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1 **Assessment of trace element contamination and effects on**
2 ***Paracentrotus lividus* using several approaches: pollution indices,**
3 **accumulation factors and biochemical tools**

4

5 El Idrissi O.^{a,b,c*}, Ternengo S.^{a,b}, Monnier B.^a, Lepoint G.^c, Aiello A.^{a,b}, Bastien R.^b,
6 Lourkisti, R^a., Bonnin M.^a, Santini J.^a, Pasqualini V. ^{a,b}, Gobert S.^{c,d}

7

8 a Université de Corse Pasquale Paoli, UMR CNRS 6134 Sciences pour
9 l'Environnement, 20250 Corte, France

10 b Université de Corse Pasquale Paoli, UAR CNRS 3514 Plateforme marine Stella
11 Mare, 20620 Biguglia, France

12 c Université de Liège, Centre MARE, Focus, Laboratoire d'Océanologie, Sart Tilman,
13 B6c, 4000 Liège, Belgium

14 d STation de REcherche Sous-marines et Océanographiques (STARESO), 20260
15 Calvi, France

16 * Corresponding author at: Université de Corse Pasquale Paoli, UMR CNRS 6134
17 Sciences pour l'Environnement, 20250 Corte, France.

18

19 E-mail address: el-idrissi_o@univ-corse.fr (O. El Idrissi)

20

21 **Abstract**

22 Among the most common contaminants in marine ecosystems, trace elements are
23 recognized as serious pollutants. In Corsica (NW Mediterranean Sea), near the old
24 asbestos mine at Canari, trace elements from the leaching of mine residues have
25 been discharged into the sea for several decades. The aim of this study was to
26 assess the levels of contamination in this area and the potential effects on
27 *Paracentrotus lividus* (Lamarck, 1816) using pollution indices, accumulation factors
28 and biochemical tools. For this purpose, the concentration of 24 trace elements was
29 measured in sea urchins (gonads and gut content), macroalgae, seawater column
30 and sediment collected at 12 stations nearby the old asbestos mine and at a
31 reference site. The bioaccumulation of trace elements occurs as follows: macroalgae
32 > gut > gonads. TEPI contribute to highlight contamination gradients which are
33 mainly due to the dominant marine currents allowing the migration of mining waste
34 along the coastline. This hypothesis was supported by TESVI, which identified
35 characteristic trace elements in the southern area of the mine. High hydrogen
36 peroxide content, associated with elevated catalase and glutathione-S-transferase
37 enzyme activities, were also identified at these sites and at the reference site. Trace
38 elements contamination as well as several abiotic factors could explain these results
39 (*e.g.* microbiological contamination, hydrodynamic events, etc.). The results obtained
40 in this study suggest that oxidative stress induced by contamination does not affect
41 the health of *Paracentrotus lividus*. This work has provided a useful dataset allowing
42 better use of sea urchins and various tools for assessing trace element contamination
43 in coastal ecosystems.

44

45 **Keywords:** *Paracentrotus lividus*; Asbestos mine; Trace Element Pollution Index;
46 Bioaccumulation; Biomagnification; Oxidative stress
47

48 **1. Introduction**

49 Coastal ecosystems are at the interface between the marine and terrestrial domains
50 and provide many goods and ecosystem services of high value (Costanza *et al.*,
51 2017). Coastal marine waters are particularly vulnerable to a wide range of
52 disturbances such as climate change effects and the release into the sea of a wide
53 variety of contaminants (Livingston *et al.*, 1994; Mostofa *et al.*, 2013). These habitats
54 are subject to an increase in anthropogenic activities that can lead to high levels of
55 contamination (Islam & Tanaka, 2004). The presence of these contaminants can
56 have a devastating effect on organisms and their ecosystems, making marine
57 pollution a global concern (Abdel-Shafy & Mansour, 2016; Torres *et al.*, 2016).

58 Among the major pollutants, trace elements are a real ecological issues because of
59 their toxicity, their persistence and their ability to accumulate in marine organisms
60 and even be biomagnified through the trophic chain (Rainbow & Luoma, 2011;
61 Bonanno & Di Martino, 2017). The inputs of trace elements in marine environment
62 derived from natural geogenic pollution (geological phenomena) and/or
63 anthropogenic sources (*e.g.* mining, agriculture, petrochemical industry, aquaculture,
64 sewage waste water) result in increased concentration levels (D'Adamo *et al.*, 2008;
65 Barhoumi *et al.*, 2014). The trace elements can be classified as essential or non-
66 essential (Jiang *et al.*, 2014). Essential elements have a biological function in
67 organisms, such as copper, iron and zinc and non-essential trace elements are not
68 involved in any metabolic mechanism, such as cadmium, mercury and lead (Amiard,
69 2011). Above a certain threshold, all trace elements present a potential threat for
70 organisms (Nordberg *et al.*, 2007). As a result of the threats caused by trace
71 elements, continuous monitoring of their presence and concentration must be
72 undertaken (Richir & Gobert, 2014).

73 The Mediterranean Sea, which represents 0.82% of the global ocean surface area, is
74 one of the main hotspots of marine biodiversity in the world, with 4–18% of identified
75 marine species (Coll *et al.*, 2010). However, the coastal areas surrounding this semi-
76 enclosed sea are subjected to the increasing synergistic effects of global changes
77 and intensive anthropogenic activities leading to significant alterations in the structure
78 and functioning of trophic webs and to the degradation of coastal and marine
79 ecosystems (Coll *et al.*, 2012; Ramírez *et al.*, 2018; Stock *et al.*, 2018a). Located in
80 the northwestern Mediterranean Sea, Corsica Island has long been considered as a
81 pristine region with low anthropogenic disturbances resulting in low levels of
82 contamination (Gobert *et al.*, 2017). However, recent studies have revealed major
83 trace elements contamination near an old asbestos mine in northern Corsica (Cary *et al.*
84 *et al.*, 2013; Ternengo *et al.*, 2018; El Idrissi *et al.*, 2020). These high levels originate
85 from untreated material directly discharged into the sea during the active period of
86 the asbestos mine between 1948 and 1965 (BRGM, 2017; Cary *et al.*, 2013). The
87 continuous leaching of these mining residues, still present on the sides of the mine,
88 contributes to the dispersal of nickel, chromium and cobalt along the coastline
89 (Galgani *et al.*, 2006; Kantin & Pergent-Martini, 2007). Apart from the threat
90 associated with the old asbestos mine, this region is characterized by relatively low
91 levels of anthropogenic pressure (*i.e.* industrial, agricultural and urban activities;
92 ASR, 2011). Because of these specificities, this sector represents a privileged study
93 area to assess the trace elements contamination from a specific source within the
94 coastal ecosystems.

95 The bioaccumulation of trace elements by marine organisms can occur through
96 various pathways such as absorption and/or adsorption directly from sediments and
97 interstitial seawater but also through ingestion, which will potentially affect other

98 species along the food chain (Adamo *et al.* 2005; Chen *et al.* 2007; Türkmen *et al.*,
99 2008). In order to assess bioaccumulation and biomagnification to clarify the toxicity
100 and fate of trace elements in organisms, it is important to determine the
101 concentrations in the different biological and environmental matrices (water column,
102 sediment, plants, animals; Jha, 2004). Echinoderms, and in particular sea urchins,
103 are particularly suitable organisms for use in bioindication studies (Parra-Luna *et al.*,
104 2020). Along the Mediterranean coast, *Paracentrotus lividus* (Lamarck, 1816) is
105 frequently considered as a good bioindicator of local pollution because of its wide
106 distribution, abundance, benthic behavior, sedentary habits, rapid response and its
107 recognized sensitivity to a variety of pollutants (Sugni *et al.*, 2007; Amri *et al.*, 2017;
108 Rouhane-Hacene *et al.*, 2017). Described as the organs that accumulate the most
109 trace elements, its gonads and gut are of interest in studies assessing contamination
110 levels in coastal marine ecosystems (Augier *et al.*, 1989; Warnau *et al.*, 1998; Geraci
111 *et al.*, 2004). *P. lividus* is also a species of economic importance due to its valued
112 consumption in several countries (Fernández-Boán *et al.*, 2013; Powell *et al.*, 2014;
113 Sun & Chiang, 2015), and ecological significance, controlling the dynamics of
114 seaweeds and seagrass through its grazing activity (Lawrence & Sammarco, 1982;
115 Boudouresque & Verlaque, 2013).

116 The use of biomarkers as bioindicators is an important approach in aquatic
117 biomonitoring to assess the relationships between exposure to environmental
118 pollutants and increased effects on individuals and populations (Bouzahouane *et al.*,
119 2018). The effects of pollutants on the marine ecosystem can be expressed in terms
120 of biochemical endpoints (Kamel *et al.*, 2014). The exposure of aquatic organisms to
121 trace elements can lead to the production of reactive oxygen species (ROS; Nieto *et*
122 *al.*, 2010), causing an imbalance between the production of ROS and endogenous

123 antioxidant activity (Beyersmann & Hartwig, 2008). Oxidative stress is involved in
124 DNA damage, protein oxidation and lipid peroxidation (Winston & Digiulio, 1991). In
125 view of this, it is relevant to assess the activities of antioxidant enzymes acting
126 against oxidative stress such as catalase (CAT), glutathione peroxidase (GPX),
127 glutathione-s-transferase (GST) and superoxide dismutase (SOD). The content of
128 malondialdehyde (MDA), a by-product of lipid peroxidation, and the level of hydrogen
129 peroxide (H₂O₂) in the tissues of organisms is also a good indicator of oxidative
130 stress intensity. Therefore, oxidative stress biomarkers are widely used in marine
131 ecotoxicology (Kamel *et al.* 2014; Benedetti *et al.*, 2015; Ghribi *et al.*, 2020).

132 The aims of this study are (i) to assess the reliability of different matrices (sea urchin,
133 macroalgae, water column and sediment) to characterize 24 trace elements
134 contamination levels; (ii) to test the effectiveness of several indices used in
135 ecotoxicological studies in order to confirm their use in the assessment of
136 contamination; (iii) to determine the bioaccumulation, biomagnification and biota-
137 sediment accumulation factor of trace elements, and (iv) to estimate the effects of
138 trace elements contamination on the oxidative stress of *P. lividus*.

139

140 2. Material and methods

141 2.1. Study area

142 The study area is located on the northwest coast of Corsica (NW Mediterranean Sea;
143 Fig. 1A; Fig. 1B). This rocky coast is characterized by the occurrence of several cliffs
144 (40 to 50 m high) formed of ophiolitic rocks (pillow-lavas and prasinites, gabbros,
145 serpentinites and peridotites) and of mineral characteristics of the rubble sand
146 belongs to the serpentine, olivine, pyroxene and amphibole groups (Bernier *et al.*,
147 1997; Lafabrie *et al.*, 2008). In this area, the old asbestos mine of Canari extends
148 along 1 km of rocky coastline (42°49'15"N, 9°19'41" E; Fig. 1C). After the discovery
149 of the asbestos deposit in 1898, the mine was industrially exploited from 1948 to
150 1965 (BRGM, 1997). During this active period of exploitation, up to 28,000 tons of
151 asbestos was produced yearly and approximately 4.5 million m³ of solid mine waste
152 was directly discharged into the Mediterranean Sea (BRGM, 1997; Méria, 2004). The
153 soils surrounding the old mine are covered by dense scrubland vegetation (upstream
154 of the mine: over 400 m) while the natural revegetation of the exploited areas
155 (downstream of the old mine) remains very reduced (BRGM, 1999).

156 Although the mine has been closed for over 50 years, the leaching of mining
157 residues, present along the coastline of the mine, still contributes to the dispersal of
158 nickel, chromium and cobalt into the marine environment (Galgani *et al.*, 2006; Kantin
159 & Pergent-Martini, 2007). Indeed, in the southern part of the shoreline, there are
160 many beaches (*e.g.* Albo, Nonza) resulting from the southward transport of
161 discharged rubble (360,000-400,000 m³ yr⁻¹) associated with the old asbestos mine
162 of Canari (Fig. 1C; Bernier *et al.*, 1997; Méria, 2004). The sediment enrichment of
163 these beaches results from two sources: a natural enrichment by coarse sediments
164 from small coastal rivers and an artificial enrichment due to discharged rubbles

165 (Pouquet, 1958; Bernier *et al.*, 1997). Greater amounts of sediment are provided by
166 artificial sources (mixture of cobbles and pebbles, sands and silts) continually eroded
167 and winnowed by wave action (Bernier *et al.*, 1997). Currently, the low levels of
168 anthropogenic activity in this area and the local sources of contamination associated
169 with the old mine provide a unique mesocosm to study the contamination in the
170 marine environment (ASR, 2011).

171 Six sampling sites located along the coast were selected (Fig. 1C): one in front of the
172 old asbestos mine (Old Mine: OM), two to the north (Punta di Canelle: PC; Canelle:
173 CN) and three to the south (Punta Bianca: PB; Albo: AB; Nonza: NZ). Samples were
174 also collected at a reference site (RF), recognized as having a low level of trace
175 elements contamination (Fig. 1D; Gobert & Richir, 2019; El Idrissi *et al.*, 2020). These
176 sites were selected to provide a basis for assessing the differences in trace elements
177 concentration in order to establish a potential gradient of contamination and also to
178 better understand the effects of these different concentrations on the organisms.

179 **2.2. Collection and processing of biological samples**

180 Individuals (n = 28) of *P. lividus* sea urchin and macroalgae species observed at
181 each sampling site were collected at two distinct depths for each site (3 m and 6 m
182 depth) in winter (Fig. 1C; Fig. 1D). As indicated by El Idrissi *et al.* (2020), the winter
183 season avoids bias due to the reproductive stage of the urchins. In total, 364 sea
184 urchins of commercial size (~50 mm) were collected and directly transported in a
185 cooler with oxygenated seawater to the laboratory. Among the macroalgae collected
186 at each site, the three most dominant taxa were retained and presented in this study
187 (*Ericaria amentacea* (C.Agardh) Molinari & Guiry, 2020, *Dictyota dichotoma* (Hudson)
188 J.V.Lamouroux, 1809 and *Ellisolandia elongata* (J.Ellis & Solander) K.R.Hind &

189 G.W.Saunders, 2013). Three samples of each macroalgae (\approx 500 g wet weight) were
190 collected at the different sites.

191 After sampling, macroalgae were rinsed with ultrapure MilliQ™ water and placed at –
192 20 °C until chemical analysis. The specimens of *P. lividus* were measured, weighed
193 and dissected. The sex ratio has been respected to avoid bias. Gonads and guts
194 collected from sea urchins (n = 16) from each station were immediately weighed and
195 stored at –20 °C. Frozen samples were lyophilized (CHRIST LCG Lyochamber
196 Guard 121550 PMMA/Alpha 1-4 LD plus) and ground with an agate mortar.
197 Approximately 0.2 g of each dried material was mineralized in Teflon digestion
198 vessels, in a closed microwave digestion labstation (Ethos D, Milestone Inc. Sorisole,
199 Italy), using nitric acid (HNO₃, 60%) and hydrogen peroxide (H₂O₂, 30%) as reagents
200 (Suprapur grade, Merck, Darmstadt, Germany).

201 **2.3. Collection and processing of sediment samples**

202 Sediment samples were collected at each station using a 0.1 m² Van Veen grab from
203 the bottom (top 20 cm). After collection, the sediments were stored at –20 °C until
204 chemical analysis. Frozen sediments were lyophilized (BenchTop 3L, VirTis
205 Company Inc.) and were eluted for 4 h at room temperature with 30 mL of HCl 1N
206 (Suprapure grade, Merck, Darmstadt, Germany), according to Townsend *et al.*
207 (2007). A 4-h extraction time in HCl 1N ensures the removal of the available
208 precipitated trace elements while not favouring the extraction of natural geogenic
209 metals (Snape *et al.*, 2004). Eluates were then diluted to an appropriate volume of 50
210 mL, centrifuged for 10 min at 2000 rotations per minute and separated from their
211 remaining culot prior to being analyzed.

212 **2.4. Trace element concentrations**

213 **2.4.1. Bioavailable concentrations in the seawater column**

214 The concentration of trace elements in the seawater column at each site was
215 measured using diffusive gradients in thin films (DGTs) devices (DGT Research Ltd.,
216 UK). DGT units, fixed with a nylon fishing line to a plastic stage buried in the
217 sediment, floated freely in the seawater column for one week. After recovery, DGTs
218 were rinsed with ultrapure MilliQ™ water and stored at 4 °C until analysis. As trace
219 metal diffusion coefficients through the diffusive layer depend on water temperature,
220 average temperatures were recorded using HOBO TidbiT® v2 loggers (accuracy: ±
221 0.21 °C).

222 **2.4.2. Trace element analysis**

223 The concentration of 24 trace elements (Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe,
224 Hg, Li, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, U, V, Zn) were determined by Inductively
225 Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology (ICP-
226 MS ELAN DRC II, Perkin Elmer), according to the method described by Richir &
227 Gobert (2014). In order to check the purity of the chemicals used, analytical blanks
228 were analyzed similarly to the samples and were performed every 40 samples.
229 Analytical accuracy was checked by analyzing Certified Reference Materials (CRM):
230 DORM-4 (fish protein), NIST1566b (oyster), NIST2976 (mussel tissue), BCR-60
231 (*Lagarosiphon major*), BCR-661 (*Platyhypnidium riparioides*) and GBW07603 (bush
232 twigs and leaves). For each TE, detection limit (LD) and quantification limit (LQ) were
233 calculated, depending on their specific blank distribution (Grinzaid *et al.* 1977; Currie,
234 1999). The total Hg (THg) content of biological samples was determined using atomic
235 absorption spectrometry at 254 nm, in a Direct Mercury Analyser (DMA 80 153
236 Milestone, Minnesota, USA). Quality assessment was operated using replicates,
237 standards (THg 100 ng g⁻¹), blanks (HCl 1%) and CRM at the beginning and the end
238 of each series. The results are expressed in milligrams of element per kilogram of dry

239 weight (mg kg^{-1} DW) for tissue and sediment, and in milligrams of element per litre
240 (mg L^{-1}). TEs with values below the detection limit were removed from the database.
241 For the others, concentrations below the LD were replaced with a value of LD/2, as
242 reported by Skrbić *et al.* (2010).

243 **2.5. Histological study**

244 The gonads of six sea urchins from different stations were fixed in formaldehyde in
245 order to perform a histological study and determine the reproductive stage. This
246 enables us to determine whether trace elements levels are influenced by the
247 reproductive stage in this study. Gonadal tissues were dehydrated using ethanol
248 (from 70 to 100%) then placed in Neo-Clear™, xylene substitute, before being
249 embedded in paraffin. After cooling the blocks, two duplicate sections (thickness: 5
250 μm) were taken using a microtome, mounted on slides, and air-dried for the
251 deparaffinization of the tissue in preparation for rehydration. After that, the sections
252 were stained with Masson's Trichrome and observed under a light microscope to
253 determine the stage of maturity based on the stages described by Byrne (1990):
254 stage 1, recovery; stage 2, growing; stage 3, premature; stage 4, mature; stage 5,
255 partly spawned; stage 6, spent.

256 **2.6. Biochemical analyses**

257 For the oxidative stress study, the gonads of six sea urchins per station were fixed in
258 liquid nitrogen before storage at $-80\text{ }^{\circ}\text{C}$. Samples were homogenized using a Potter-
259 Elvehjem homogenizer in chilled phosphate buffer (100 mM, pH 7.4; 25 mg w/w per
260 mL of buffer) containing 20% glycerol and 0.2 mM phenylmethylsulfonyl fluoride as a
261 serine protease inhibitor. The homogenates were centrifuged at $15,000 \times g$ for 30
262 min at $4\text{ }^{\circ}\text{C}$ and the supernatant was used for biochemical assays. Protein
263 concentration was measured as described in Bradford (1976) and was used to

264 normalize the final unit for biomarker responses. Biomarkers including superoxide
265 dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione-S-
266 transferase (GST) and determination of malondialdehyde (MDA) content were
267 assessed as described by Greani *et al.* (2017). The hydrogen peroxide (H₂O₂)
268 concentration was determined using a PeroxiDetect Kit (Sigma, Aldrich, St. Louis,
269 MO, United States) as described by Lourkisti *et al.* (2020).

270 **2.7. Elemental and isotopic analyses**

271 Gut content of *P. lividus* (n = 12 per site) and food sources (*D. dichotoma*, *E.*
272 *elongata* and *E. amentacea*) were analyzed through elemental and isotopic analyses.
273 The sea urchins and macroalgae samples were dried at 60 °C for 48-96 h and
274 successively ground to a homogenous powder with agate mortar. As acidification is
275 known to alter N stable isotope ratios (Mateo *et al.*, 2008), acidified samples (*E.*
276 *elongata*) were analyzed twice: once for stable C isotopic ratios, using decarbonated
277 material, and once for N isotopic ratios, using native material. Samples of food
278 source (1-5 mg) and gut content (2-3 mg) were subsequently loaded into tin capsules
279 (8 x 5 mm, Elemental Microanalysis). Stable isotope ratio measurements were
280 performed *via* continuous flow-elemental analysis-isotope-ratio mass spectrometry
281 (CF-EA-IRMS) at University of Liège, using a Vario Micro Cube elemental analyser
282 (Elementar Analysensysteme GmbH, Hanau, Germany) coupled to an Isoprime 100
283 mass spectrometer (Isoprime, Cheadle, UK). Isotopic ratios of C and N were
284 expressed conventionally (Coplen 2011), using standard delta (δ) notation relative to
285 their respective international standards, Vienna-Pee Dee Belemnite (VPDB) and
286 atmospheric N₂. Certified reference materials (CRM) of sucrose (IAEA-C6, $\delta^{13}\text{C} = -$
287 $10.8 \pm 0.5\text{‰}$) and ammonium sulphate (IAEA-N2, $\delta^{15}\text{N} = 20.3 \pm 0.2\text{‰}$) obtained from
288 the International Atomic Energy Agency (IAEA, Vienna, Austria), were used for the

289 measurement of isotopic ratios. These CRM, procedural blanks and internal
290 replicates (*i.e.*, glycine and in-house seagrass reference materials) were used to
291 assess the analytical precision. Standard deviations on multi-batch replicate
292 measurements were 0.1‰ for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{15}\text{N}$.

293 The relative contribution of macroalgae to the diet of the sea urchin *P. lividus* was
294 estimated with a Bayesian isotopic mixing model (MixSIAR, Stock and Semmens,
295 2016; Stock *et al.*, 2018b). The application of the mixing model provided accurate
296 information concerning the contribution of macroalgae species to the sea urchin
297 tissues and recognized the main components of the diet under different conditions
298 (Peterson, 1999; Fry, 2006; Wing *et al.*, 2008; Cabanillas-Terán *et al.*, 2016). The
299 model runs included the isotopic signatures of each individual, isotopic compositions
300 of food sources (mean \pm SD) and trophic enrichment factors (TEFs; mean \pm SD)
301 corresponding to the isotopic composition difference of consumer tissues and the
302 potential food sources (Mascart *et al.*, 2018). Though the use of suitable TEFs is
303 required to run mixing model, there are no specific TEF for the taxa studied here, and
304 we used widely applicable values (*i.e.*, $0.4 \pm 1.2\text{‰}$ for C, $2.3 \pm 1.6\text{‰}$ for N) from
305 McCutchan *et al.* (2003). The model was run using the MixSIAR 3.1.12 package in R
306 4.1. The results of the mixing model showing the calculated sea urchin dietary
307 proportions were represented using mean \pm SD and 95% credible intervals (CI₉₅)
308 indicating the intervals of probability density function distributions.

309 **2.8. Numerical procedures**

310 In order to compare the contamination levels at the different sites, the Trace Element
311 Pollution Index (TEPI) was calculated for each site. The TEPI allows a reliable
312 comparison of study sites, regardless of trace elements or the biological model used
313 (Richir and Gobert, 2014). The index was calculated with concentrations measured in

314 gonads, digestive tract and macroalgae to confirm its robustness. The data were
315 standardized by mean normalization as recommended for the calculation by Richir
316 and Gobert (2014). TEPI values were calculated using the following formula:

$$317 \quad TEPI = (Cf_1 * Cf_2 * \dots * Cf_n)^{1/n}$$

318 where Cf_n is the mean normalized concentration of the trace element at each site and
319 n is the number of trace elements examined. The higher the TEPI value, the more
320 contaminated the site is. In order to classify and compare the trace elements
321 according to their spatial variability on all the studied sites, the Trace Element Spatial
322 Variation Index (TESVI) was determined for each element according to Richir and
323 Gobert (2014). For each trace element, TESVI was calculated as follows:

$$324 \quad TESVI = [(x_{max} / x_{min}) / (\sum (x_{max} / x_i) / n)] * SD$$

325 where x_{max} and x_{min} are the maximum and minimum mean concentrations recorded
326 among the n sites, x_i are the mean concentrations recorded at each of the n sites,
327 and SD is the standard deviation of the mean ratio $\sum (x_{max} / x_i) / n$. For a given trace
328 element, the higher the value of the TESVI, the more its environmental levels will
329 vary overall across the study area. Therefore, the higher the TESVI, the more
330 representative it is of a site.

331 Bioaccumulation is generally referred to as a process in which the chemical
332 concentration in an organism achieves a level that exceeds that in the environment,
333 the diet, or both (Gobas *et al.*, 2009). The extent to which chemicals bioaccumulate is
334 expressed by several values, including bioaccumulation factor (BAF),
335 biomagnification factor (BMF) and biota-sediment accumulation factor (BSAF). BAF
336 is ratio of the steady chemical concentrations in an aquatic water-respiring organism
337 (CB, g kg⁻¹ WW) and the water (CW, g L⁻¹) determined from field data in which

338 sampled organisms are exposed to a chemical in the water and in their diet. BAF is
339 calculated using the following formula: CB / CW . BFM is the ratio of the steady state
340 chemical concentrations in an organism ($CB, g\ kg^{-1}\ WW$) and the diet of the organism
341 ($CD, g\ kg^{-1}\ WW$) determined from field data. Lastly, BSAF is the ratio of the chemical
342 concentrations in an organism ($CB, g\ kg^{-1}\ DW$) and the sediment ($CS, g\ kg^{-1}\ DW$).

343 Data were expressed as mean \pm standard error (SE) and analyzed using XLSTAT
344 software. The data were transformed in order to meet the conditions of application of
345 the parametric tests and to reduce the effect of outliers skewing the data distribution.
346 Analyses of variance (ANOVA) followed by post-hoc Tukey's honestly significant
347 difference (HSD) tests were performed. The relationship between enzymatic activities
348 and trace elements was measured by Pearson correlation coefficient. A significant
349 difference was considered a p-value less than 0.05.

350

351 **3. Results**

352 **3.1. Distribution of trace element content**

353 The levels in Be and Bi levels were below the detection limit in all matrices analyzed,
354 so they were not considered in the statistical analyses of the results. In addition, Hg
355 concentrations were also below the detection limit in the sediments and the seawater
356 column. For the sake of clarity, only 14 representative examples chosen among the
357 24 studied trace elements are graphically illustrated throughout the paper, and
358 discussion mainly revolves around these selected examples. The mean trace
359 element concentrations measured in the different matrices (sea urchins, macroalgae,
360 sediment and seawater column) are presented in Fig. 2.

361 Trace element concentrations follow the sequence: Fe> Zn> Al> Ni> Cr> Mn> Cu>
362 V> Se> Co> Mo> Cd> Ag> Pb in sea urchin gonads; Fe> Al> Cr >Ni> Zn> Mn> Cu>
363 V> Se> Co> Cd> Mo> Ag> Pb in sea urchin guts; Fe> Al> Ni> Cr> Mn> Cu> V> Zn>
364 Co> Pb> Mo> Cd> Ag in macroalgae; Fe> Pb> Al> Ni> V> Zn> Se> Mn> Cr> Cu>
365 Co> Mo> Cd> Ag in seawater column and Fe > Al> Ni> Cr> Mn> V> Co> Cu> Zn>
366 Se> Pb> Mo> Cd> Ag in sediment (Fig. 2).

367 **3.2. Spatial variations of trace elements**

368 There is no significant difference in trace elements concentrations between the
369 samples collected at 3 m depth and those collected at 6 m depth. The TEPI was
370 calculated with concentrations in sea urchin gonads, sea urchin guts and macroalgae
371 (Fig. 3). In gonads and guts, a gradient of contamination clearly appeared, with
372 higher contamination to the south of the old asbestos mine, particularly at Albo
373 (gonads: 1.367, guts: 1.172) and Nonza (gonads: 1.161, guts: 1.201; Fig. 3A; Fig.
374 3B). In contrast, the reference site is the least contaminated, with a TEPI of 0.456 for
375 the gonads and 0.529 for guts (Fig. 3A; Fig. 3B).

376 The TEPI calculated with macroalgae showed a more contrasting gradient with the
377 Canelle site, located north of the mine, similarly contaminated to Nonza (0.902 and
378 0.907, respectively; Fig. 3C). The four trace elements with the highest TESVI in
379 gonads, guts and macroalgae are Co, Cr, Fe and Ni (Table 1). The highest
380 concentrations were noted at Albo for the four trace elements (Table 1). Therefore,
381 Co concentrations are 10 to 20-fold higher at Albo than at the reference site, Cr 96 to
382 172-fold, Fe 2 to 9-fold and Ni approximately 38-fold.

383 **3.3. Bioaccumulation, biomagnification and biota-sediment accumulation** 384 **factors**

385 The BAF and BSAF were calculated with the concentrations in the gonads and guts
386 of the sea urchin and with concentrations measured in macroalgae (Table 2). Most
387 trace elements were most bioaccumulated in macroalgae then the guts and finally the
388 gonads (Al, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V; Table 2). Only Zn had a higher
389 accumulation in the gonads in comparison to the guts and macroalgae (Table 2).

390 Among the macroalgae studied, the isotopic analysis highlighted that sea urchins
391 collected fed mainly on *E. elongata* ($96.6 \pm 2.3\%$; CI_{95} : 90.8-99.6%) and very little on
392 *D. dichotoma* ($2.5 \pm 2.2\%$; CI_{95} : 0.1-8.4%) and *E. amentacea* ($0.9 \pm 0.9\%$; CI_{95} : 0-
393 3.2%; Fig. 4). In order to calculate the BMF, a weighted average of macroalgae was
394 used for accuracy. Therefore, the formula used in this study was:

$$395 \quad CB / (CD_1 * 0.966 + CD_2 * 0.025 + CD_3 * 0.009)$$

396 where, CB is the concentration measured in guts, CD_1 the concentration in *E.*
397 *elongata*, CD_2 in *D. dichotoma* and CD_3 in *E. amentacea*. The highest BMFs were
398 obtained for Ag, Cd and Mo, elements for which the guts accumulate more than the
399 other matrices (Table 2).

400 **3.4. Biotic factors of the sea urchin**

401 The histological analysis highlighted that sea urchins were mostly at stage 4 (mature)
402 and stage 5 (partly spawned; Fig. 5). However, variations were observed at Punta
403 Bianca where ~30% of the sea urchins had already spawned and at the reference
404 site where 50% of the sea urchins had reached the premature stage (Fig. 5).
405 Significant differences were noted between the concentrations measured in the male
406 and female gonads. Females exhibited higher concentrations of Cd, Mn, Se, and Zn
407 while high Mo, Pb, and V levels were found in males (p -value < 0.05). In contrast, no
408 significant differences were found for biomarkers of stress. In the guts, the only
409 significant difference between males and females is measured for Zn with a
410 concentration 1.5-fold higher in females (p -value < 0.001).

411 **3.5. Biochemical analyses**

412 There was no significant difference between sites regarding MDA content and
413 specific activities of SOD and GPX (Fig. 6). At the same time, the specific activity of
414 CAT and the H₂O₂ levels were higher in sea urchins collected at Albo, Nonza and at
415 the reference site (Fig. 6). Finally, the highest specific activity of GST was measured
416 in sea urchins at Albo and three sites (Reference site, Nonza and Old Mine; Fig. 6).
417 Specific activities of CAT and GST as well as H₂O₂ levels in sea urchin gonads
418 showed significant positive correlation (p -value < 0.001, Table A.1). In addition, there
419 were significant positive correlations for several trace elements with CAT and H₂O₂
420 (Table A.1). Accordingly, increased concentrations of Al, Cr, Cu, Fe, Mn, Pb, and Se
421 resulted in higher specific CAT activity and H₂O₂ levels in the gonads (p -value <
422 0.05).

423

424 **4. Discussion**

425 The study of environmental contamination requires a deep knowledge of the
426 distribution of pollutants and their concentrations in biotopes and organisms (Lagadic
427 *et al.*, 1998; Ramade, 2007). For this purpose, trace element concentrations were
428 analyzed in sea urchins (gonads and guts), three macroalgae species, the seawater
429 column and sediment. The trace elements follow an almost similar sequence
430 regardless of the environmental matrices studied, although there are some notable
431 differences. Fe is the predominant trace element resulting from its essential character
432 (Phillips & Rainbow *et al.*, 1989; Lohan & Tagliabue, 2018) or from the contamination
433 of the ecosystem as described in the literature (Brik *et al.*, 2018). Fe is an essential
434 trace element playing a major role for many species due to its role in various
435 physiological processes (*e.g.* photosynthesis, enzymatic activity, reproduction, etc.)
436 and it is also included in geochemical processes (Sunda, 2001; Thuróczy *et al.*,
437 2011). However, in contrast to the literature, Fe concentrations exceeded Zn
438 concentrations in the gonads (Strogyloudi *et al.*, 2014; Ternengo *et al.*, 2018; El
439 Idrissi *et al.*, 2020). This is due to the remarkably high concentrations of Fe, 2 to 8-
440 fold higher than in many studies (Warnau *et al.*, 1998; Storelli *et al.*, 2001; Soualili *et*
441 *al.*, 2008; Strogyloudi *et al.*, 2014). According to Blum *et al.* (2006), the serpentinites
442 of Cap Corse could generate significant Fe content explaining these high contents at
443 the studied sites. Although Fe is an essential requirement for marine organisms, an
444 excessive concentration could be harmful to their health and must therefore be
445 monitored (Fosmire, 1990). Zn is the second element measured in high concentration
446 in the gonads. It is the only trace element with a higher accumulation factor in the
447 gonads than in the guts and macroalgae. This accumulation could be due to its
448 intervention in gametogenesis and in particular in ovogenesis explaining its high

449 content in females compared to males (Unuma *et al.*, 2003, 2007). In contrast to the
450 sediment, macroalgae, gonads and guts of sea urchins where Pb is among those
451 with the lowest concentrations, this trace element is the second most abundant in the
452 distribution sequence of the seawater column. It is one of the most abundant and
453 common non-essential trace elements in the environment (Mishra *et al.*, 2006). Its
454 concentration in the seawater column ($2.17 \pm 0.83 \text{ mg L}^{-1}$) is considerably higher than
455 previously reported in the literature (Lin *et al.*, 2000; Bruland & Lohan, 2003). This is
456 may be due to point contamination of the water column during the period of this study
457 or contamination of DGTs during material handling. Seawater column analysis is
458 reported to be less relevant in the literature for trace element monitoring due to its
459 high fluctuations caused by several factors such as hydrodynamic energy (Jahan &
460 Strezov, 2019). Moreover, it is not always possible to analyze all the contaminants in
461 this biotope, the concentration levels being too low and the analytical techniques not
462 being sensitive enough in this study for Hg.

463 Calculation of the TEPI through measurement of trace element contents in the
464 gonads and guts of the sea urchin enabled us to determine a gradient of
465 contamination with a higher concentration to the south of the old asbestos mine, in
466 particular at Albo and Nonza. The occurrence of a similar north-south gradient within
467 this area has already been demonstrated in other matrices such as mussels (Kantin
468 *et al.*, 2015). In the literature, it has been established that asbestos wastes were
469 accumulated to the south of the old asbestos mine (BRGM, 1997; Cary *et al.*, 2013).
470 These materials migrated along the coastline due to the effect of swell and prevailing
471 marine currents (BRGM, 1997). As a result, the wastes expanded the coastline
472 southward for 5 km from the old mine, progressively filling the beach at Albo and
473 creating the beach at Nonza (about 1 500 m long; BRGM, 1997). A total of one

474 million tons of excavated material was transported to the artificial beaches during the
475 35 years of operation of the site (Boulmier *et al.*, 1999; Hervé *et al.*, 1997; Cary *et al.*,
476 2013). This observation highlights the important role that the marine environment
477 plays in the diffusion and distribution of contaminants in the environment. Hence, it is
478 necessary to take care during the selection process of the sampling site for
479 ecotoxicological studies and to consider which can contribute to the variability of
480 contaminants at local scale. Moreover, sites at 3 and 6 m depth are similarly
481 impacted by diffusion, indicating that the contamination affects the whole of the
482 studied bathymetric range.

483 The Albo and Nonza beaches are characterized by black pebbles constituted by
484 serpentine (BRGM, 1997). The serpentinites are characterized by high levels of Fe
485 and Mg (Morrison *et al.*, 2009) which may explain the high Fe concentrations found at
486 Albo with a high TESVI whatever the matrices studied. Co, Cr, and Ni are also
487 present in high concentrations at Albo with significant concentration differences
488 compared to the reference site. Previous works have also highlighted a high
489 contamination of Ni, Cr and Co in marine sediments in the area adjacent (15 km of
490 coastline) to the old asbestos mine at Canari (Andral & Tomasino, 2007; Kantin &
491 Pergent-Martini, 2007) and an accumulation of these elements in several marine
492 organisms (Bouchoucha & Andral, 2010; El Idrissi *et al.*, 2020). According to several
493 authors, in addition to Fe, serpentinites are naturally enriched in other trace elements
494 such as Cr, Ni and Co (Morrison *et al.*, 2009; Siebecker, 2010; Tashakor *et al.*,
495 2011), explaining these high concentration levels in the region and particularly to the
496 south of the old mine. The general formula of serpentine is $Mg_5 \{Si_2O_4\} (OH)_4$ and
497 substitution of Mg by Fe (II), Fe (III), Cr, Al, Ni and Mn may occur (Mével, 2003). In
498 the same way, large amounts of Mg and/or Fe are reported, disproportionate

499 richness in Ni, Cr and Co and poorness in Ca in the serpentinite (Lee *et al.*, 2004; Pal
500 *et al.*, 2006). Thus, even though mine drainage was the main cause of these
501 contamination variations, there is a general sensitivity related to a particularly
502 concentrated geochemical background. The assessment of ecosystem quality
503 requires good knowledge of the natural geochemical context in order to distinguish
504 the trace elements naturally present in the environment from those resulting from
505 anthropogenic activities.

506 Serpentine soils are considered a source of geogenic pollution by many researchers
507 because of the trace elements they contain and their potential deleterious effect at
508 high concentrations in the environment (Caillaud *et al.*, 2009; Tashakor *et al.*, 2011).
509 In this regard, recent experiments have been performed to determine the impacts of
510 concentrations measured in this area on *Paracentrotus lividus* but no significant
511 effect were highlighted on the larvae, even in the presence of chronic contamination
512 (El Idrissi *et al.*, 2022a, 2022b).

513 The TEPI of macroalgae also indicated strong contamination at Albo but no north-
514 south contamination gradient has been highlighted. Indeed, the macroalgae located
515 in the northern part of the mine exhibited contamination levels similar to those located
516 in the south, resulting in a gradient around the mine rather than north-south. This
517 result may be related to a reversal phenomenon of the littoral drift (from south to
518 north) during a strong storm in 1973 causing a transport of sediment from the rubble
519 discharge towards the north (Bernier *et al.*, 1997).

520 Macroalgae may be better able to accumulate than sea urchins as indicated by the
521 different accumulation factors calculated in this study. Thus, the macroalgae can be
522 particularly useful to determine the presence of a contaminant at the specific location.
523 However, it is necessary to be careful when interpreting contamination levels at the

524 risk of overestimating them (Richir, 2013). Moreover, if the aim of the study is to
525 identify the magnitude of well-integrated contamination, the sea urchin may be a
526 better tool for assessing a contamination gradient.

527 It is commonly recognized that the accumulation of contaminants in marine
528 organisms occurs through three compartments: the seawater column, food and
529 sediments (Bouzahouane *et al.*, 2018). Accumulation can lead to high internal
530 concentrations resulting in toxicity, even when external concentrations are low
531 (ECHA, 2017). According to some authors, sediment often plays the role of a local
532 sink for contaminants, which can increase the concentrations of benthic organisms
533 that indiscriminately ingest sediment particles while feeding (Li *et al.*, 2019; Jacobs,
534 1998). In the present study, the BSAFs are mostly low (less than 1) indicating low
535 bioaccumulation (Warnau *et al.*, 1998). These low values are notably related to high
536 trace element concentrations in the sediment. Only Ag, Cd, Mo, Se and Zn have
537 BSAFs higher than 1 with concentrations in organisms equivalent to those recorded
538 in the literature (*e.g.* Guendouzi *et al.*, 2017; Rouane-Hacene *et al.*, 2017; Warnau *et*
539 *al.*, 1998). A high BSAF does not necessarily indicate a risk and rather suggests high
540 concentrations in the organism due to its essential role in physiological processes
541 and low concentrations in sediments, as with Zn for instance. Therefore, the
542 efficiency of trace element absorption from different sources may vary according to
543 ecological needs, organism metabolism and concentrations in different compartments
544 (Bouzahouane *et al.*, 2018). Variations in trace elements within the same species can
545 therefore have different origins. The reproductive stage of the sea urchin is known as
546 a parameter influencing trace element concentrations (El Idrissi *et al.*, 2020). In the
547 present study, the sea urchins are almost all at the same stage of reproduction
548 except at Punta Bianca where about 30% of the sea urchins have already spawned

549 (stage 5) and at the reference site where 50% of the gonads are premature (stage 3).
550 This information probably indicates a low overestimation of gonadal contamination
551 levels at Punta Bianca and the reference site compared to the other investigated
552 sites where a dilution effect occurs (El Idrissi *et al.*, 2020). In this study, BAFs
553 indicate that the majority of trace elements are most bioaccumulated in macroalgae
554 then the gut and later the gonads. Therefore, the use of guts might be more
555 interesting than gonads in studies assessing local variations in trace element
556 contamination. Indeed, this compartment of the sea urchin bioaccumulates more
557 making concentrations higher and therefore the analytical analysis more accurate. In
558 addition, in contrast to the gonads, the majority of the trace elements do not vary
559 according to the sex. Investigations should be conducted to assess whether
560 reproductive stages and the source of food have an impact on the concentrations in
561 the guts. In the present study, we estimated BMF using the guts and macroalgae that
562 the urchin could potentially feed on. Although in some conditions, *P. lividus* can be
563 omnivorous (Wangensteen *et al.*, 2011), it remains mainly herbivorous (Agnetta *et*
564 *al.*, 2013; Boudouresque & Verlaque, 2013). Several factors such as seasonality
565 (Verlaque, 1987), abiotic parameters or resources available within the biotope (Paine
566 & Vadas, 1969; Frantzis *et al.*, 1988) can influence its choice. Therefore, for this
567 work, three species of macroalgae were collected because of the presence of *P.*
568 *lividus* in their beds and their high abundance in the sites. Isotopic analyses identified
569 *E. elongata* as the main food source compared to *D. dichotoma* and *E. amentacea*.
570 Frantzis & Grémare (1992) have already demonstrated a preference of *P. lividus* for
571 this macroalgae which is consistent with our results. The most biomagnified trace
572 elements (Ag, Cd and Mo) are those measured at low concentrations and are among
573 the last elements in the distribution regardless of the matrices studied. Luy *et al.*

574 (2011) have also studied these elements and, according to them, the levels of Ag
575 and Mo measured in seagrass reflect the background level of agriculture in this area.

576 Several studies suggest that exposure to trace element contamination can induce a
577 cascade of events such as ROS production (Farombi *et al.*, 2007; Nieto *et al.*, 2010).
578 Thus, antioxidant defense mechanisms are crucial to maintain the redox balance
579 between pro-oxidants and antioxidants in aerobic organisms (Limon-Pacheco &
580 Gonsebatt, 2009; Chan & Wang, 2019). In order to estimate the effects of trace
581 element contamination on the oxidative stress of *P. lividus*, analyses were performed
582 on the gonads of sea urchins collected at different locations of the contamination
583 gradient (north to south). The highest specific activity of CAT, GST and H₂O₂ was
584 reported in the south of the old asbestos mine where the contamination is the
585 highest. A positive correlation between the specific activity of CAT, H₂O₂ and some
586 trace elements is noted confirming an effect of contaminants on the oxidative stress
587 of sea urchin and on the reliability of biomarkers used in this study. In the
588 mitochondrial respiratory chain, H₂O₂ can react directly with metal ions such as Fe or
589 Cu, by the Fenton reaction, and form the hydroxyl radical which is a powerful oxidant
590 (Regoli & Giuliani, 2014; Mejdoub *et al.*, 2017). Consequently, the elimination of H₂O₂
591 is a key strategy of organisms against oxidative stress (Regoli *et al.*, 2002a; Mejdoub
592 *et al.*, 2017). According to Giarratano *et al.* (2013), the simultaneous induction of
593 GST and CAT activities suggests a similar pattern for hydrogen peroxide removal.
594 Therefore, the increase in these enzymes suggests an activation of detoxification
595 processes, probably reflecting high stress (Louiz *et al.*, 2016). A large number of
596 other studies have shown higher activity of CAT and GST in organisms collected
597 from contaminated sites (Bougherira *et al.*, 2015; Keblouti *et al.*, 2015; Bouzenda *et*
598 *al.*, 2017). Regoli *et al.* (2002b) consider CAT a sensitive and important biomarker of

599 oxidative stress superior to SOD, explaining the invariance of specific SOD activity in
600 this study. These high specific activities of CAT and GST as well as this high H₂O₂
601 content were also measured at the reference site which however has a low
602 concentration of trace elements. The main difficulty in using biomarkers in the natural
603 environment is the interference with other environmental factors (Lagadic *et al.*,
604 1998). In contrast to experiments under controlled conditions, various factors can
605 lead to responses of biochemical parameters in the natural environment (Lagadic *et*
606 *al.*, 1998; Ramade, 2007). As a result, many factors such as meteorological
607 conditions, interactions with other contaminants than those studied, or interspecific
608 relationships can complexify the interpretation of oxidative stress responses (Lagadic
609 *et al.*, 1998). In this case, the same habitat at the reference site was chosen as for
610 the sites near the old asbestos mine in order to minimize variation; it thus appears
611 that other factors led to these high values. Further study would be of interest to
612 determine the source of this significant oxidative stress response. Finally, despite the
613 higher levels of H₂O₂ in sea urchin gonads collected to the south of the mine and at
614 the reference site, the level of MDA, a by-product of lipid peroxidation, did not
615 significantly vary, suggesting that the antioxidant enzyme system of *P. lividus*
616 prevented oxidative damage from occurring (Amri *et al.*, 2017; Ding *et al.*, 2018).

617

618 **5. Conclusion**

619 This study assessed the reliability of sea urchins and macroalgae in the assessment
620 of contamination in coastal ecosystems. The sea urchin gut appears to be a good
621 bioindicator tool for assessing trace element levels. In this context, it would be
622 interesting to perform complementary studies in order to understand the influence of
623 several parameters such as the season and the food sources of the sea urchin. TEPI

624 allowed identification of the different levels of contamination by highlighting the
625 gradients of contamination in the old asbestos mine using sea urchins and
626 macroalgae. TESVI enabled us to determine with efficiency the trace elements
627 characteristic (Co, Cr, Fe, Ni) of the Albo site. The high levels of these trace
628 elements in the different matrices are due to the discharges of the old mine swept
629 along by the sea current and the geology of the region composed by serpentinites.

630 The contamination generated by the old asbestos mine causes oxidative stress very
631 well regulated by the antioxidant mechanisms of the sea urchin. This study also
632 highlighted the need for caution when interpreting biomarkers of stress under
633 environmental conditions due to the different factors involved. Finally, this research
634 shows that the effects of the old asbestos mine are still present and in order to
635 prevent them, it is essential to stabilize the surrounding environment and soils
636 through revegetation action and to maintain monitoring of the marine environment.
637 Currently, a large-scale project to remediate and to rehabilitate the area is underway.
638 A study of the effects of this project on the surrounding marine ecosystems would be
639 relevant at short-term, because of the increased risk of contamination from this
640 rehabilitation work, and in the long-term to monitor potential beneficial effects.

641

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Table Captions

1134 **Table 1.** Trace Element Spatial Variation Index (TESVI) of 22 trace elements
1135 examined in sea urchins (gonads and guts) and macroalgae from six sites near the
1136 old asbestos mine (Corsica, NW Mediterranean Sea; PC: Punta di Canelle; CN:
1137 Canelle; OM: Old Mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: Reference site).
1138 The higher the TESVI value, the greater the spatial variation of that element among
1139 the sampling locations. In dark grey, the higher values.

1140 **Table 2.** Bioaccumulation (BAF), biomagnification (BMF) and biota-sediment
1141 accumulation (BSAF) factor of 14 trace elements. TE: Trace elements; SW:
1142 Seawater.

1143

1144 **Table 1.**

1145

Trace elements	Sea urchins				Macroalgae	
	Gonads		Guts		TESVI	Site x_{max}
	TESVI	Site x_{max}	TESVI	Site x_{max}		
Ag	1.622	NZ	3.964	CN	0.216	PC
Al	4.896	AB	1.321	AB	0.776	AB
As	0.704	AB	0.962	OM	2.357	AB
Ba	0.710	PC	0.182	PB	n.a	n.a
Cd	1.354	PC	0.370	CN	0.639	PC
Co	8.243	AB	31.789	AB	10.124	AB
Cr	194.926	AB	406.518	AB	13.335	AB
Cu	0.589	NZ	0.750	NZ	4.422	AB
Fe	16.854	AB	15.238	AB	10.913	AB
Hg	1.703	AB	0.576	RF	n.a	n.a
Li	0.160	AB	0.247	PB	n.a	n.a
Mn	3.078	AB	6.650	AB	3.897	AB
Mo	0.454	PC	0.652	RF	1.871	AB
Ni	56.107	AB	50.454	AB	17.229	AB
Pb	0.296	RF	0.488	RF	0.422	CN
Sb	0.794	CN	0.304	RF	0.185	AB
Se	0.790	NZ	3.457	NZ	3.451	AB
Sn	1.002	AB	2.190	RF	2.893	AB
Sr	0.960	AB	0.520	PB	0.237	PC
U	3.293	CN	0.120	PB	n.a	n.a

V	3.735	AB	1.310	AB	2.000	OM
Zn	1.429	PC	0.287	PC	0.204	CN

1146

1147

1148 **Table 2.**

1149

TE	BAF			BMF		BSAF	
	gonads/ SW	guts/ SW	macroalgae/ SW	guts/ macroalgae	gonads/ sediment	guts/ sediment	macroalgae /sediment
Ag	50.3526	163.6319	29.8274	5.5936	38.7261	167.7989	22.9402
Al	11.2258	46.8450	117.4314	0.1401	0.0038	0.0210	0.0395
Cd	33.4045	181.6255	13.1774	6.9715	23.6158	171.2038	9.3159
Co	5.5608	18.1816	41.7896	0.1351	0.0205	0.0894	0.1541
Cr	7.5379	45.2430	98.6556	0.1514	0.0086	0.0691	0.1131
Cu	5.0336	14.3122	21.8451	0.2092	0.2221	0.8419	0.9638
Fe	13.1917	59.3457	148.6595	0.1393	0.0086	0.0516	0.0970
Mn	3.7815	18.2950	36.8864	0.1556	0.0109	0.0702	0.1061
Mo	3.1471	13.0912	2.7865	3.2869	2.5737	14.2747	2.2788
Ni	2.2670	11.1937	32.2017	0.1214	0.0081	0.0532	0.1149
Pb	0.0097	0.0361	0.1022	0.1169	0.0794	0.3938	0.8352
Se	170.7810	502.6702	958.6628	0.1535	1.5232	5.9777	8.5503
V	0.7858	1.8492	4.5681	0.1535	0.0720	0.2259	0.4185
Zn	38.8205	10.4672	5.4270	0.8050	8.7230	3.1360	1.2195

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1151

Figure Captions

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1153 **Figure 1.** Location of the study area in Corsica Island (A, B) and the sampling sites
1154 (C, D). PC: Punta di Canelle; CN: Canelle; OM: Old Mine; PB: Punta Bianca; AB:
1155 Albo; NZ: Nonza; RF: Reference site

1156 **Figure 2.** Distribution of trace element (TE) concentrations in sea urchins *P. lividus*
1157 (gonads: A, guts: B), macroalgae (C), seawater column (D) and marine sediments
1158 (E).

1159 **Figure 3.** Trace Element Pollution Index (TEPI) determined using sea urchins
1160 (gonads: A, orange points, guts: B, purple points) and macroalgae (C, green points),
1161 of the seven sites (PC: Punta di Canelle; CN: Canelle; OM: Old Mine; PB: Punta
1162 Bianca; AB: Albo; NZ: Nonza; RF: Reference site). No macroalgae samples were
1163 collected at RF.

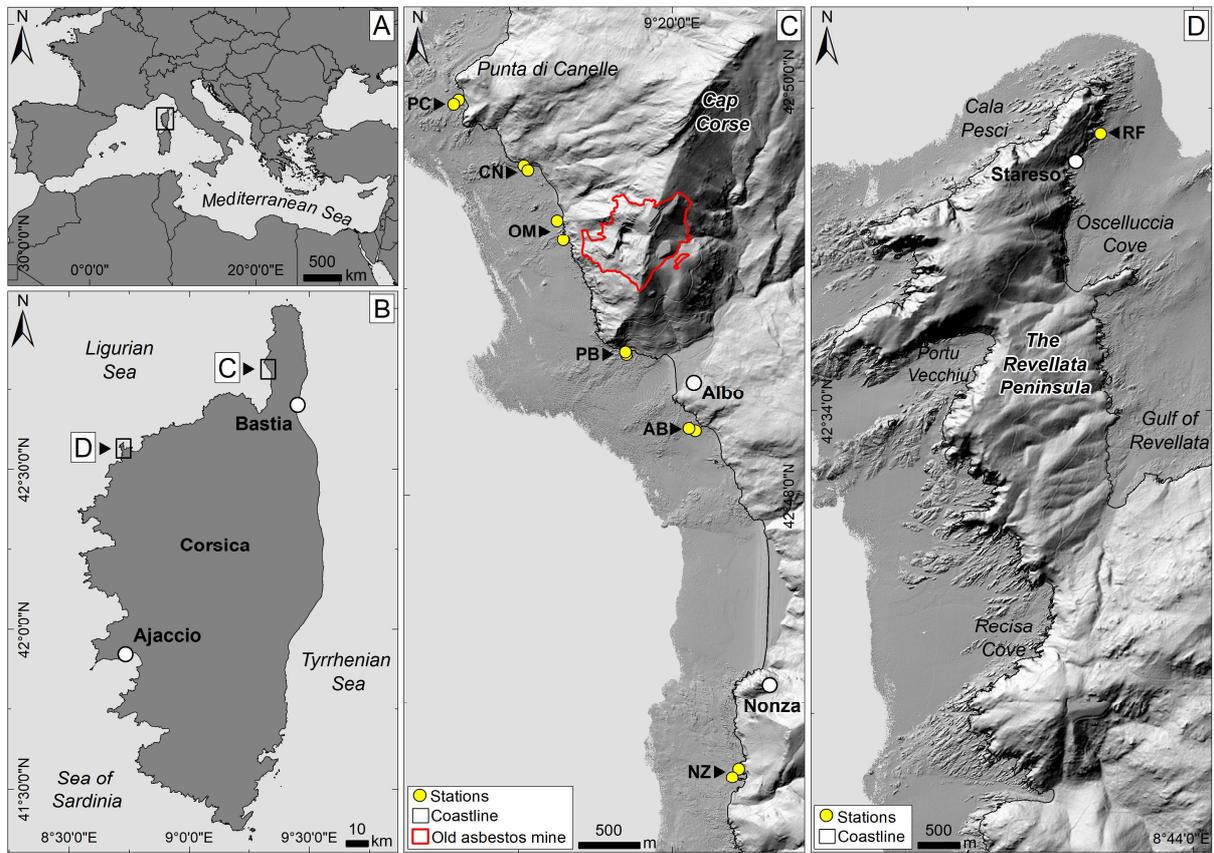
1164 **Figure 4.** Relative contributions of three food sources (*Dictyota dichotoma*,
1165 *Ellisolandia elongata* and *Ericaria amentacea*) to the diet of *P. lividus* individuals
1166 sampled near the old asbestos mine at Canari (Corsica, NW Mediterranean).

1167 **Figure 5.** Maturity stage of sea urchin gonads (%) at each site (PC: Punta di Canelle;
1168 CN: Canelle; OM: Old Mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: Reference
1169 site).

1170 **Figure 6.** Changes in specific activities of (A) superoxyde dismutase (SOD), (B)
1171 catalase (CAT), (C) glutathione peroxidase (GPX), (D) glutathione-S-transferase
1172 (GST) and changes in (E) hydrogen peroxide (H₂O₂) and (F) malondialdehyde (MDA)
1173 contents in gonads of *P. lividus* collected at seven sites (PC: Punta di Canelle; CN:
1174 Canelle; OM: Old Mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: Reference site).
1175 Dissimilar letters denote significant differences between groups (p-value < 0.05).

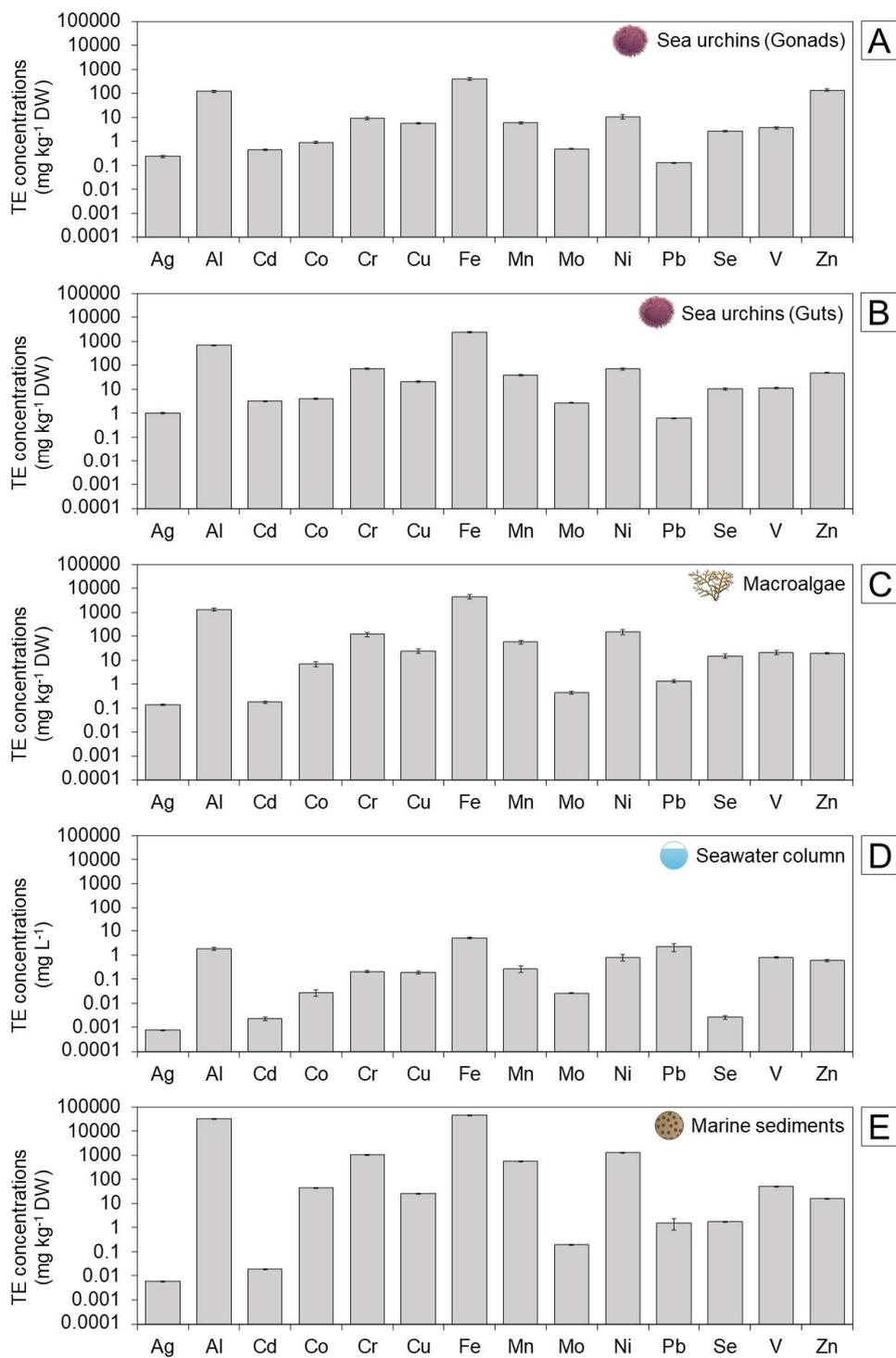
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1177 **Figure 1.**



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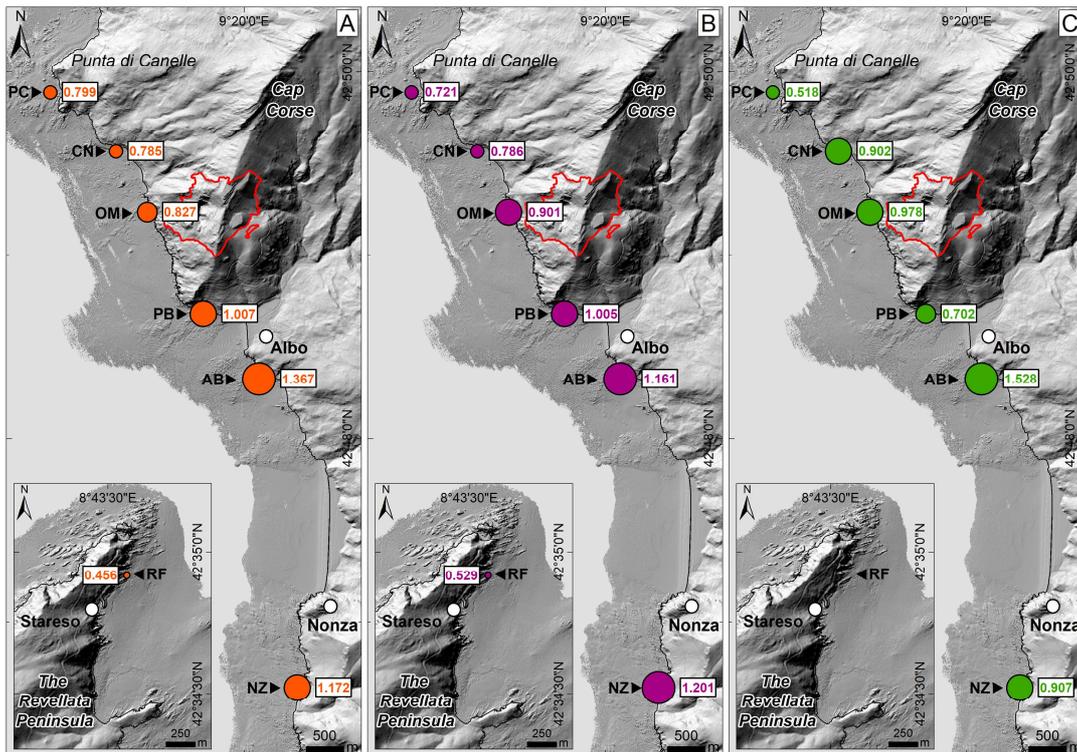
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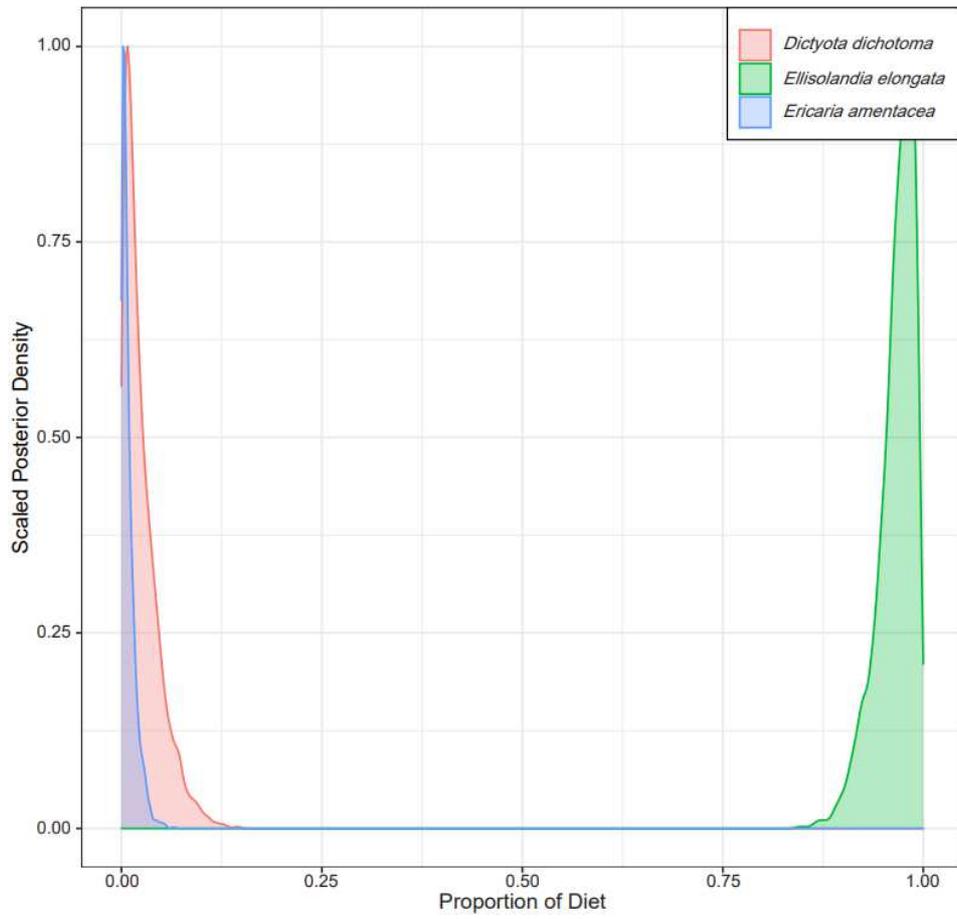
1183 **Figure 3.**



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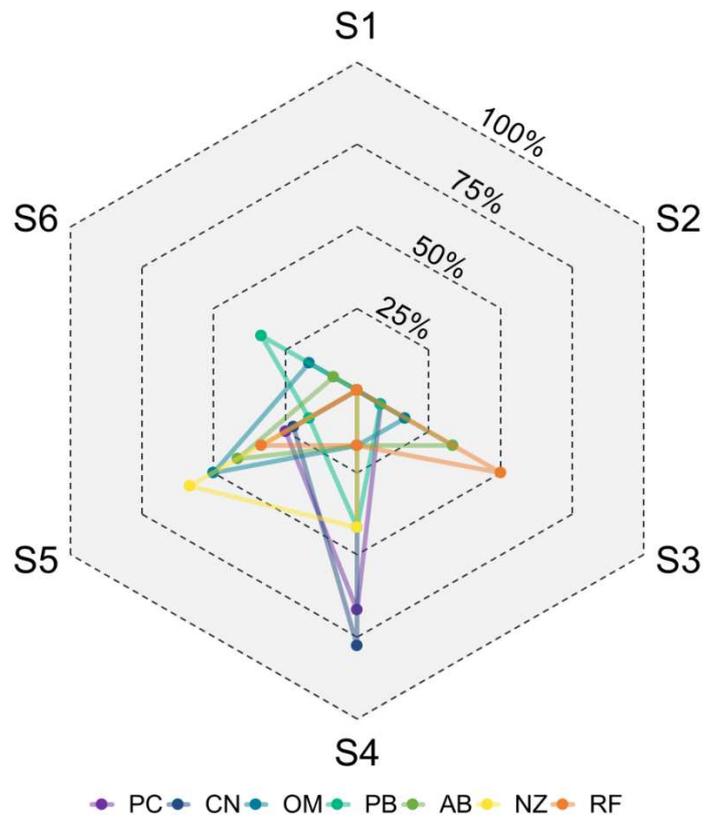
1186 **Figure 4.**



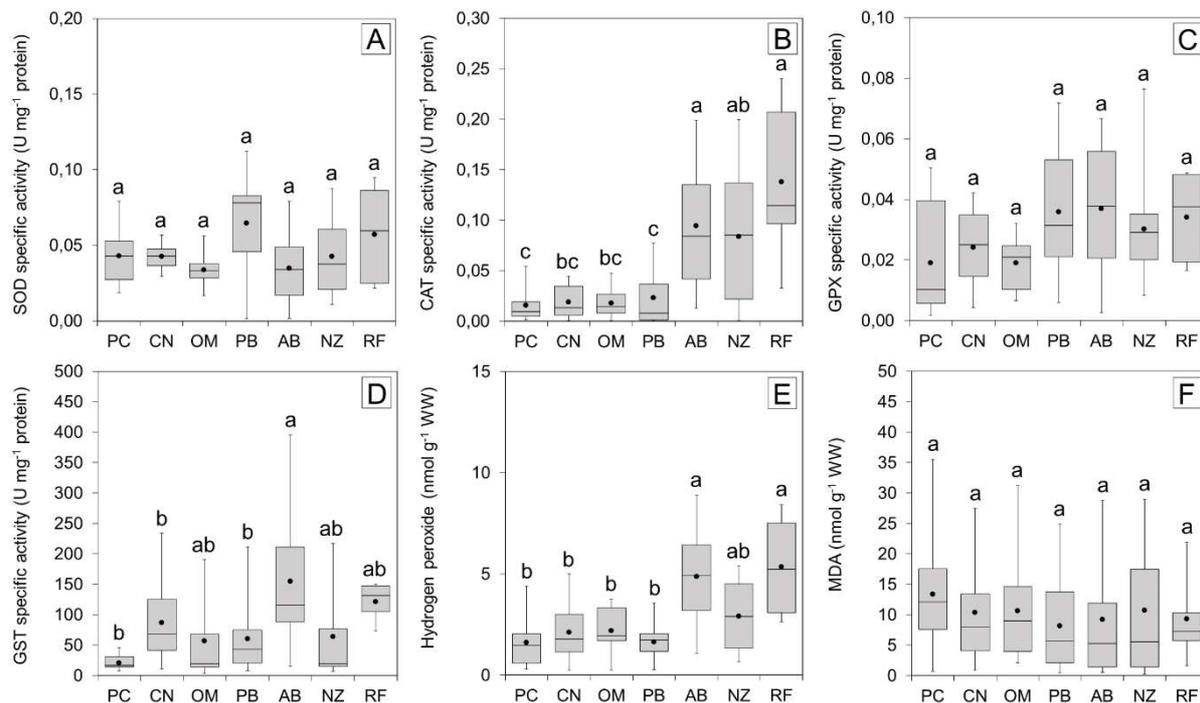
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1189 **Figure 5.**



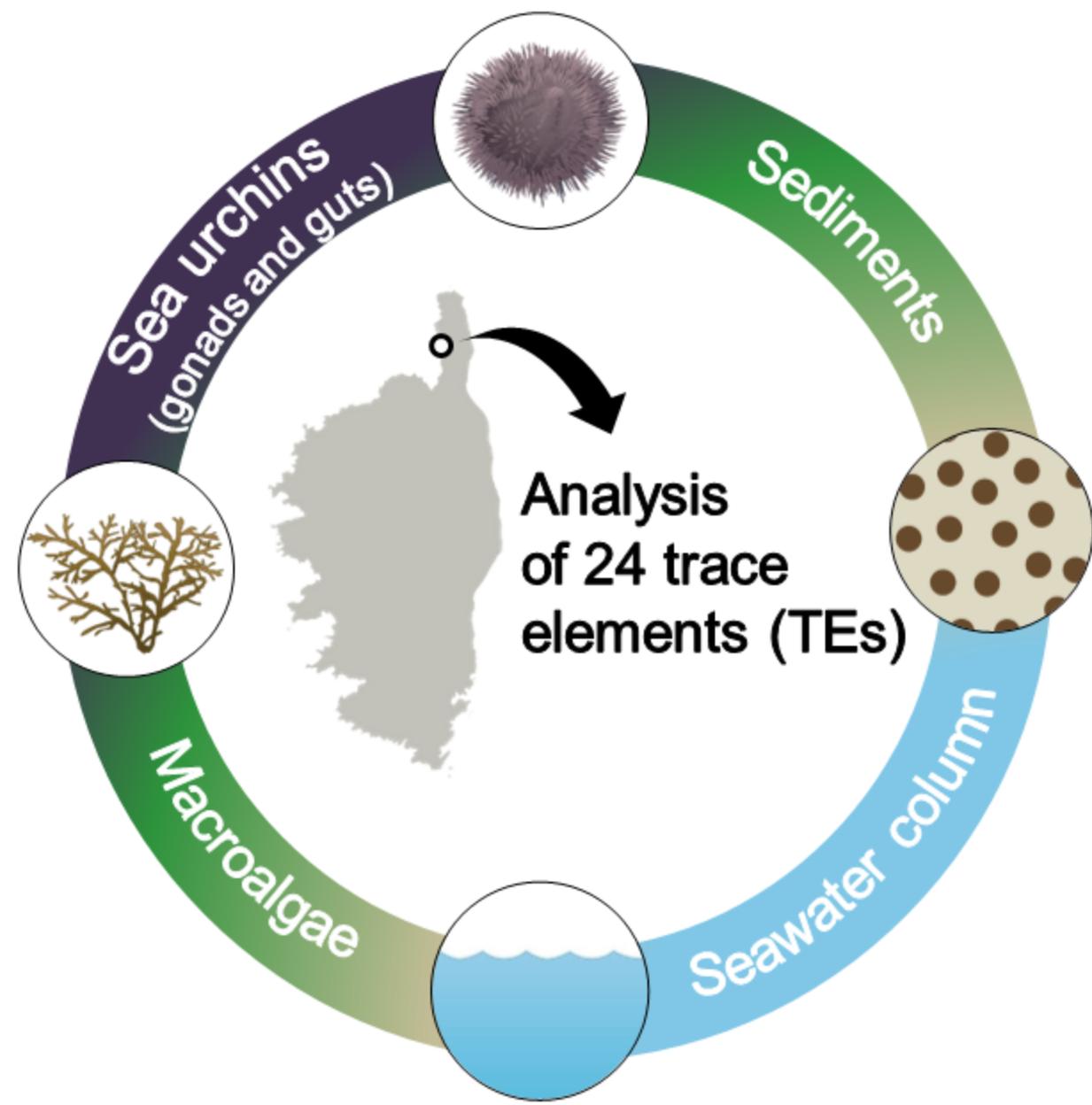
1192 **Figure 6.**



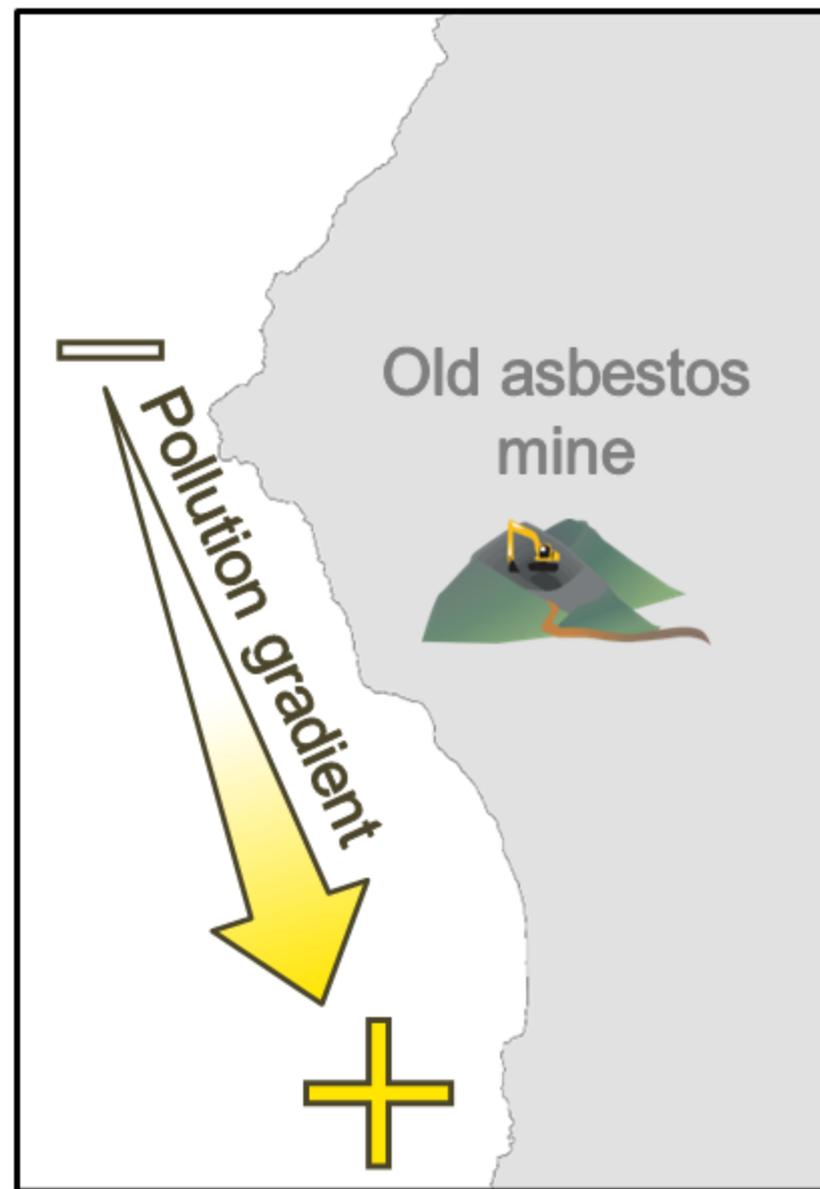
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Assessment of contamination



Spatial distribution



Stress effects on sea urchins

