentrations of Ni (210-320 ng/L) previously measured in the
373 Ligurian sea located at the East of the Fourmigues site as suggested by (Migon et al., 2020).

374

375 **4.3. *Oscarella lobularis* as a valuable bioindicator for metallic contamination**

376 Finally, the inter-site variability does not question the relevance of *Oscarella lobularis* as
377 valuable biomonitor: its homogeneous metal bioaccumulation profile makes it a good
378 candidate as a bioindicator of metallic pollution in Mediterranean Sea. The choice of sentinel
379 or bioindicator species should be guided by several criteria: i) preferring sessile species or
380 species displaying limited dispersion (Azizi et al., 2018; Vieira et al., 2021), ii) opting for filter
381 feeders (Batista et al., 2014; Milanese et al., 2003), iii) identifying, capturing and collecting
382 easily a species with a sufficient population size to study spatial variations of ecosystems
383 (Berthet, 2013). *Oscarella lobularis* fits all these criteria. Even if, several sponges have been
384 proposed as bioindicator species (mostly Demospongiae but also one species of Calcarea
385 *Paraleucilla magna*) (Batista et al., 2014), as far as we know, only one species (*Haliclona fulva*
386 (Topsent, 1893)) has been showed as a generalist species in the Mediterranean Sea (Orani,
387 Barats, Vassileva, et al., 2018), which appears to be a crucial criterion for choosing a sentinel
388 organism.

389 **Conclusions**

390

391 This study is the first one i) reporting such a dataset concerning the bioaccumulation of metals
392 in the bay of Marseille using a homoscleromorph sponge; ii) comparing different
393 bioaccumulation profiles between sponge species at the Maire site in order to objectively
394 compare the relative relevance of sponge species for environmental assessment.

395 According to its numerous features fitting the ideal characteristics required for the use of a
396 relevant model to biomonitor the metallic contamination in marine coastal waters, our work
397 suggests *Oscarella lobularis* as a new bioindicator of contaminants in Mediterranean coastal
398 environments. Consequently, *O.lobularis* and *Haliclona fulva* may be useful to implement a
399 “Sponge Watch Program” for the western part of the Mediterranean Sea, as it was proposed
400 in Denmark with the demosponge *Halichondria panicea* (Hansen et al., 1995; Olesen & Weeks,
401 1994).

402 The present results are also expected to be useful for future ecotoxicological studies. Indeed,
403 *Oscarella lobularis* is now one of the best described Mediterranean sponge species at
404 chemical, physiological, morphological, reproductive, cellular and molecular levels (Belahbib
405 et al., 2018; Ereskovsky et al., 2015; Fierro-Constain et al., 2017; Renard et al., 2021; Rocher
406 et al., 2020; Vernale et al., 2021), thereby enabling integrative approaches.

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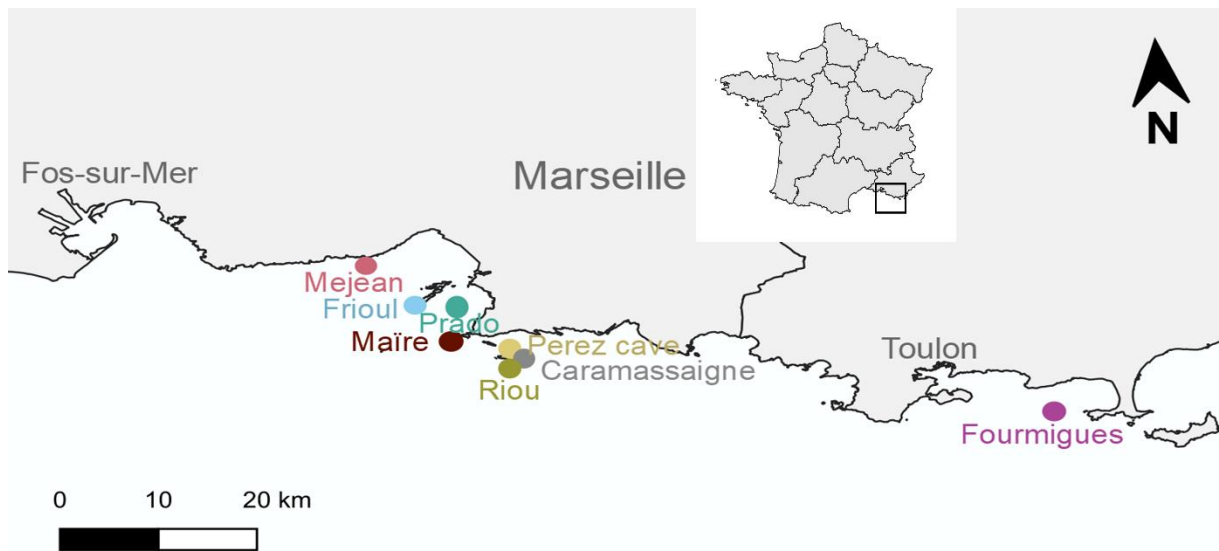
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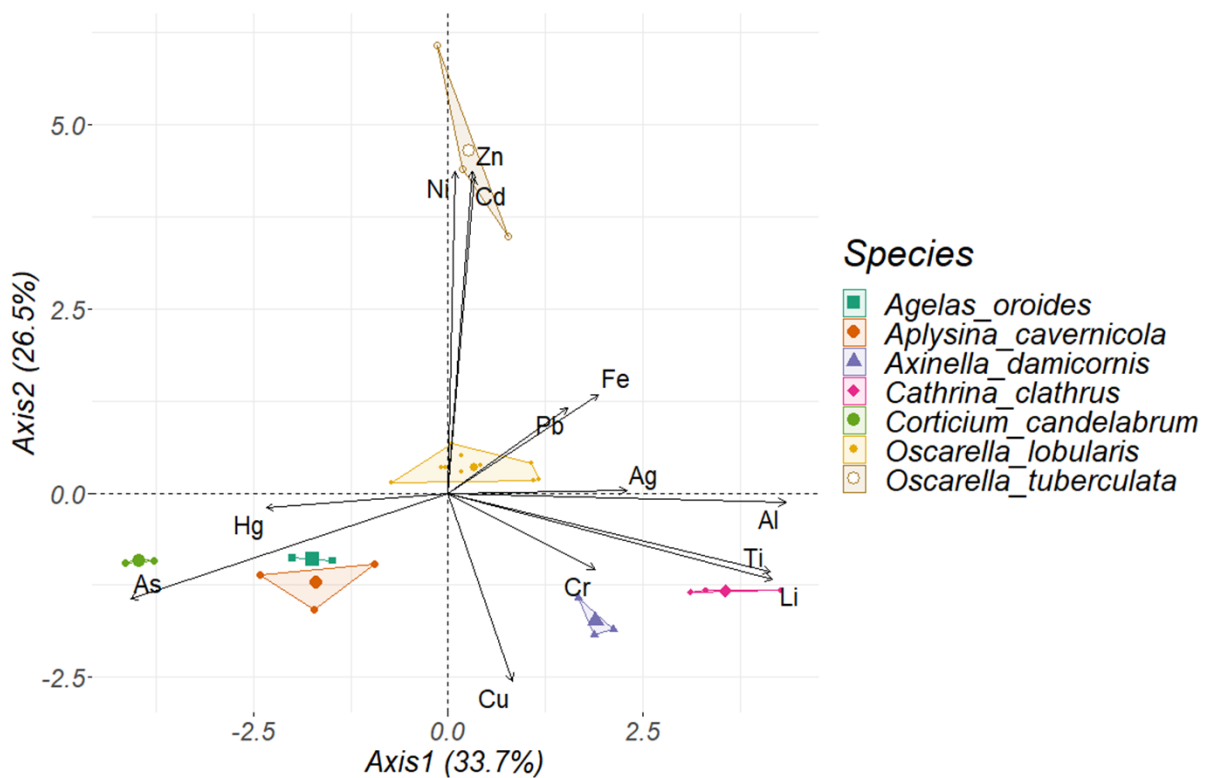
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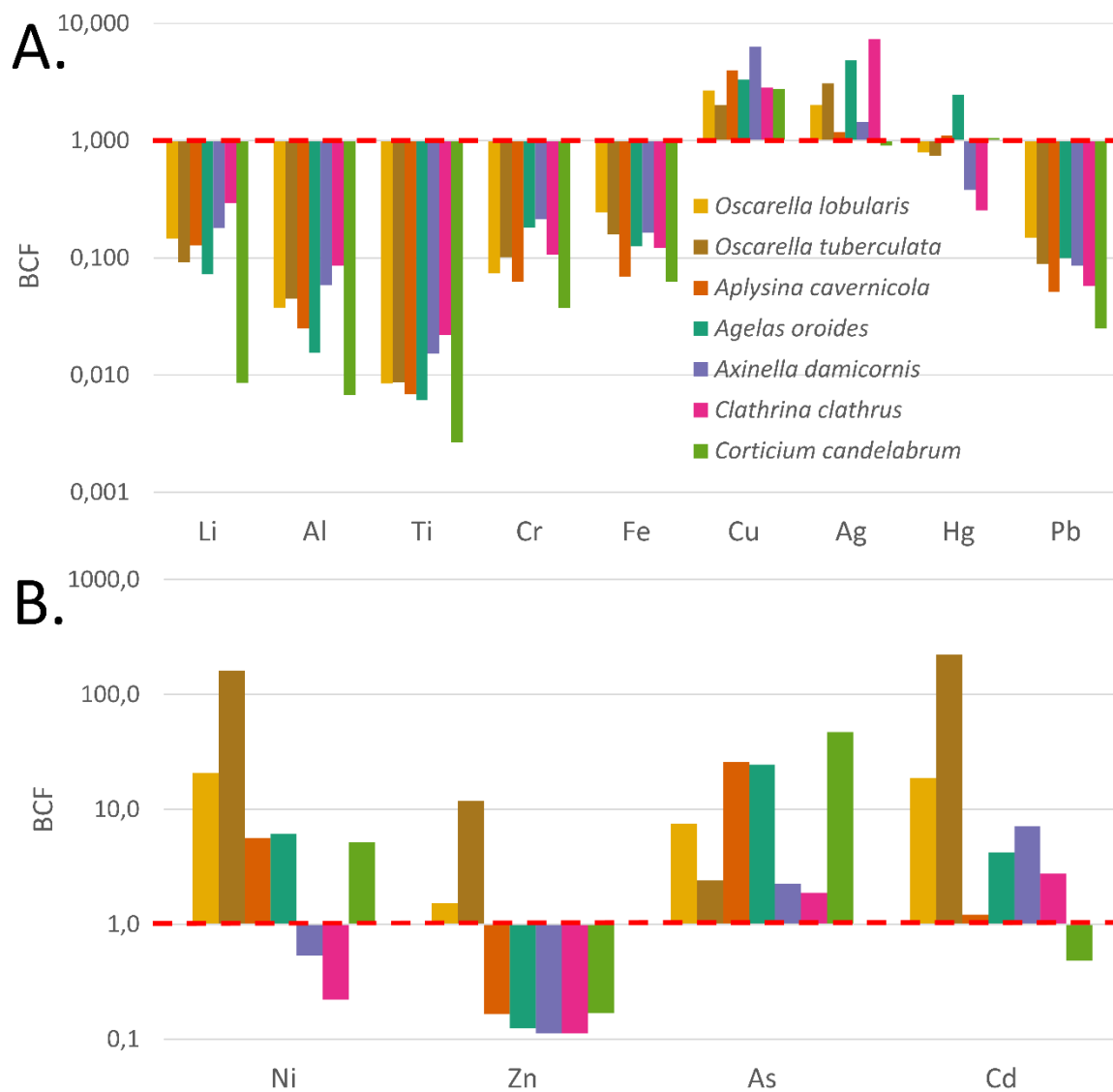
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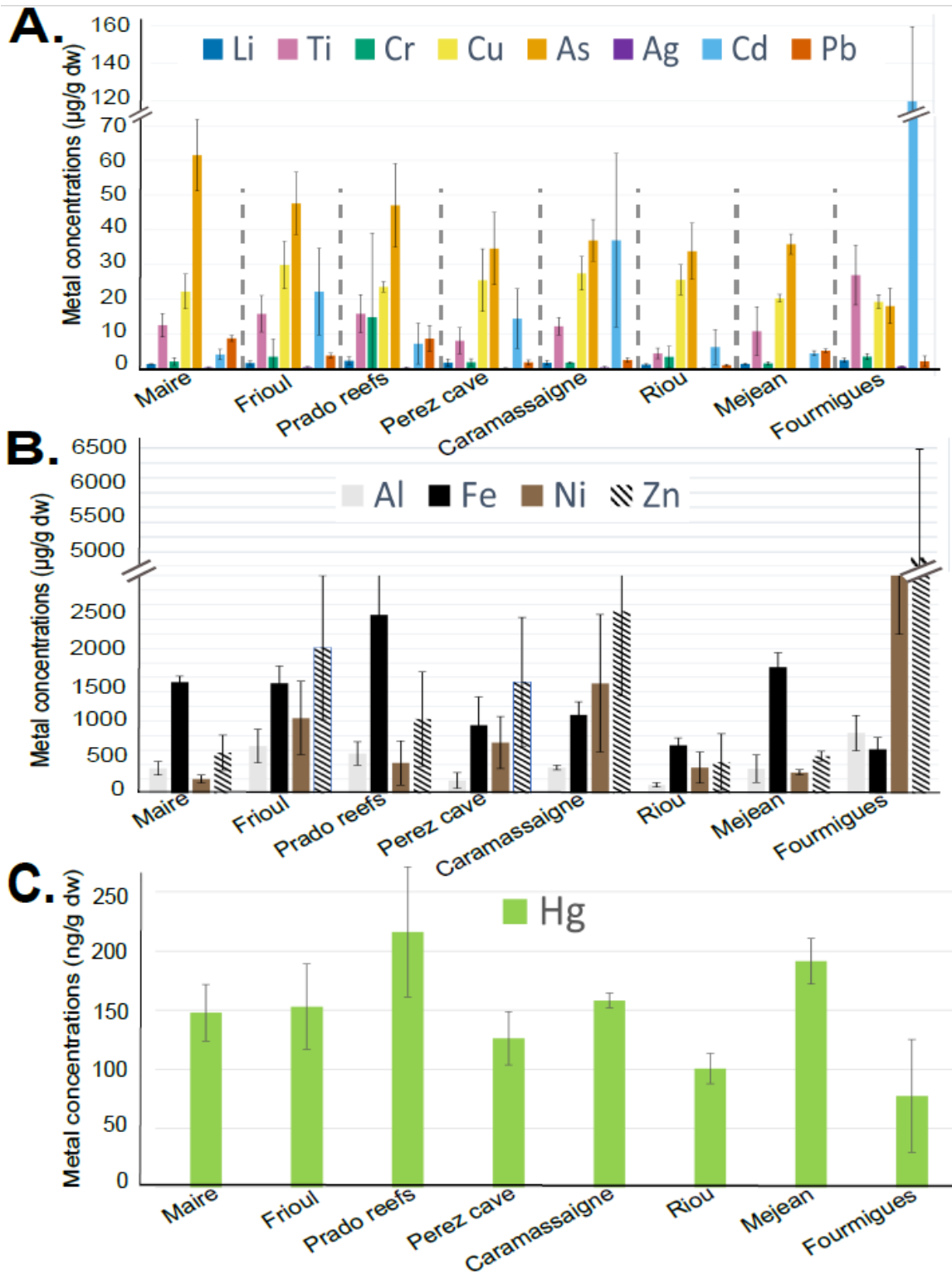
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 788 Figure 1: Location of the eight sampling sites (south of France; North-Western Mediterranean Sea).



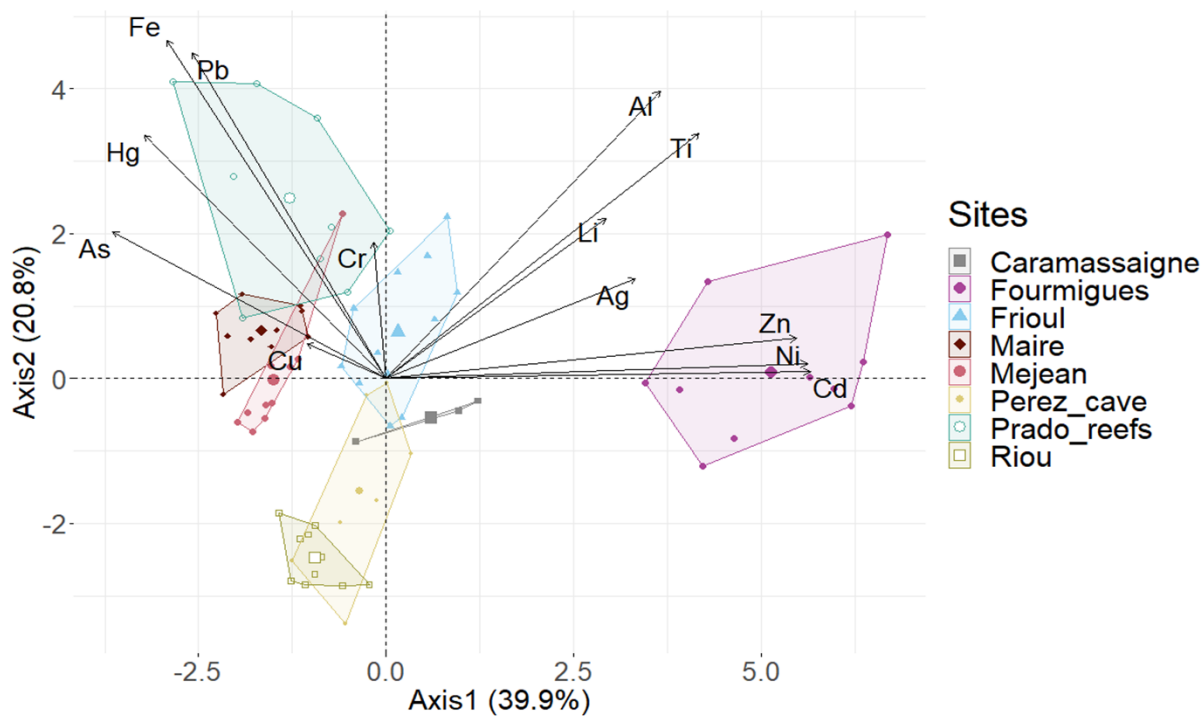
789
 790 Figure 2: Principal component analysis (PCA) of metal concentrations in seven distinct marine sponge
 791 species collected in the Maire site. Convex ellipses group heavy metal concentrations from the seven
 792 species.



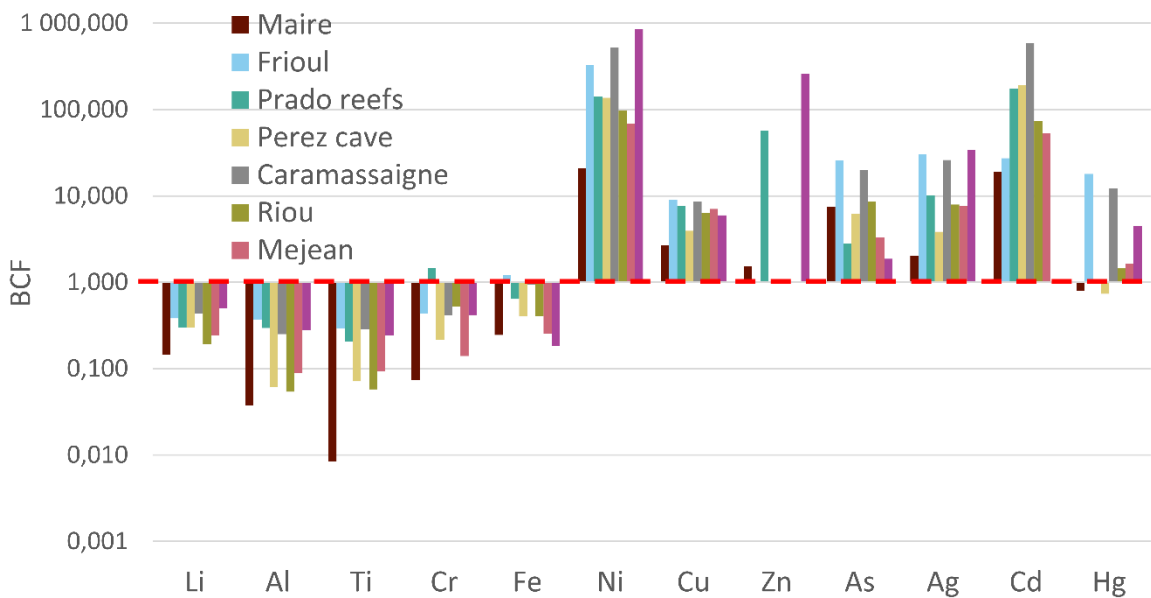
793
 794 Figure 3: Bioconcentration factors calculated for all metals in seven marine sponges. BCFs of (A) Li, Al,
 795 Ti, Cr, Fe, Cu, Ag, Hg, Pb and (B) Ni, Zn, As, Cd. Values are available in table A.6.



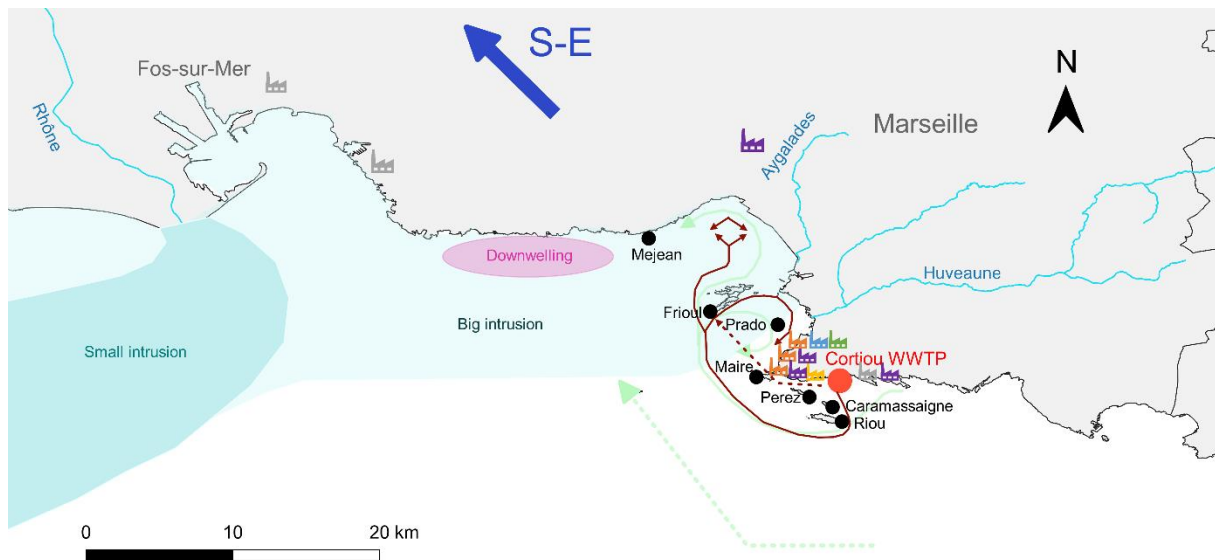
796
 797 Figure 4: Metals concentrations in *Oscarella lobularis* according to the various sampling sites. Values
 798 are means \pm SD. Concentrations of (A) Ag, As, Cd, Cr, Cu, Li, Pb, Ti, (B) Al, Fe, Ni, Zn and (C) Hg were
 799 measured in tissues.



800
 801 Figure 5: Principal component analysis (PCA) plot describing inter-site variation of metal contents
 802 present in *Oscarella lobularis* tissues.



803
 804 Figure 6: Bioconcentration factors (BCF) calculated for all metals in *Oscarella lobularis* individuals
 805 sampled at different sites (using metal concentrations measured in sediments as reference). Values
 806 are available in table A.6.



807
 808 Figure 7: Hydrodynamics in the bay of Marseille in presence of south-east winds. Including the North
 809 Current intrusion (dotted green arrow for the surface current and green arrow for the subsurface), the
 810 downwelling formation and the intrusions of the Rhône River. Red arrows indicate the subsurface
 811 particles transported from the WWTP area (10-30 m depth) and red point indicates the WWTP outlet.
 812 The factory patterns represent the former lead factories established in the bay of Marseille in the 19th
 813 century. Figure adapted (Arnaud et al., 2021; Fraysse et al., 2014; Millet et al., 2018) with lead and
 814 silver factories in orange; glass factories in blue; oil factories in grey; tartaric acid factories in green;
 815 sulfur factories in yellow; and soda factories in purple.

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Appendix A. Supplementary material

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833 **Supplemental Table 1.** Number of sponge adult individuals collected at different sites and depths.

<i>Sites</i>	<i>GPS coordinates</i>	<i>Depth (m)</i>	<i>Number of individuals</i>	<i>Date of sampling</i>
Maire Island	43°12.760'N, 5°19.800'E	12	12	02/2019 (Oscarellidae) ; 01/2021 (other sponges phyla)
Prado's Reef	43°15.745'N, 5°20.360'E	27	10	04/2019 ; 01/2020
Frioul	43°15.703'N, 5°17.273'E	8	13	04/2019 ; 02/2020
Riou	43° 10.362'N, 5° 23.432'E	20	10	07/2020
Perez Cave	43°11.190'N, 5°23.470'E	13	8	01/2020 ; 05/2020
Caramassaigne	43°10.592'N, 5°23.621'E	13.5	3	01/2020 ; 02/2021
Mejean	43° 19.688'N, 5° 13.338'E	25-30	10	07/2020
Fourmigues	43°02.3630'N, 06°04.1796'E	30	14	01/2021

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835 **Supplemental Table 2.** Limit of quantification (LOQ) in µg/L for trace metal elements measured by
836 ICP-MS and in ng for mercury measured with AMA-254.

LOQ												
Li	Al	Ti	Cr	Fe	Ni	Cu	Zn	As	Ag	Cd	Hg	Pb
0,003 µg/L	0,15 µg/L	0,05 µg/L	0,009 µg/L	0,3 µg/L	0,006 µg/L	0,014 µg/L	0,05 µg/L	0,037 µg/L	0,002 µg/L	0,017 µg/L	0,003 ng	0,005 µg/L

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843 **Supplemental Table 3.** Samples measured with AMA-254 and ICP-MS to determine the metal
 844 concentration at the various sites. Each sample was replicated before measurement.

Type of sample	Site	ICP-MS		AMA-254	
		No. Individuals	No. technical replicates	No. individuals	No. technical replicates
<i>Oscarella lobularis</i>	Maire	11	3	12	1-5
	Frioul	12	3	13	1-2
	Prado's Reef	9	3	10	2
	Perez Cave	7	3	8	2
	Caramassaigne	3	3	3	1-2
	Riou	10	3	10	2
	Mejean	10	3	10	2
	Fourmigues	10	3	10	1-2
<i>Oscarella tuberculata</i>	Maire	3	3	3	1
<i>Aplysina cavernicola</i>	Maire	3	3	3	1
<i>Agelas oroides</i>	Maire	2	3	2	1
<i>Axinella damicornis</i>	Maire	3	3	3	1
<i>Cathrina clathrus</i>	Maire	3	3	3	1
<i>Corticium candelabrum</i>	Maire	3	3	3	1
Sediment	Maire		3		1
	Frioul		3		3
	Prado's Reef		3		2
	Perez Cave		3		3
	Caramassaigne		3		3
	Riou		3		2
	Mejean		3		2
	Fourmigues		3		3

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847 **Supplemental Table 4.** Relative standard deviation (RSD) in seven sponges' species collected at the
 848 Maire site. Values for each heavy metal are expressed in %.

Sponge species (Maire site)	RSD (%)												
	Li	Al	Ti	Cr	Fe	Ni	Cu	Zn	As	Ag	Cd	Hg	Pb
<i>O.lobularis</i>	8,9	26,8	27,2	54,8	5,4	26,1	22,5	42,7	16,8	68,5	36,5	16,1	9,9
<i>O.tuberculata</i>	8,5	10,7	27,8	88,7	12,3	35,0	14,1	20,9	9,5	40,1	13,0	10,6	18,1
<i>A.cavernicola</i>	21,4	39,9	36,8	58,4	19,6	12,8	28,8	9,9	10,3	8,7	8,8	4,5	47,0
<i>A.oroides</i>	11,0	7,1	24,4	61,6	41,3	24,4	13,6	18,6	15,0	46,3	15,8	76,3	60,4
<i>A.damicornis</i>	9,4	4,7	6,1	36,1	8,0	44,3	14,9	3,0	5,5	4,0	6,8	7,1	27,6
<i>C.clathrus</i>	3,1	16,6	11,7	11,3	11,0	16,4	12,1	3,6	34,9	32,2	19,7	5,8	27,3
<i>C.candelabrum</i>	62,2	30,1	23,6	37,4	14,8	11,9	1,4	1,4	3,3	12,0	19,0	9,5	24,8

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854 **Supplemental Table 5.** Concentration of metals ($\mu\text{g/g}$ of dry weight) in seven sponges' species and on
 855 surface sediment collected in the different sites. Values are means \pm SD. Different letters indicate a
 856 significant difference between sites for *O. lobularis* samples (Kruskall-Wallis test followed by a Dunn
 857 post hoc test; $p < 0,05$)

Samples	Sites	Li	Al	Ti	Cr	Fe	Ni	Cu	Zn	As	Ag	Cd	Hg	Pb
<i>Oscarella lobularis</i>	Maire	1,32 \pm 0,12 ^(ab)	349 \pm 93 ^(ab)	12,5 \pm 3,4 ^(abc)	2,04 \pm 1,12 ^(a)	1536 \pm 83 ^(abc)	201 \pm 53 ^(a)	22,2 \pm 5 ^(ab)	564 \pm 241 ^(ab)	61,5 \pm 10,3 ^(a)	0,36 \pm 0,2 ^(abc)	4,1 \pm 1,5 ^(a)	0,148 \pm 0,024 ^(a)	8,76 \pm 0,87 ^(a)
<i>Oscarella lobularis</i>	Frioul	1,62 \pm 0,69 ^(abc)	655 \pm 232 ^(cd)	15,8 \pm 5,2 ^(a)	3,46 \pm 5,09 ^(abc)	1518 \pm 237 ^(abc)	1040 \pm 505 ^(bcd)	29,9 \pm 6,8 ^(c)	2011 \pm 987 ^(cd)	47,6 \pm 9 ^(abc)	0,41 \pm 0,3 ^(bc)	22,2 \pm 12,5 ^(bc)	0,153 \pm 0,036 ^(a)	3,79 \pm 0,73 ^(bc)
<i>Oscarella lobularis</i>	Prado reefs	2,3 \pm 1,14 ^(cd)	552 \pm 162 ^(bcd)	15,8 \pm 5,5 ^(abc)	14,8 \pm 24,3 ^(c)	2460 \pm 912 ^(c)	422 \pm 305 ^(abcd)	23,6 \pm 1,5 ^(bc)	1026 \pm 650 ^(bcd)	47,1 \pm 12 ^(abcd)	0,28 \pm 0,2 ^(b)	7,23 \pm 5,9 ^(abcd)	0,216 \pm 0,055 ^(b)	8,69 \pm 3,75 ^(a)
<i>Oscarella lobularis</i>	Perez cave	1,76 \pm 1,09 ^(abc)	182 \pm 108 ^(bc)	8,05 \pm 3,76 ^(cd)	1,87 \pm 1,02 ^(b)	943 \pm 389 ^(a)	703 \pm 358 ^(abcd)	25,5 \pm 9 ^(abc)	1537 \pm 888 ^(cd)	34,6 \pm 10,4 ^(cd)	0,21 \pm 0,1 ^(b)	14,4 \pm 8,6 ^(abcd)	0,126 \pm 0,023 ^(abc)	1,82 \pm 0,71 ^(bcd)
<i>Oscarella lobularis</i>	Caramassaigne	1,73 \pm 0,7 ^(abcd)	359 \pm 33 ^(abcd)	12,2 \pm 2,5 ^(abcd)	1,75 \pm 0,29 ^(abc)	1085 \pm 179 ^(abc)	1518 \pm 946 ^(abcd)	27,5 \pm 4,9 ^(abc)	2508 \pm 1165 ^(cd)	37 \pm 6 ^(abcd)	0,38 \pm 0,3 ^(abcd)	37 \pm 25 ^(abcd)	0,158 \pm 0,006 ^(abc)	2,51 \pm 0,67 ^(bcd)
<i>Oscarella lobularis</i>	Riou	1,18 \pm 0,26 ^(b)	121 \pm 30 ^(c)	4,4 \pm 1,53 ^(c)	3,41 \pm 3,06 ^(a)	666 \pm 101 ^(a)	358 \pm 214 ^(abc)	25,6 \pm 4,4 ^(abc)	433 \pm 389 ^(b)	33,8 \pm 8,1 ^(c)	0,18 \pm 0,04 ^(b)	6,22 \pm 5 ^(abc)	0,101 \pm 0,013 ^(c)	1,02 \pm 0,28 ^(c)
<i>Oscarella lobularis</i>	Mejean	1,38 \pm 0,3 ^(ab)	344 \pm 189 ^(abc)	10,8 \pm 7,1 ^(bc)	1,54 \pm 0,47 ^(a)	1740 \pm 197 ^(bc)	294 \pm 37 ^(abc)	20,3 \pm 1,1 ^(b)	516 \pm 68 ^(b)	35,9 \pm 2,9 ^(c)	0,1 \pm 0,01 ^(c)	4,44 \pm 0,74 ^(abc)	0,191 \pm 0,019 ^(b)	5,21 \pm 0,51 ^(abc)
<i>Oscarella lobularis</i>	Fourmigues	2,45 \pm 0,6 ^(c)	834 \pm 243 ^(c)	27 \pm 8,6 ^(c)	3,48 \pm 0,92 ^(bc)	610 \pm 160 ^(c)	3277 \pm 1084 ^(c)	19,3 \pm 2 ^(b)	4920 \pm 1467 ^(c)	18,1 \pm 5,1 ^(c)	0,68 \pm 0,1 ^(c)	120 \pm 39 ^(c)	0,0778 \pm 0,0476 ^(c)	2,14 \pm 1,55 ^(bcd)
<i>Oscarella tuberculata</i>	Maire	0,833 \pm 0,071	418 \pm 45	12,8 \pm 3,6	2,8 \pm 2,48	996 \pm 122	1564 \pm 548	16,7 \pm 2,4	4394 \pm 918	19,9 \pm 1,9	0,546 \pm 0,219	48,5 \pm 6,3	0,138 \pm 0,015	5,23 \pm 0,95
<i>Aplysina cavernicola</i>	Maire	1,16 \pm 0,25	231 \pm 92	10,1 \pm 3,7	1,74 \pm 1,01	433 \pm 85	54,4 \pm 6,9	32,8 \pm 9,4	61,3 \pm 6,1	211 \pm 22	0,209 \pm 0,018	0,264 \pm 0,023	0,207 \pm 0,009	3 \pm 1,41
<i>Agelas oroides</i>	Maire	0,657 \pm 0,073	143 \pm 10	9,05 \pm 2,21	5,01 \pm 3,09	786 \pm 325	59,5 \pm 14,5	27,6 \pm 3,8	46,5 \pm 8,6	200 \pm 30	0,86 \pm 0,398	0,921 \pm 0,146	0,46 \pm 0,351	5,86 \pm 3,54
<i>Axinella damicornis</i>	Maire	1,64 \pm 0,15	541 \pm 25	22,6 \pm 1,4	5,92 \pm 2,14	1032 \pm 82	5,2 \pm 2,31	52,2 \pm 7,8	41,7 \pm 1,2	18,5 \pm 1	0,257 \pm 0,01	1,55 \pm 0,11	0,0707 \pm 0,005	5,05 \pm 1,39
<i>Cathrina clathrus</i>	Maire	2,67 \pm 0,08	795 \pm 132	32,7 \pm 3,8	2,96 \pm 0,33	759 \pm 84	2,13 \pm 0,35	23,4 \pm 2,8	42 \pm 1,5	15,5 \pm 5,4	1,31 \pm 0,4	0,602 \pm 0,118	0,0473 \pm 0,0028	3,39 \pm 0,93
<i>Corticium candelabrum</i>	Maire	0,0783 \pm 0,0485	63,2 \pm 19	3,93 \pm 0,93	1,04 \pm 0,39	393 \pm 58	50,3 \pm 6	22,9 \pm 0,3	62,4 \pm 0,9	387 \pm 13	0,162 \pm 0,019	0,105 \pm 0,02	0,197 \pm 0,019	1,47 \pm 0,36
Sediment	Maire	9,10 \pm 4,6%	9275 \pm 2%	1480 \pm 2%	27,7 \pm 3,5%	6262 \pm 2,6%	9,67 \pm 3%	8,29 \pm 9,6%	371 \pm 8,3%	8,19 \pm 26%	0,178 \pm 3,8%	0,217 \pm 146,8%	0,18601539	58,8 \pm 1,3%
Sediment	Frioul	4,2 \pm 2,1%	1762 \pm 5,6%	54,1 \pm 5,1%	7,99 \pm 0,9%	1250 \pm 2,1%	3,18 \pm 3%	3,33 \pm 1%	0	1,86 \pm 11%	0,0135 \pm 7,8%	0,815 \pm 6,3%	0,0085 \pm 11%	19,1 \pm 1,5%
Sediment	Prado reefs	7,67 \pm 3%	1872 \pm 5,7%	76,7 \pm 2%	10,1 \pm 3,4%	3838 \pm 6,1%	3 \pm 3,8%	3,07 \pm 6%	18,1 \pm 3,5%	16,8 \pm 1,5%	0,0278 \pm 7,7%	0,0410 \pm 7,1%	0,227 \pm 10,59%	20,8 \pm 5,8%
Sediment	Perez cave	5,92 \pm 2,2%	2959 \pm 3,2%	113 \pm 19,2%	8,63 \pm 0,7%	2347 \pm 2,8%	5,15 \pm 0,3%	6,47 \pm 2,8%	0	5,58 \pm 5,9%	0,0546 \pm 3,9%	0,0746 \pm 11,7%	0,172 \pm 14%	20,6 \pm 0,8%
Sediment	Caramassaigne	3,97 \pm 0%	1431 \pm 8%	42,8 \pm 2,4%	4,20 \pm 4,3%	1156 \pm 1%	2,91 \pm 0,8%	3,18 \pm 3%	0	1,86 \pm 3,7%	0,0146 \pm 1,3%	0,0633 \pm 17,2%	0,013 \pm 18%	10,6 \pm 1,1%
Sediment	Riou	6,21 \pm 2%	2241 \pm 8%	76,6 \pm 17%	6,55 \pm 1,9%	1640 \pm 1,3%	3,66 \pm 0,3%	4,02 \pm 4%	0	3,96 \pm 4,3%	0,0227 \pm 2,6%	0,0852 \pm 40%	0,0697 \pm 3%	12,3 \pm 0,9%
Sediment	Mejean	5,66 \pm 1,4%	3871 \pm 3%	115 \pm 4,3%	11,0 \pm 1,4%	6866 \pm 1,1%	4,30 \pm 2,3%	2,88 \pm 4,7%	0	10,8 \pm 4,1%	0,0131 \pm 8,9%	0,0834 \pm 0,9%	0,116 \pm 21%	18 \pm 2,3%
Sediment	Fourmigues	9,10 \pm 13%	3008 \pm 3,2%	111 \pm 1,3%	8,32 \pm 1,7%	3336 \pm 1,4%	3,86 \pm 11,1%	3,23 \pm 3,6%	19 \pm 4,5%	9,67 \pm 1,5%	0,02 \pm 2%	0	0,0172 \pm 19,31%	11,3 \pm 4,3%

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860 **Supplemental Table 6.** Average bioconcentration factors (BCF) calculated for seven sponge species.
 861 Values were obtained for each metal in *Oscarella lobularis* collected at eight sites and in six other
 862 sponge species collected at the Maire site.

Samples	Sites	BCF													
		Li	Al	Ti	Cr	Fe	Ni	Cu	Zn	As	Ag	Cd	Hg	Pb	
<i>O.lobularis</i>	Maire	0,1	0,0	0,0	0,1	0,2	20,8	2,7	1,5	7,5	2,0	18,9	0,8	0,1	
	Frioul	0,4	0,4	0,3	0,4	1,2	326,7	9,0		25,7	30,3	27,3	18,0	0,2	
	Prado reefs	0,3	0,3	0,2	1,5	0,6	140,7	7,7	56,7	2,8	10,1	176,3	1,0	0,4	
	Perez cave	0,3	0,1	0,1	0,2	0,4	136,6	3,9		6,2	3,8	193,3	0,7	0,1	
	Caramassaigne	0,4	0,3	0,3	0,4	0,9	522,0	8,6		19,9	26,0	584,1	12,1	0,2	
	Riou	0,2	0,1	0,1	0,5	0,4	98,0	6,4		8,5	7,9	73,0	1,4	0,1	
	Mejean	0,2	0,1	0,1	0,1	0,3	68,3	7,1		3,3	7,6	53,3	1,7	0,3	
	Fourmigues	0,5	0,3	0,2	0,4	0,2	849,9	6,0	259,3	1,9	34,0		4,5	0,2	
<i>O.tuberculata</i>	Maire	0,1	0,0	0,0	0,1	0,2	161,8	2,0	11,9	2,4	3,1	223,4	0,7	0,1	
<i>A.cavernicola</i>	Maire	0,1	0,0	0,0	0,1	0,1	5,6	4,0	0,2	25,8	1,2	1,2	1,1	0,1	
<i>A.oroides</i>	Maire	0,1	0,0	0,0	0,2	0,1	6,2	3,3	0,1	24,5	4,8	4,2	2,5	0,1	
<i>A.damicornis</i>	Maire	0,2	0,1	0,0	0,2	0,2	0,5	6,3	0,1	2,3	1,4	7,2	0,4	0,1	
<i>C.clathrus</i>	Maire	0,3	0,1	0,0	0,1	0,1	0,2	2,8	0,1	1,9	7,4	2,8	0,3	0,1	
<i>C.candelabrum</i>	Maire		0,0	47,2	0,0	0,1	5,2	2,8	0,0		0,9	0,5	0,2	1,1	

ACCEPTED VERSION