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Decision-making criteria for plant-species selection for phytostabilization: Issues of biodiversity and functionality

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21 In polluted protected areas, using phytoremediation raises the question of the choice of the
22 plant species to select. As an example, *Atriplex halimus* has been identified as a proliferative
23 plant species that needs to be eradicated in the Calanques National Park (PNCa). Since it has
24 been proven that the spontaneous populations of this plant species could phytostabilize shore
25 waste deposits generated by past industrial activities within the PNCa territory, its status
26 seems controversial, presenting a dilemma between biodiversity management of a protected
27 area and ecological solutions for pollution management. To address this issue, we assessed
28 the ability of *A. halimus* to grow on different soils from this territory, in order to estimate the
29 potential invasiveness of this plant in this territory. Petri dish germinations and pot-growth
30 experiments showed 50% germination of seeds collected on local individuals from the most
31 polluted PNCa soil and 20% growth reduction of seedlings. Soil analysis showed that
32 limitation of growth was caused by high pH value and sparsely available micronutrients as
33 well as metal and metalloid contamination. Our results suggested that local populations of *A.*
34 *halimus* may stabilize the highly metal and metalloid polluted salt-affected soils of the PNCa,
35 with low seed germination potential lowering the eventuality of a propagation over the PNCa
36 territory. As a consequence of this study, the administration of the PNCa decided not to
37 remove *A. halimus* populations along the polluted coastline until another solution to prevent
38 pollution dispersal had been found. This laboratory approach may be extended to other similar
39 situations where plant species may be evaluated not only in term of phytoremediation
40 potential but also in term of biodiversity preservation.

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42 Keywords: *Atriplex halimus*, ecological management, metals and metalloids,
43 phytostabilization, potential invasiveness.

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1. Introduction

Problem statement

Most of the papers dealing with *in-situ* soil phytoremediation examine persistence of selected agronomic plant species in polluted sites and mainly use efficiency of the pollution management as the major criteria of success (Vangrosveld et al., 2009; Kidd et al., 2015). Apart from this mainstream approach, the use in phytoremediation of native plants to avoid introduction of non-native and potentially invasive species that may result in decreasing regional plant diversity has been discussed (Mendez and Maier, 2008) and pilot assays using local plant assemblages for phytostabilization in polluted protected areas have been incremented (Heckenroth et al., 2016). However in certain cases, the status of invasiveness or even more of indigeneity is still controversial for some plant species and new criteria of evaluation of the compliance of these plants for phytoremediation of protected polluted areas have to be investigated. Moreover, although the great majority of invasive species are introduced, occasionally native plant species may become invasive, spreading rapidly into previously unoccupied habitats according to Simberloff (2011), these new habitats may correspond to recently polluted habitats. In France, many national parks host polluted soils in their territory (Desrousseaux and Ugo, 2016) and worldwide this situation occurs regularly (Mazurek et al., 2017; Armendáriz-Villegas et al. 2015). Amongst invasive plant species, some of them are really good candidates for phytoremediation such as *Miscanthus X giganteus* or alimurgic species (Bandiera et al., 2016). However, in protected areas, phytoremediation approach favour phytostabilization i.e. use of plant cover to reduce pollutant mobility in soils rather than phytoextraction i.e. use of the ability of some plants for metal translocation in their aerial parts involving the removal of the aerial parts. Thus, determining the benefit of ecological services vs the proliferation risk of these controversial plant species in polluted protected areas is important to assess and also better understanding

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70 the local parameters and specially soil characteristics that enable or not their proliferation and
71 their ability for metal phytostabilization.

72 *Atriplex halimus*: example of a controversial species in a protected area

73 To illustrate this situation, the plant species *Atriplex halimus* (Amaranthaceae), a
74 xerohalophyte that mainly grows in salt-affected nitrophilous and/or degraded soils with a
75 high tolerance to drought (Walker et al., 2014), may be an expressive case study in the
76 Mediterranean area. This plant species has a high tolerance to metal and metalloid elements
77 (MM) and numerous papers dealing with its potential use for phytoremediation have recently
78 been published, showing the growing interest in this plant species (Caçador and Duarte, 2015;
79 El-Bakatoushi et al., 2015; Manousaki and Kalogerakis, 2009; Márquez-García et al., 2013;
80 Pardo et al., 2014; Pérez-Esteban et al., 2013; see reviews by Lutts and Lefèvre, 2015; Walker
81 et al., 2014). *Atriplex halimus* has been widely cultivated as forage or wind barrier in much of
82 the world (Otal et al., 2010; Walker et al., 2014), although it originated in the Mediterranean
83 Basin (Ortiz-Dorda et al., 2005; Walker et al., 2014). In South East France (Mediterranean
84 coast), spontaneous populations of *A. halimus* are found in polluted coastal soils of a
85 protected area i.e. the Calanques National Park (PNCal). Though part of the populations
86 present on the site may be native of Ibero-Provençal origin, according to Ortiz-Dorda et al.
87 (2005), the Mediterranean National Botanical Conservatory (MNBC), a French authority for
88 plant conservation, considers that many ornamental individuals of this species planted as
89 hedges near housing might have escaped from neighbouring gardens and originated most of
90 the populations currently occurring along the coast of the PNCal on the basis of
91 phytosociological criteria. Therefore the MNBC suggested the eradication of *A. halimus* from
92 the territory of the PNCal in the same way as the notorious invasive species *Carpobrotus*
93 *edulis* in Mediterranean (Affre et al., 2010). However, the composition of the soil near the
94 coastal road in the PNCal was definitely altered by slag deposits from past industrial activities

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95 from the 19th to the mid-20th century ([Laffont-Schwob et al., 2011](#); [Daumalin and Laffont-](#)
96 [Schwob, 2016](#)), and it has been demonstrated that stands of spontaneous *A. halimus*
97 phytostabilize these polluted soils, preventing soil erosion, diffuse pollution and
98 contamination of the food web ([Rabier et al., 2014](#)).

99 This turns to a cornelian dilemma for the PNCal between plant conservation including
100 invasive plant eradication and maintaining pollution management to prevent pollutant transfer
101 to terrestrial and sea biocoenosis. Therefore, a set of effective parameters has been examined
102 to provide decisional criteria for the PNCal.

103 Methodological approach

104 A genetic approach for the study of the PNCal *A. halimus* populations would be too
105 expensive, time-consuming and probably not effective for determining the autochthonous
106 status or not of these populations. In addition to showing high genetic variability ([Abbad et al.](#)
107 [2004](#); [Ortíz-Dorda et al. 2005](#)), local populations may have been subject to microevolution
108 processes due to the driving force of high MM pollution, as has been recently proven for
109 another pseudo-metallophyte ([Słomka et al., 2011](#)).

110 An ecological approach on life traits may be more accurate to evaluate the potential of
111 invasiveness of this species on a specific territory. *A. halimus* mainly spreads via seeds that
112 may be transported by animals, air or water and, to a lesser extent, can propagate vegetatively
113 ([Walker et al., 2014](#)). A potential spread of this species at the expense of other native plant
114 species, especially rare ones, needs to be taken into account. Therefore the invasiveness
115 potential may be evaluated as the ability of this species to germinate and grow in soil
116 conditions differing from those in which its currently develop. While the tolerance of *A.*
117 *halimus* to MM in coastal areas has been demonstrated previously on older *in situ* individuals,
118 no information is available on its capacity to establish on the more or less polluted soils of

119 this territory. More scientific information on the ecological preferendum of *A. halimus* is thus
120 necessary. As a first step, the ability of local *A. halimus* populations to germinate and grow on
121 various soils from the PNCal territory needs to be assessed. Therefore, an ex-situ experiment
122 was conducted in laboratory on the germination, seedling growth and MM accumulation
123 capacities and on chemical and ecological traits of *A. halimus* in various PNCal soils. The
124 results are discussed in a global perspective to propose a methodological approach for the
125 selection or keep up of non-native or potentially invasive plant species in polluted protected
126 areas.

2. Materials and methods

2.1. Study area and soil sampling

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129 The current PNCal territory was the site of metallurgical and chemical industrial activities
130 from the mid-19th century until the beginning of the 20th century. Silver–galena ore was
131 treated by pyrometallurgical processes during this period (Daumalin and Raveux, 2016). The
132 former Escalette factory (in operation from 1851 to 1924) was the one that had the most
133 intensive activity in the area. The factory, located on the lower slopes of a hill, was
134 characterized by a horizontal smelter chimney as the condensing system. The ruins of this
135 chimney are still present today. In well-constructed flues, the deposit could yield from 2 % to
136 3 %, while loss dispersed in the smoke could amount to around 10 % of the lead produced in
137 the ore, depending on its quality (Percy, 1870). Slag was deposited on the old factory site, but
138 was also scattered along the coast in several main deposits and as roadfill. Six sites were
139 selected for this study (supp. data 1), located in the peri-urban area of Marseille, south-east
140 France, i.e. Calanque de Saména (SA, 43°13.749' N; 5°20.960' E) and Calanque des Trous
141 (TR, 43°13.233' N; 5°20.766' E), corresponding to soils from moderately polluted seashore
142 sites where *A. halimus* grows spontaneously; Escalette Chimney (E.C, 43°13.584' N; 5°21.32'

144 E) and Escalette Slagheap (E.S, 43°13.454' N; 5°21.126' E), corresponding to soils from the
145 former Escalette smelter heavily polluted by two different steps of the smelting process; Cap
146 Croisette (CC, 43°12.812' N; 5°20.899' E), a site exposed to very low pollution and seaspray;
147 Sormiou (SO, 43°12.806' N; 5° 24.964' E), a site exposed to very low pollution and no
148 seaspray; and in order to have a reference condition, was chosen as a control substrate a
149 loamy horticultural soil (CN, mixture of peat moss, sphagnum peat moss, wood fiber and
150 plant cultivation support composted with NPK 8-2-7, Botanic®). The areas were chosen on
151 the basis of previously published data on metal and metalloid contamination in the PNCal
152 area (Laffont-schwob et al., 2016) and according to similar physico-chemical characteristics
153 of the soils (except level of MM contamination). All soils are pooled samples of five sub-
154 samples collected on the top soil: for SA, TR and CC, soils are from the root zone of *Atriplex*
155 populations as previously described (Rabier et al., 2014); for E.C., E.S and SO, soils samples
156 were collected under matorral plant cover (Testiati et al., 2013; Affholder et al., 2013).

157 On each of the six selected sites, soil samples were collected from the top 15 cm after removal
158 of the litter. In order to obtain representative samples of each site, a composite soil sample
159 was achieved by mixing 5 subsamples of equal weight. These 5 subsamples were collected 2
160 m apart in a cross pattern at each site, and were sieved on site to 2 mm mesh. Each composite
161 soil sample was stored in a plastic bag until returned to the laboratory.

162 An *ex-situ* assay by pot experimentation in the laboratory was conducted with these
163 composite soils, to assess the germination and growth capacities of *A. halimus* from seeds
164 collected on spontaneous individuals in the area on these MM and salt-affected soils.

166 2.2. Soil physico-chemical analyses

167 In the laboratory, soil composite aliquots were air-dried at room temperature and then ground
168 to pass through a 0.2 mm titanium sieve (RETSCH zm 1000 with tungsten blades) before
169 analyses. Physico-chemical parameters of soil, i.e. salinity, pH, conductivity, texture, total
170 organic carbon and total Kjeldahl nitrogen content, and trace and major element
171 concentrations, were determined on 3 analytical replicates of each soil composite sample per
172 site.

173 Measurements of pH (ISO 10390, 2005) and salinity (calculated from conductivity) were
174 determined by potentiometry in a 1:5 soil:water suspension using a Multi 3420 SET B-WTW
175 pH meter and ECmeter (Baize, 2000).

176 Total organic carbon (TOC) quantifications were carried out with a Jena Analytic TOC-
177 N/C2100S, coupled with HT1300 solid module (ISO 10694, 1995). Total Kjeldahl nitrogen
178 content was measured with Büchi 323 digester and distillation units (ISO 11261, 1995).

179 For MM analyses, soil samples were mineralized in a microwave mineralizer (Milestone Start
180 D) using *aqua regia* (1/3 HNO₃ + 2/3 HCl). The solutions obtained for soil mineralization
181 were filtered with a 0.45 µm mesh and the metal levels were determined by ICP-AES
182 (JobinYvon Horiba, Spectra 2000) for As, Cu, Fe, Mn, Pb, Sb and Zn (Lotmani et al., 2011);
183 flame AES (Thermo Scientific ICE 3000) was used for Na and K measurements. Cd was not
184 taken into account since results showed concentration levels far below 1ppm. Quality
185 assurance controls and accuracy were checked using standard soil reference materials (CRM
186 049-050, from RTC-USA) with accuracies within 100 ± 10 %. Results are presented in supp.
187 data 2.

188 The following analyzes were performed by the Laboratoire Développement Méditerranée at
189 Alès (Gard, France). The soil texture was measured on five non-decarbonated fractions (NF X
190 31-107, 2003), the exchangeable P (P₂O₅) was determined according to the Joret Hébert

191 method (ISO 11263, 1995), the cation exchange capacity (CEC) was measured according to
192 the Metson method (ISO 23470, 2007), extraction of the exchangeable cations was carried out
193 with ammonium acetate (ISO 23470, 2007).

195 *2.3 Seed collection*

196 *A. halimus* seeds were collected in September 2013 from 5 individuals of the spontaneous
197 population at Escalette (highly polluted site, supp. data 1), then pooled and stored in paper
198 bags at room temperature until use. No information is available on this population regarding
199 its potential ornamental origin, but it was confirmed that the chromosome number of this
200 populations was diploid ($2n = 2x = 18$) (Snow, 1963), corresponding to the phenotype of the
201 subsp. *halimus* originating from France and Spain, according to Le Hou  rou, 1992; McArthur
202 and Sanderson, 1984; Talamali et al., 2004; Walker et al., 2005. This population is thus
203 characteristic of the particular conditions of *A. halimus* populations in the area.

205 *2.4. Seed germination experiment*

206 Seeds were removed from their bracts by dragging them over sandpaper. The seeds were
207 surface-sterilised just before use by immersion in 5 % (v/v) sodium hypochlorite for 10 min
208 and rinsed three times in sterile water, a treatment that does not affect germination parameters
209 or seedling characteristics (Mu  oz-Rodr  guez et al., 2012).

210 The seeds were germinated under controlled conditions with 12/12 h of day/night at 24/20   C,
211 respectively, constant humidity 60 %, that have been proven to be appropriate conditions to
212 enhance the percentage of germination in *A. halimus* (Mu  oz-Rodr  guez et al., 2012). The
213 Petri dishes were put in a plant growth chamber (SANYO MLR-351H). The light was

214 provided by fluorescent lamps that produce an average photosynthetic photon flux density of
215 300 $\mu\text{mol}/\text{m}^2/\text{s}$. Germination tests were carried out in Petri dishes filled with 40 ml of every
216 soil type (corresponding to 20 g of composite soil (sieved to 2 mm) from each site and 5 g of
217 control substrate (CN), due to the different substrate densities). These different soils were
218 watered with distilled water to near field capacity. Ten replicates of 30 seeds were used for
219 each soil type. Germination was monitored every 2-days by counting the number of
220 germinated seeds per Petri dish (Keiffer and Ungar, 1997). Seeds were considered to have
221 germinated after radicle emergence.

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223 *2.5. Growth conditions and plant morphology*

224 The six composite soils (sieved to 2 mm) and the control (CN) were distributed in individual
225 120 ml-paper pots (100 ml of dried soil per pot from the six PNCal sites or CN) in order to
226 get 10 pots for each soil type. These pots were placed in a plant growth chamber under the
227 same previously described conditions and were sprayed with distilled water until soil water-
228 holding capacity (WHC) was reached. WHC had been previously determined for each soil
229 type.

230 Seedlings from the Petri dishes were transplanted into the pots, being careful to keep the
231 seedlings from one type of soil in the Petri dish in the same soil in the pot (7 types of soils i.e.
232 6 sites and CN, 5 plants per pots, 10 replicates per type of soil). Seedlings were grown for 63
233 days and they were watered with distilled water every two days to restore the initial WHC.

234 Plant height (shoots) and number of leaves were measured every 7 days.

235 At the end of the experiment, the plants were carefully removed and then separated into roots,
236 stems and leaves. Roots were thoroughly washed with tap water to clean off soil particles and

237 were rinsed with deionized water (three successive rinses of 30 s each), then gently blotted
238 between paper towels. Morphological parameters (root length, shoot length, and leaf number)
239 were measured on each plant.

240 Leaf surface areas were determined using a scanner and ImageJ software (URL:
241 <http://rsbweb.nih.gov/ij/>).

242 Shoot and root fresh weights (FW) were determined immediately. The pooled root samples
243 per pot were oven-dried at 40 °C for 48 h to determine the root dry weight (DW). Dry weight
244 determination was done identically from pooled shoot samples per pot.

245 Hydration rates (HR) were calculated on shoots and roots of plants grown on the different
246 soils, as described elsewhere ([Rabier et al. 2007](#))

247 The root and shoot dry material was ground separately for metal analysis. Finally, MM
248 concentrations were analyzed in root and shoot parts.

249 *2.6. Non-destructive plant physiological index measurements*

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251 At three different stages (35, 55 and 63 days), plant physiological indices were estimated
252 optically using a Multiplex® 3 non-destructive measurement equipment (FORCE-A, Orsay,
253 France; [Agati et al., 2011](#)). This portable fluorimetric device uses fluorescence technology
254 with multiple excitations to measure constitutive and induced epidermal phenols, flavonols,
255 anthocyanins, chlorophylls and a chlorophyll-to-flavonoid ratio referred to as nitrogen
256 balance index (NBI). Different combinations of the blue-green, red and far-red fluorescence
257 signals at the various excitation bands could be used as indices of the different compounds
258 ([Agati et al., 2011](#); [Cerovic et al., 2008](#)). Though we used the term 'anthocyanin indices'
259 because the apparatus was developed for relative measurement of anthocyanin, the red

260 pigments of *A. halimus* are betalains, as for other members of the Amaranthaceae with a
261 similar absorbance spectrum (Lavene, 1995; LoPresti, 2015). For each type of soil, the
262 average of five measurements was made per pot and was repeated for the 10 pot replicates.
263 Before starting the measurement by Multiplex, we covered the soil of each pot with pieces of
264 blackboard wall stickers to avoid fluorescence of organic matter from soil.

266 2.7. Plant elemental analysis

267 Before analysis, dried shoot and root plant samples from the 10 replicates were pooled
268 separately. They were then ground to pass a 0.2 mm mesh titanium sieve and three aliquots
269 were analyzed by sample. About 0.5 g of dry matter was digested with the microwave
270 digestion system Milestone start D with a HNO₃, H₂O₂ and ultra-pure H₂O mixture (volume
271 proportion ratio 2:1:1). Extracts were analyzed for pseudo-total metals and metalloids (MM)
272 i.e. Cu, Fe, Mn, Pb, and Zn content, using inductively coupled plasma-atomic emission
273 spectroscopy (ICP-AES, JY 2000 Jobin Yvon Horiba) and by graphite furnace AAS (Thermo
274 Scientific ICE 3000) for As and Sb, while flame AES (Thermo Scientific ICE 3000) was used
275 for Na and K measurements. Three analytical replicates were performed for each type of plant
276 sample. Standard plant reference materials (DC 73349) from China National Analysis Centre
277 for Iron and Steel (NSC) were analyzed as part of the quality control protocol (accuracies
278 within 100 ± 10 %).

279 To evaluate the phytoremediation ability of the plants, bioaccumulation factors (BCF) and
280 translocation factors (TF) in each soil were computed according to the following formula
281 (Yoon et al., 2006):

$$282 \text{BCF} = C_{\text{root}}/C_{\text{soil}} \text{ (1)}$$

283 $TF = C_{shoot}/C_{root}$ (2)

284 Where C_{soil} , C_{root} , C_{shoot} are average concentrations of trace elements in soils, roots and shoots,
285 respectively. These factors indicate the importance of pollutant transfer from soils to roots
286 (BCF) and from roots to shoots (TF).

287 A quantitative approach of the multi-element contamination can be made based on the
288 pollution load indices (PLI) calculated for each soil following [Rashed \(2010\)](#):

289 $PLI = \sqrt[5]{CF_{As} \times CF_{Cu} \times CF_{Pb} \times CF_{Sb} \times CF_{Zn}}$ (3)

290 with $CF_{MM} = [MM]_{considered\ soil}/[MM]_{SO}$

291 CF being the soil contamination factor and Sormiou (SO) being considered as the reference
292 area for this study since MM soil concentrations at this site are very close to the local
293 background of contamination in the PNCal area ([Affholder et al., 2014](#)). A PLI value strictly
294 above 1 indicates that the soil may be considered as polluted.

296 2.8. Root symbiosis observations

297 Before the final harvest, an individual from each pot was removed for root symbiosis analysis
298 (10 replicates per soil type). The roots of these plants were rinsed first under tap then
299 deionized water, pooled in three replicates for each soil type and stored in alcohol (60 %, v/v)
300 at room temperature until proceeding. To limit the use of highly toxic products, we used the
301 method described by [Vierheilig et al. \(1998\)](#) for staining fungal structures before observation.
302 First roots were soaked for 3 min in 10 % KOH bath at 80 °C then rinsed and stained with
303 Pelikan ® blue ink, in a 5 % acetic acid solution at 90 °C for 3 min. The roots were mounted
304 on slides. For each sample, five slides were prepared, each containing 10 root fragments of 1
305 cm length. Therefore the results are based on the observation of 50 fragments of roots per

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5 306 replicate. The arbuscular mycorrhizal (AM) structures were sorted into mycelium (M),
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8 307 vesicles (V) and arbuscular (A) structures, using optical microscopy at 100 and 400
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10 308 magnifications.

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13 309 The structures of dark septate endophytes (DSE) i.e. septate melanized mycelia and
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15 310 microsclerotia were observed. The percentages of each symbiotic structure (A, M, V and
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17 311 DSE) observed in the samples were independently estimated for each soil type, using the
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19 312 formula of [Zhang et al. \(2010\)](#):

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21 313 Percentage of fungal structures observed = $100 * \text{number of fragments where the structure}$
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23 314 $\text{was observed} / \text{number of observed fragments}$. Information about root symbioses may
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25 315 indicate the ability of the plant to interact with edaphic micro-organisms, playing a role in
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27 316 plant pollutant tolerance but also giving an indication of the potential occurrence of symbionts
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29 317 in polluted soils (biological characteristic of soils).

30 31 32 318 33 34 35 319 *2.9. Statistical analysis*

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38 320 Statistical analyses and control charts were performed for all data using JMP 11 statistical
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40 321 software (SAS Institute, Cary, North Carolina, USA). *A. halimus* germination percentages,
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42 322 MM concentrations in plant parts and soils, plant stress indices and fungal root colonization
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44 323 percentages were compared using non-parametric Wilcoxon rank sum test (Kruskal-Wallis
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46 324 test) and Wilcoxon each pair test.

47 48 49 50 51 325 52 53 54 326 **3. Results**

55 56 57 58 327 *3.1. Soil characterisation*

328 Except for the control substrate (CN) with a pH value ca. 6, all soils of the PNCal area were
329 alkaline including the technosols (E.C and E.S), being characteristic of local limestone soils
330 (Fig. 1). SO had the lowest conductivity and SA the highest, but lower than 2000 $\mu\text{S}/\text{cm}$.
331 Therefore, according to natural soil saline classification and conductivity data, all the PNCal
332 soils, as well as CN, may be considered as non-saline.

333 TOC values in all PNCal soils were very low except for SO, with an average value of 110
334 mg/g. The TOC values were 14 (CC) to 3.5-fold lower (SO) than CN. No NTK differences
335 were observed for SA and SO compared to CN, although other NTK values were up to 7-fold
336 lower (E.C, E.S) than CN. The fertility of these soils could be thus considered as low.

337 The texture of soils from the coastal sites (SA, TR and CC) was sandy loam as well as for
338 CN, but with less coarse sand for the latter (Fig. 1). The granulometry of SO soil
339 corresponded to clay loam while the technosols (E.C, E.S) were sandy clay loam with coarser
340 sand for E.S.

341 According to agronomic criteria (Fig. 1), soils from the coastal sites contained highest
342 concentrations of exchangeable Na but also of the major fertility elements (P, Ca, Mg, K),
343 with less exchangeable P for TR. The technosols (E.C, E.S) and the soil from SO had a low
344 content of exchangeable Na but also of exchangeable P, Ca, Mg, K. As expected, CN
345 presented good fertility indices.

346 The E.S and E.C technosols had the highest Pb and Zn contents whose varied from 1 to 3 %,
347 and a very high content of As in E.C (about 0.3 %), but also high values of Fe, i.e. 1 to 5 % as
348 for all PNCal soils (Fig. 2 and supp. data 2).

349 We used the PLI to compare the relative load of the mixed pollution of the various soils. PLI
350 for E.C and E.S soils were at least 20-fold higher than all the other soils i.e. $\text{PLI}_{\text{E.C}} = 216$ and
351 $\text{PLI}_{\text{E.S}} = 203$ whereas $\text{PLI}_{\text{SA}} = 6$, $\text{PLI}_{\text{TR}} \& \text{PLI}_{\text{SO}} = 4$, $\text{PLI}_{\text{CC}} = 1.4$ since $\text{PLI}_{\text{CN}} = 1.3$. This

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5 352 revealed a potentially high ecotoxicological risk associated with both technosols i.e. ingestion
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7 353 or inhalation of soil particles may expose biocoenosis to non negligible effects of trace
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9 354 elements.

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12 355 Following these analyses, the loamy horticultural soil (CN) could be considered as control
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14 356 soil and the soil of CC and SO were the least PNCal polluted soils, being also the least
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16 357 seaspray-affected of the four seashore soils.

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19 359 *3.2. Germination test*

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21 360 The percentage of germination after 14 days was 80 % in the CN (Fig. 3), while significantly
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23 361 lower for all the other soils tested (it varied from 20 to 60 %). However, the germination of
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25 362 this plant species was mostly affected by the combination of alkalinity and salinity (TR, Fig.
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27 363 1). The germination percentage at 4 days was negatively correlated both with soil pH and
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29 364 conductivity (supp. data 3). The results also showed a weak negative correlation after 14 days
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31 365 with Zn ($r_s = - 0.45$, p-value < 0.0001) and Cu ($r_s = - 0.45$, p-value < 0.0001). Very weak
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33 366 negative correlations were observed for As ($r_s = - 0.30$, p-value < 0.0001) and Pb ($r_s = - 0.28$,
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35 367 p-value < 0.0001). The negative correlation between germination and pH increased with time,
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37 368 while the correlation with exchangeable Na and conductivity decreased. The negative
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39 369 correlation between germination and soil Zn concentration increased with time. These results
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41 370 can be interpreted as a rapid osmotic effect for germination inhibition by conductivity, with
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43 371 possibly a partially transitory priming effect for Na (Capron et al., 2000) and a progressive
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45 372 inhibition by MM, mainly Zn.

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48 373 These results demonstrated the ability of *A. halimus* to germinate up to 50 % even in the case
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50 374 of soils containing up to 2 500 mg/kg of As and up to 30 000 mg/kg of Pb near the horizontal
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1 375 chimney (E.C), and the limitation of germination potential by pH alone or combined with
2 376 salts for all the PNCal soils.
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7 8 9 378 *3.3. Growth traits*

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12 379 Overall, the growth traits expressed as percentages compared to control can be classified by
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14 380 increasing order of effects, as follows: survival (given by the number of individuals) < shoot
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16 381 and root lengths < shoot and root fresh weights < leaf surface area (Fig. 4). In other words,
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18 382 amongst growth traits, leaf surface, length and plant weight were the most significantly
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20 383 inhibited with 60-90 % reduction compared to control. After germination, individual loss
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22 384 continued during plantlet growth up to 10-20 % for TR whereas no significant losses were
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24 385 observed for the four other PNCal soils. Reductions of all parameters except survival for E.C
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26 386 and SO soils were significantly greater than those for SA soil. The growth traits were not
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28 387 significantly inhibited for SA and only concerning leaf surface and shoot fresh weight for CC.
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32 388 The growth traits not significantly inhibited were survival, shoot and root length for E.S and,
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34 389 shoot length and root fresh weight for TR.
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43 391 *3.4. Plant chemical traits*

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46 392 After 63 days, the highest indices of flavonol, betacyanin (referred as antocyanin index for
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48 393 Multiplex® index) and phenol were obtained for plants grown in SO soil, whereas phenol and
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50 394 betacyanin indices were the lowest in CN soil. Chlorophyll indices were the most stable, with
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52 395 significantly lower values in E.S, E.C and CC soils than in other soils.
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57 396 Monitoring of plant chemical traits with non-destructive index measurements (Fig. 5) enabled
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59 397 observation of a time-dependent response. As a first step, betacyanin indices decreased in
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398 basal leaves and stems in CN and SA and increased in SO soils, immediately followed by an
399 increase in chlorophyll index in all soils except SO, then in phenol indices in all soils and
400 finally a decrease in flavonol indices. The latter was concomitant with the appearance of
401 necrosis in the basal leaves in E.C and E.S. Moreover, there were no specific visual symptoms
402 of toxicity and/or necrosis on the other leaves and heterogeneity of betacyanin pigmentation
403 developed in the same way from rib to limb on CN soil as on the PNCal soils. The general
404 trend of the curves (Fig. 5) showed that this physiological succession was faster for the
405 control. Thus part of the response of chemical traits may be due to the physiological stage of
406 the seedlings.

3.5 Metal and metalloid transfer to plants

409 Concerning non-essential elements, the highest accumulations of Pb and Sb in roots were
410 measured in E.C and E.S soils and for As in roots, only in E.C soil (Fig. 2). For CC and SO
411 soils, Sb and As concentrations in roots were similar to those of the control whereas Pb
412 concentrations were still above those of CN. In some of the PNCal soils, Pb, As and Sb
413 concentrations in roots were not those of normal tissue concentrations but those of possibly
414 harmful concentrations (Markert, 1994; Prasad et al., 2006). The major pollutants in root
415 tissues were As and Pb in E.C soil, with 3 439 mg/kg and 24 444 mg/kg respectively, in
416 accordance with the composition of the slag deposit from the smelter industrial activity.
417 Metals and metalloids (As, Pb, Sb, Zn, Cu) present in soils were weakly transferred to the
418 roots ($BCF < 1$) (supp. data 4), except in the case of E.C for As, Pb and Zn, of CN for Cu, of
419 SA for Cu and Sb, of CC for As, Cu and Zn, and of SO for Cu. Even in the soil of E.C, there
420 was a selective transfer of each of these elements between the soil and the root parts, as Pb
421 was better transferred to root than Sb and Zn (Fig. 2). The transfer coefficients from soil to

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422 aerial parts were always < 1 for As, Cu, Fe and Pb (supp. data 4). TF values were more than 1
423 in the case of K and Na for all conditions. TF values were only > 1 for Zn in the case of CN,
424 CC and SO. Mn was present in soils at moderate to high concentrations, with higher
425 concentrations in E.S, SO and SA soils. However, its transfer to shoots was particularly low
426 in E.C, E.S and SA soils, although Mn TF value was high for the CN.

427 The elements that are regulators of osmotic pressure, e.g. Na and K (Fig. 2), were
428 preferentially accumulated in shoots in all the soils tested. However, the concentration ratios
429 between Na and K in shoots were strictly above 1 for TR and SA, and below 0.5 in all the
430 other PNCal soils, while this ratio was equal to 0.51 for CN (supp. data 5).

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3.6. *Root fungal colonization*

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433 Two types of fungal structure were observed in *A. halimus* roots i.e. arbuscular mycorrhizal
434 fungi (AMF) and dark septate endophytes (DSE). Both kinds of symbiotic microorganisms
435 were present in all root systems for each of the PNCal soils. A discriminant analysis was
436 applied on the percentages of each fungal structure (Fig. 6). Along the first dimension, there
437 was a separation between CN and CC, with a higher occurrence of AM colonization, and E.C
438 and E.S with more DSE. In the second dimension, more arbuscules were observed in roots
439 from CC and SO soils. With this analysis, three main groups may be identified: (i) CN, (ii)
440 CC, TR, SA and SO, (iii) E.C and E.S. A strong correlation was detected between DSE
441 colonisation percentages and As, Pb and Zn concentrations in soils, with Spearman
442 coefficients of 0.70, 0.63 and 0.56, respectively (p -value ≤ 0.01). DSE colonisation
443 percentage was also negatively correlated with available P in soil (Spearman coefficient of -
444 0.55 at p -value ≤ 0.01).

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446 4. Discussion

447 4.1 Do PNCal soils have good agronomic properties?

448 The metal and metalloid contamination of PNCal soils has previously been partially studied
449 and the diffuse pollution of these soils has been discussed in separate publications ([Affholder
450 et al., 2013](#); [Heckenroth et al., 2016](#); [Rabier et al., 2014](#); [Testiati et al., 2013](#)). However, in
451 this study, soil characterization was completed by fertility parameters for the seashore sites.
452 Soils were alkaline with pH ranging from 7.5 to 8.5, typical of calcareous areas, but the
453 highest exchangeable Ca and K values found for the soils from less polluted areas of the
454 seashore were associated with the presence of scrap materials such as lime mortar for Ca, and
455 for K an origin that could be linked to bricks and tiles with aminosilicate of potassium as the
456 active substance ([Testiati et al., 2013](#)). The soils from the seashore were mainly sandy clay
457 soils, while the granulometry of the technosols from E.C and E.S had no relation with the
458 mineralogy of natural soils. There was more fine dust in E.C without coarse sand and coarser
459 sand and less fine dust in E.S soil. Technosol from E.C seemed to be very compact when dry,
460 like real clay soil.

461 Concerning soil pollution, the strong correlation between As and Pb, particularly for E.C soil,
462 was probably related to their deposition as lead arsenate, while the correlation between As
463 and Sb was explained by their belonging to the same group in the periodic table in relation to
464 the same behaviour during the ore treatment and dust deposit in soils ([Testiati et al., 2013](#)). Sb
465 concentrations were low in soils except for E.S and E.C, where As, Pb, Zn and Cu, whose
466 toxicity is more well-known, were also present at high concentrations.

467 Zn concentration was locally important ([Testiati et al., 2013](#)), which could be explained by
468 differences in ore origin during the smelter activity and use of pyrite in the former sodium
469 carbonate factories at the vicinity of the smelter factory ([Daumalin and Raveux, 2016](#)).

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5 470 The results of Pb and TOC for SO were a little above those of natural soils, but SO was not
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8 471 exempt from previous anthropogenic activities that took place at the beginning of the 19th
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10 472 century (Fressoz, 2013; Daumalin and Raveux, 2016).

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13 473 In summary, the PNCal soils have low fertility with high pH, high Fe content and low nutrient
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15 474 and TOC contents. The anthropogenic addition of materials maintained or increased pH and
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17 475 Fe concentration, but lowered TOC. Fertility was slightly increased for seashore sites in
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19 476 relation with domestic anthropogenic nitrogen deposition and a covering layer of excavated
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21 477 loam (Rabier et al., 2014). The positive common denominator of these soils is that high pH,
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23 478 Fe and Mn oxides and low TOC lowered MM mobility and limited their transfer into plants
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25 479 and into marine ecosystems (Bert, 2012; Dang et al., 2014; Lenoble et al., 2015; Lin et al.,
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27 480 2008; Martínez-Sánchez et al., 2011). The higher fertility level compared to the other PNCal
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29 481 soils of the coastal area where spontaneous *A. halimus* populations are exclusively localized
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31 482 for the moment may be a driving parameter for *A. halimus* development.

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34 35 36 484 4.2. Is *A. halimus* able to germinate in all the PNCal soils?

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40 485 The results showed that pH, Na and Zn were involved in *A. halimus* germination inhibition.
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42 486 The data of pH and soil composition were in accordance with those of studies of germination
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44 487 inhibition whose differentiated the effect of alkalinity from salinity (Chen et al., 2010; Ma et
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46 488 al., 2015). Sodium soil concentration may explain the delay of germination shown by the
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48 489 characteristic S-shaped aspect of germination for seashore soils which has been observed for
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50 490 some other *Atriplex* species and other Chenopodiaceae (Capron et al., 2000; Katembe et al.,
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52 491 1998). The effect of multiple metals and metalloids is difficult to appreciate because they
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54 492 occur simultaneously in the field and their presence is correlated with other factors such as
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56 493 salinity and alkalinity. The correlation between Zn and germination rate was weak, which is
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494 in accordance with other studies under controlled conditions showing no effect of separated
495 Cu, Pb or Zn treatments for *A. halimus* sub. *schweinfurthii* (Lotmani et al., 2011) and
496 separated Cu or Zn treatments for *A. halimus* sub. *halimus* (Márquez-García et al., 2013).

497 Finally, our results indicated that the potential for widespread dissemination of *A. halimus* by
498 seed germination is very limited in the PNCal soils. Previous test of germination with an
499 agronomic plant species i.e. *Raphanus sativus* showed a percentage of germination of 83 ± 6 ,
500 72 ± 9 and 90 ± 3 on E.C, SO and CN soils respectively (unpublished data), confirming the low
501 capacity of *A. halimus* to develop on other soils than the type on which it spontaneously
502 develops compared to an agronomic species.

504 4.3. *A. halimus* growth inhibition in PNCal soils

505 Reduced additional individual losses were shown during plant growth monitoring for TR.
506 However, a growth inhibition of as much as 60-80 % for the less polluted soils was observed.
507 This demonstrated a negative impact on growth of these mainly oligotrophic soils,
508 independently of their pollution level. This is in accordance with the studies by other authors
509 on *A. halimus* (Martínez-Fernández and Walker, 2012), which have shown that nutrient
510 supply rather than heavy metals (Pb, Zn) limits growth of *A. halimus*. Moreover, *A. halimus*
511 grows well in nutrient-rich solution (Lutts et al., 2004) and is described as halo-nitrophilous
512 (Muñoz-Rodríguez, 2012; Walker et al., 2014). Coastal sites of the PNCal are popular and
513 often crowded during summer, generating illicit waste deposits. Moreover, permanent
514 indigenous human presence at the site of Marseilleveyre can be dated back at least to 600 AC
515 (Bouffier and Garcia, 2014). This could coincide with the introduction of *A. halimus* species
516 among the halonitrophilous taxa of the Chenopodiaceae, though older evidence of the
517 presence of *Atriplex* species has been found in pollen from Calanques sediment (Romey et al.,

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518 2015 and 2014). However, it is not possible to distinguish between the species by their
519 palynological character (Abel-Schaad et al., 2014; Fernández-Illescas et al., 2010). Though a
520 potential spread of *A. halimus* at the expense of native leguminous plant species does exist in
521 theory, especially considering *Astragalus tragacantha*, a protected native plant species on the
522 seashore and, *Coronilla juncea*, another native plant species, at the chimney site, it is limited
523 by the nitrogen contribution of these species which is probably negligible in comparison with
524 the anthropogenic nitrogen sources. These results are in accordance with our previous results
525 as *A. halimus* is spontaneously present on disturbed soils from the seashore and inland in
526 presence of waste from building, but not on the more polluted part of the former smelter nor
527 in undisturbed matorral. Moreover, it has been previously demonstrated that *A. tragacantha*
528 shows no difference of growth in a soil analogous to TR compared to a loamy reference soil
529 after two years of culture (Laffont-Schwob et al., 2011) although *A. halimus* had a
530 significantly reduced growth in TR compared to CN.

531 Consequently, for the PNCal, if it seeks to manage *A. halimus* dispersion due to its potential
532 invasiveness, the priority action would be to reduce dog droppings and organic matter
533 deposits resulting from human practices that favour preferential habitats for *A. halimus* rather
534 than to eradicate *A. halimus* individuals themselves.

535 536 4.4. Root symbiosis: the secret weapon to success for *A. halimus*?

537 Symbionts were ubiquitous in all root systems, whatever the PNCal soil tested, indicating
538 their occurrence in soils, in contrast to what it is observed on more recently disturbed soils
539 with reduced fungal inoculum (Brundrett, 2009). As previously confirmed from aerial
540 photography archives, the soils from these sites, heavily contaminated and disturbed during
541 the industrial period, have been less disturbed since 1960 for the seashore sites and ca. 1950

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542 for E.C as the roof of the smelter horizontal chimney had clearly been destroyed (Rabier et
543 al., 2014; IGN 1943; IGN 1950). It was probably destroyed by bombing during the battle of
544 Marseille in August 1944 (Duchêne and Contrucci, 1998). It appears that a sufficient period
545 of time has passed for a spontaneous symbiont spore bank to be formed in these polluted
546 soils. Moreover, antimony microbial tolerance of soils from the same site has been shown by
547 respiration measurements, demonstrating an adaptation of the PNCal soil microbial
548 populations (Guillamot et al., 2014). Even if the genus *Atriplex* was previously quoted as
549 nonmycorrhizal (Brundrett, 2009), recent papers showed the occurrence of root symbioses in
550 many species belonging to this genus (Sonjak et al., 2009). The occurrence of symbionts in
551 the roots of *A. halimus* has already been proven *in natura* in the Calanques National Park
552 (Laffont-Schwob et al., 2011; Rabier et al., 2014) suggesting an old occurrence of this plant
553 species in the area. As previously discussed, though these sites are peri-urban, which explains
554 the spontaneous presence of nitrophilous species such as *A. halimus*, the perturbation of the
555 entisol was sufficiently old to allow the development of a spontaneous fungal spore bank. The
556 positive correlation between MM and DSE is in accordance with several authors (Ban et al.,
557 2012; Xu et al., 2015; Zhang et al., 2008), and a previous work on *Rosmarinus officinalis* at
558 E.C (Affholder et al., 2014). However contrasting results can be found in the literature and are
559 related to the plant's strategy to increase its tolerance to MM (see review by Göhre and
560 Paszkowski, 2006). Recently, there has been a surge in research focused on how DSE could
561 restrict the uptake of metals by their host plants and especially the interactions of metal ions
562 with melanins (Felix et al., 1978; Fogarty and Tobin, 1996; Mandyam and Jumpponen, 2005;
563 Mugerwa et al., 2013; Stainsack et al., 2003). However, the ability of fungal symbionts to
564 limit metal transfer from soil to *A. halimus* may not be the only factor involved. It has
565 recently been proven that *A. halimus*, with its own biosynthesis of enzymes and antioxydants,
566 may bind, sequesterate and reduce the harmful effect of trace metals (El-Bakatoushi et al.,

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2 567 2015). Consequently the occurrence of this plant species in coastal polluted soils may not
3 568 increase the potential transfer of MM to the food chain and may favour phytostabilization.
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9 570 *4.5. Decision-making criteria for the PNCal to maintain or eradicate A. halimus*

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12 571 In response to the question of whether *A. halimus* is able to grow on more or less polluted or
13 572 salt-affected soils from the PNCal - in other terms, in soils different from those in which it
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15 573 spontaneously grows, - the answer is yes. However, a high loss of 50 % at the germination
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17 574 stage was observed. The results also showed 80% growth reduction for the surviving plants in
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19 575 the two technosols (E.C and E.S.). Moreover, the controlled conditions used mimicked the
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21 576 field conditions except for xericity, providing more favourable conditions than the *in natura*
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23 577 ones. The nitrophilous character of *A. halimus* also limits its potential for the colonization of
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25 578 new territory. Thus, the oligotrophic and less polluted soils of CC and SO showed a
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27 579 significant reduction of growth, while that of SA exhibited better growth results and higher
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29 580 chlorophyll indices associated with more widely available micronutrients, despite its
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31 581 significant pollution.
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40 582 Given the low translocation factors, our results confirm that the individuals of *A. halimus*
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42 583 growing naturally on polluted soils along the coast do not present a risk of transfer of
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44 584 pollutants to aerial parts and the food chain and are tolerant to MM. It would appear that the
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46 585 germination and growth of this species require disturbed soil sites with increased
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48 586 macronutrient availability and associated symbionts. On the basis of these results, the PNCal
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50 587 is considering maintaining *A. halimus* stands in the polluted soils of the coastal area until a
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52 588 better ecological solution for pollution containment is found.
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590 **5. Conclusion**

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4 591 From this case study, we wanted to reconsider the simplistic vision of pollution treatment vs
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6 592 biological conservation in polluted protected areas. In various cases, plants spontaneously
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8 593 growing in polluted areas are adapted to pollution and may prevent pollutant transfer. The
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11 594 potential invasiveness of these pollutant-tolerant plants in such field may be easily
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13 595 experienced by analysing biotic and abiotic factors favouring their germination and growth in
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16 596 ex-situ assays and give decisional tools for the protected area managers.
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868 **Figures**

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4 870 **Fig. 1:** Physico-chemical characteristics of the PNCal soils and control. Parameters: EC:
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6 871 electrical conductivity, TOC: total organic carbon, NTK: total Kjeldahl nitrogen, Exch. P₂O₅:
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8 872 exchangeable P mg/kg P₂O₅, Exch. Na, K, Mg, Ca: exchangeable Na, K, Mg, Ca (mg/g),
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11 873 CEC: cationic exchange capacity (Metson cmol+/kg). Sites: CN: control; SA: Calanque de
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13 874 Saména; TR: Calanque des Trous; CC: Cap Croisette; E.C: Escalette Chimney; E.S: Escalette
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16 875 Slagheap; SO: Sormiou.

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21 877 **Fig. 2:** Average metal and metalloid concentrations (logarithmic scale, mg/kg of dry weight,
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23 878 DW) in soils, shoots and roots of *A. halimus* (n=3, p ≤0.05). Sites: CN: control; SA: Calanque
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25 879 de Saména; TR: Calanque des Trous; CC: Cap Croisette; E.C: Escalette Chimney; E.S:
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27 880 Escalette Slagheap; SO: Sormiou. Different letters above mean values (n=3, p ≤0.05) mean
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29 881 significant difference (Wilcoxon test).

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35 883 **Fig. 3:** Effect of PNCal soil contamination and/or salinity on germination of *A. halimus*
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37 884 during the 14 days of the experiment. Different letters above mean values (n = 10, p ≤0.05)
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39
40 885 indicate significant difference (Wilcoxon test).

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45 887 **Fig. 4:** Growth traits and survival (as percentage of control) for *A. halimus* in the PNCal soils,
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47 888 n= 10, p ≤0.05). Sites: CN: control; SA: Calanque de Saména; TR: Calanque des Trous; CC:
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49 889 Cap Croisette; E.C: Escalette Chimney; E.S: Escalette Slagheap; SO: Sormiou. Different
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52 890 letters above mean values (± standard error (SE)) indicate significant difference for the same
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55 891 parameter (Wilcoxon test).

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893 **Fig. 5:** Monitoring of chlorophyll, flavonol, anthocyanin and leaf epidermal phenol indices in
894 *A. halimus* leaves depending on PNCal soils types (n=10, $p \leq 0.05$). Different letters following
895 curves indicate significant difference for the same parameter (Wilcoxon test).

897 **Fig. 6:** Canonical plot of results of discriminant analysis with the percentages of each fungal
898 structure (A: arbuscule, M: mycelium, V: vesicle and DSE: dark septate endophytes), and the
899 7 soil types as parameters (n=3 and $F_{(24,39)}=7.67$, $P<0.0001$; Wilk's Lamda = 0.002;
900 Canonical1=59.9%, Canonical2=28.3%). For each category, inner and outer circles represent
901 the 95% confidence intervals for the means and the 50% prediction intervals, respectively.
902 The seven soil types explain 59.9% and 28.3% of the observed variance in fungal occurrence
903 on axis 1 and 2, respectively.

905 **Supplementary data:**

906 **Supp. data 1:** Map of soil sampling locations (SA: Calanque de Saména; TR: Calanque des
907 Trous; CC: Cap Croisette; E.C: Escalette Chimney; E.S: Escalette Slagheap; SO: Sormiou)
908 and seed sampling location (E) in the Calanques national park (PNCal).

909 **Supp. data 2:** Average metal and metalloid concentrations (mg/kg of dry weight) in soil
910 samples from each soil type. Different letters following means \pm standard error (SE) in a
911 column indicate significant difference (n=3, $p \leq 0.05$, Wilcoxon test).

912 **Supp. data 3:** Evolution of Spearman correlations between germination percentages and soil
913 parameters up to 15 days after imbibition (n= 10, $p \leq 0.05$). $\rho_{X,Y}$: Spearman correlation,
914 X=G: germination percentage, Y=pH, EC, TOC, (Na): Na concentration, (Zn) Zn
915 concentration. The Line of Fit element shows a linear regression with confidence intervals in
916 shady colors.

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2 917 **Supp. data 4:** Bioaccumulation factor (BCF) and translocation factor (TF) values for each
3 918 metal and metalloid in *A. halimus* growing on the different soil types. Different letters
4 following means \pm standard error (SE) in a column indicate significant difference (n=3,
5 919 p \leq 0.05, Wilcoxon test).
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10 921 **Supp. data 5:** Na/K ratio in different soil types, and in root and shoot of *A. halimus* growing
11 on these different soils. Different letters following means \pm standard error (SE) in a column
12 922 indicate significant difference (n=3, p \leq 0.05, Wilcoxon test).
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Figure1

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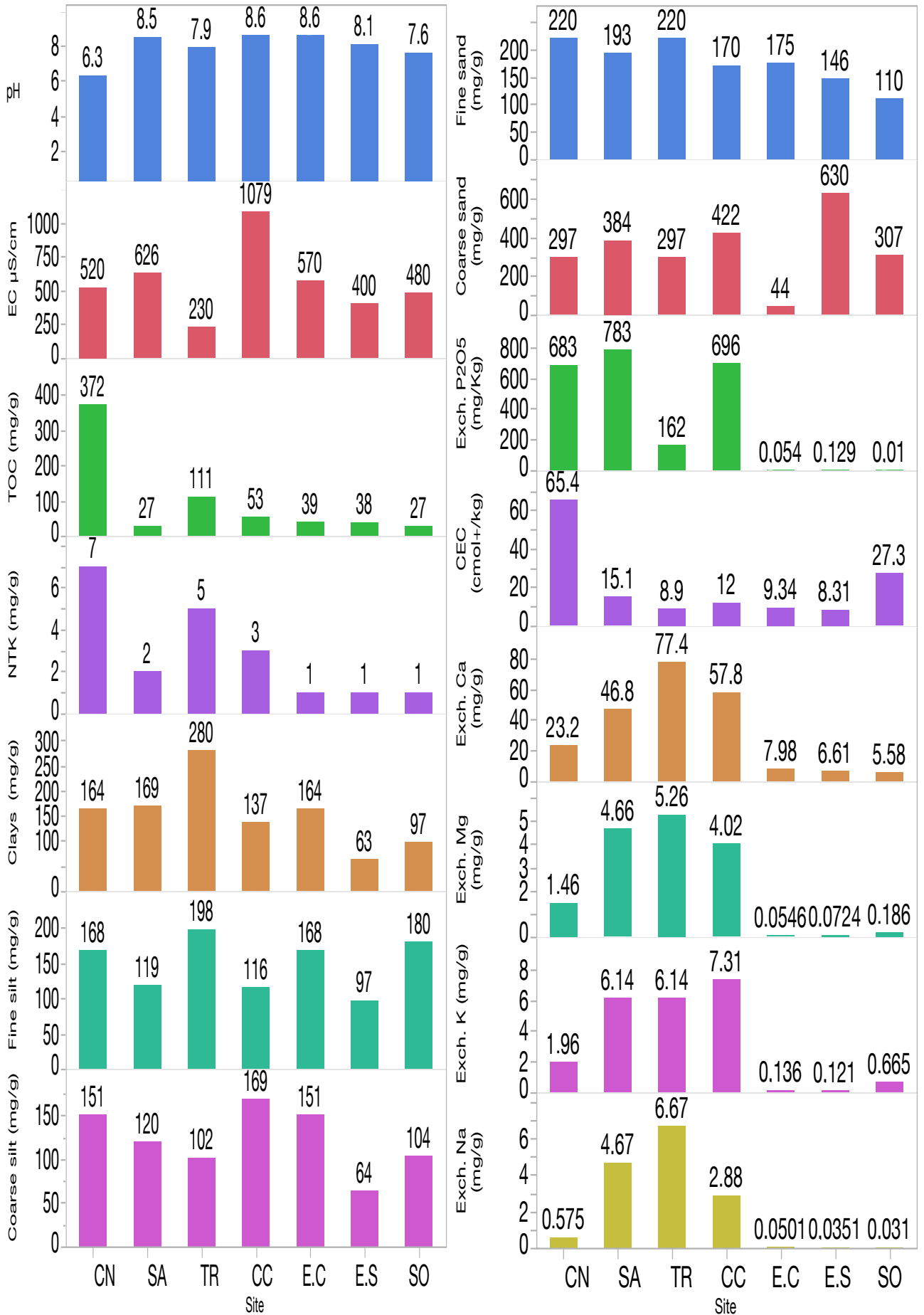


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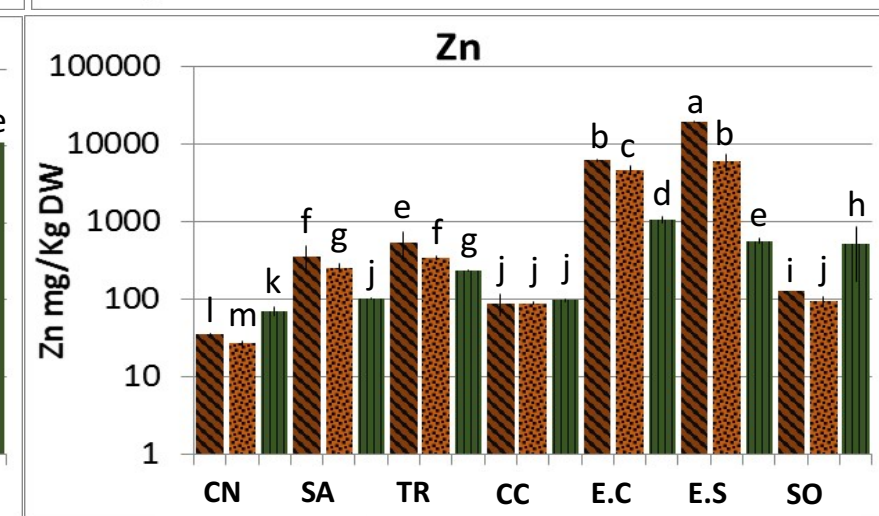
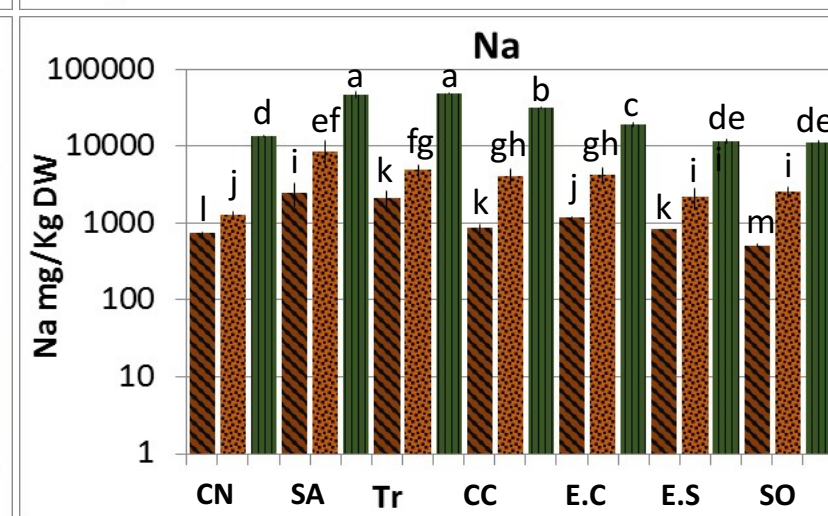
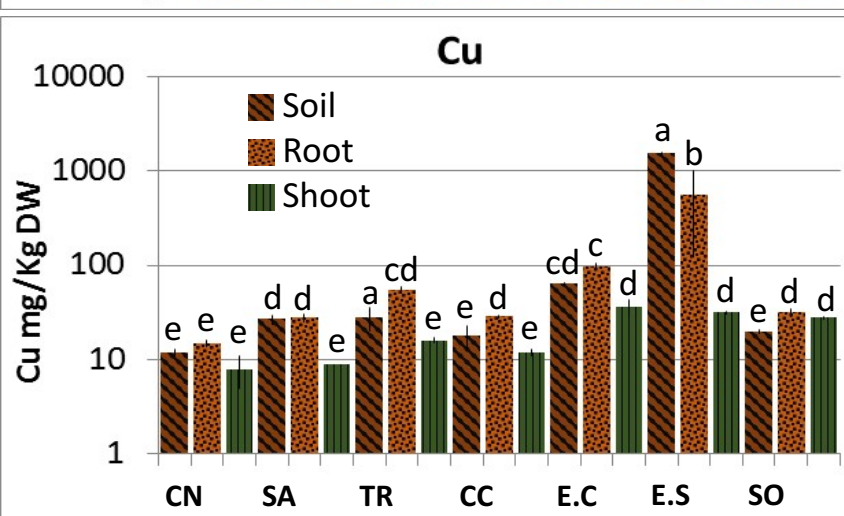
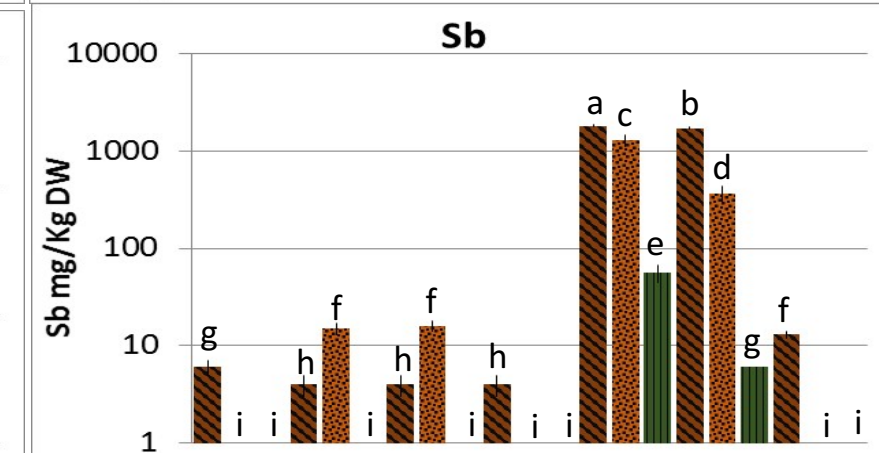
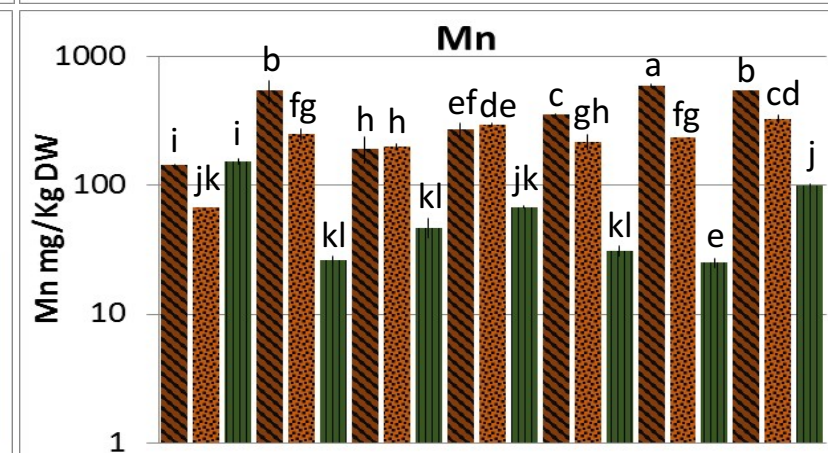
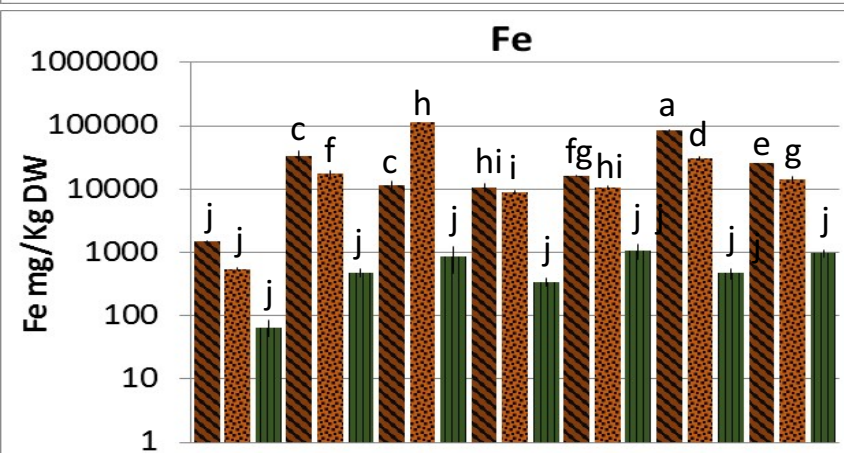
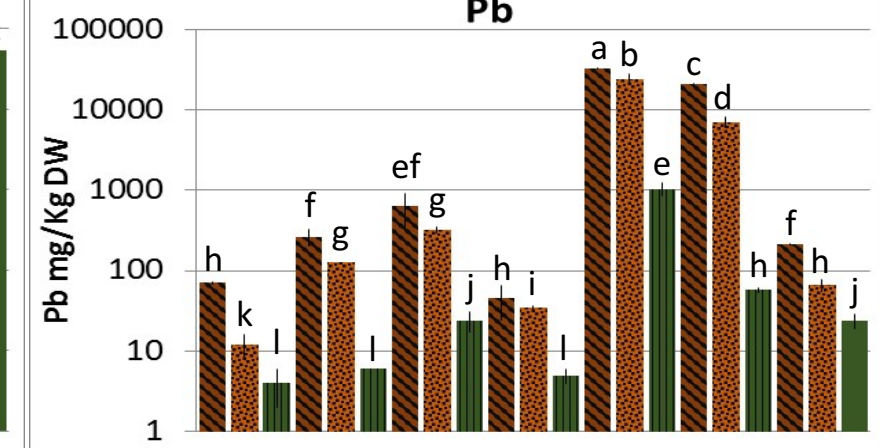
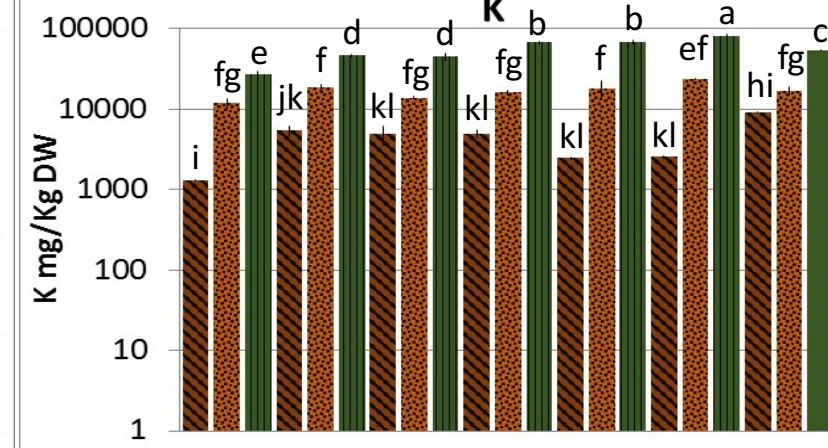
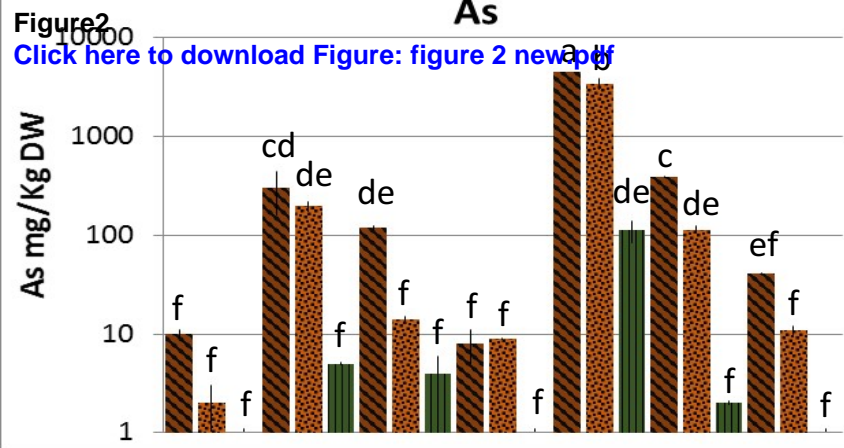


Figure3
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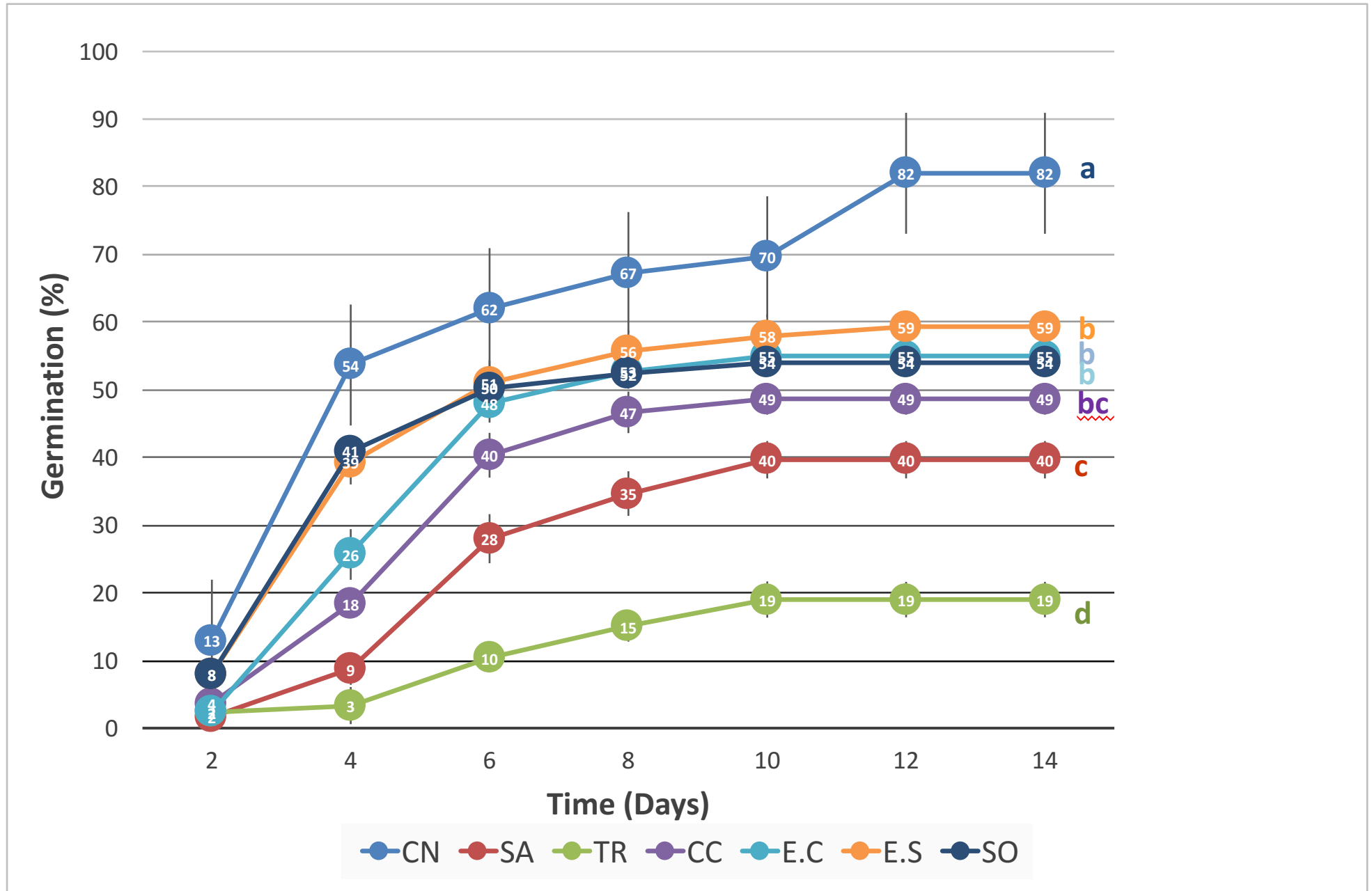


Figure4

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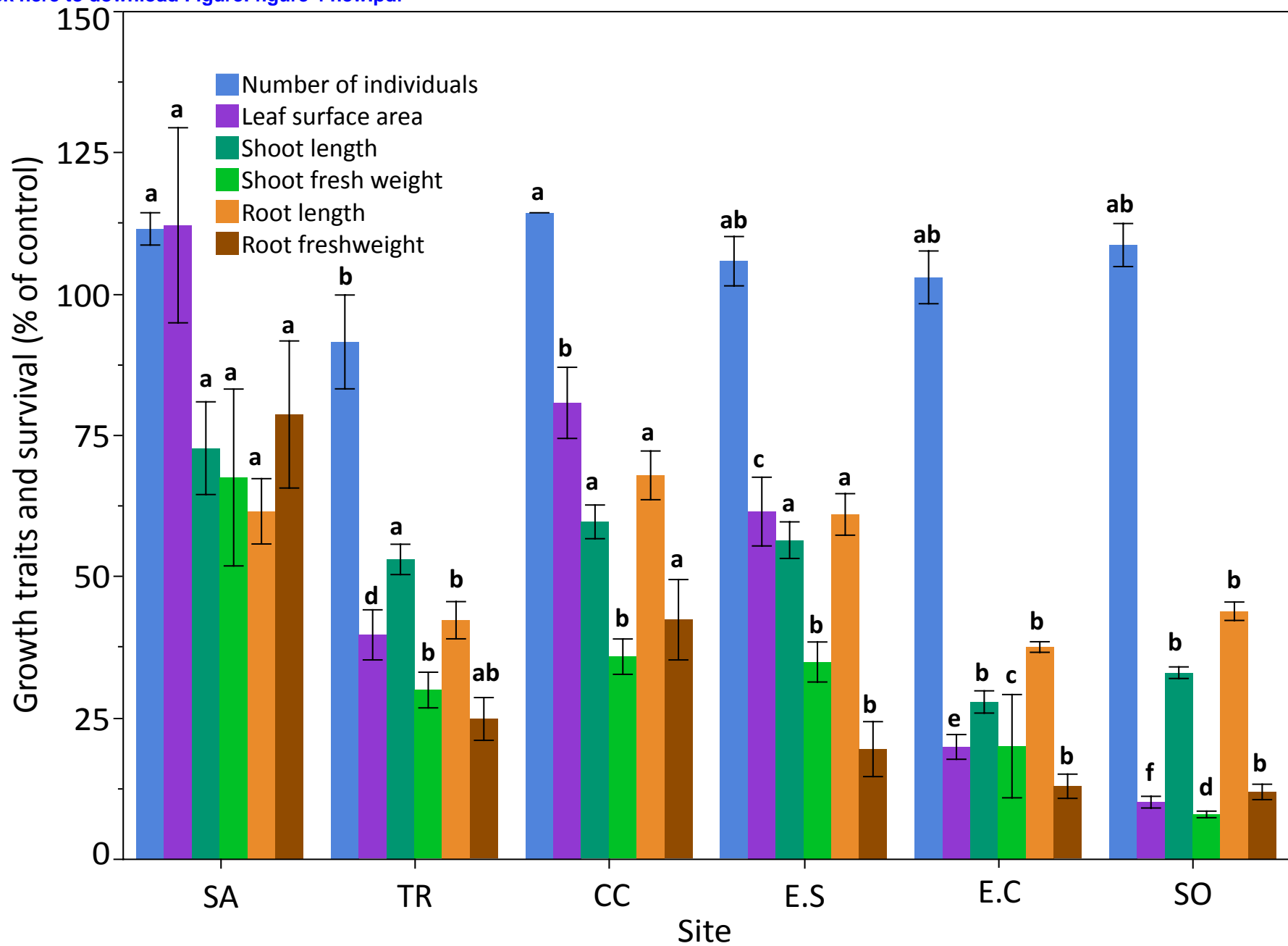
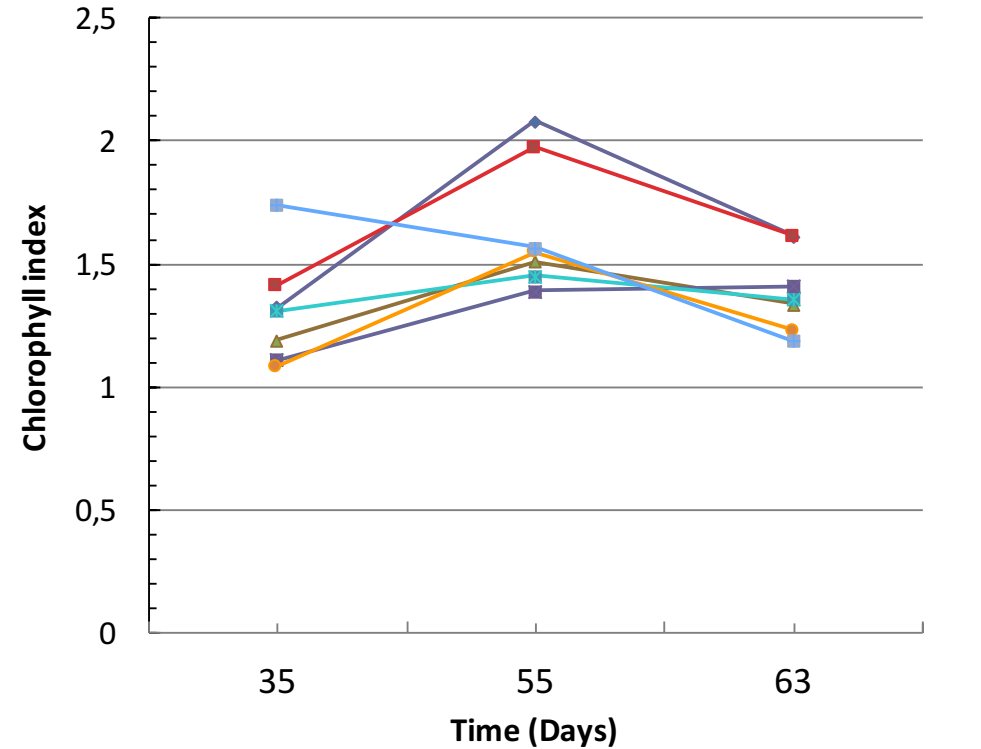
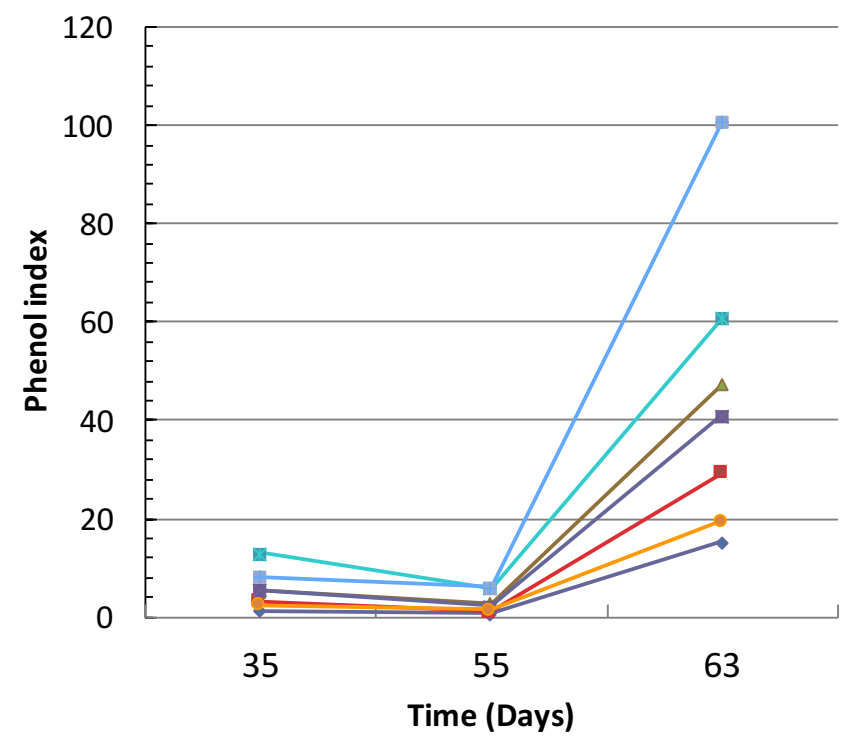
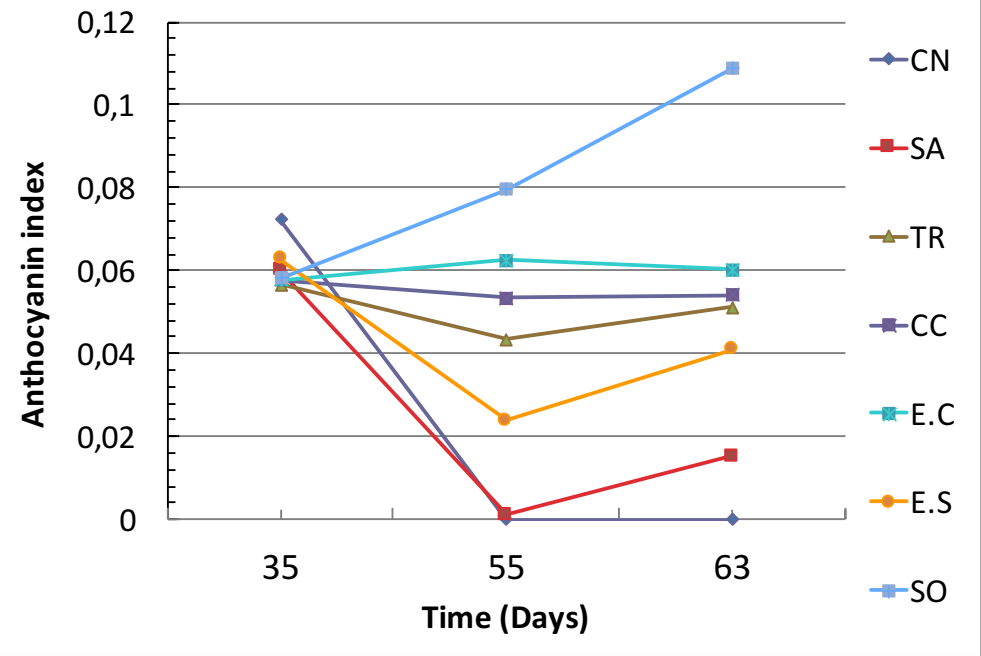
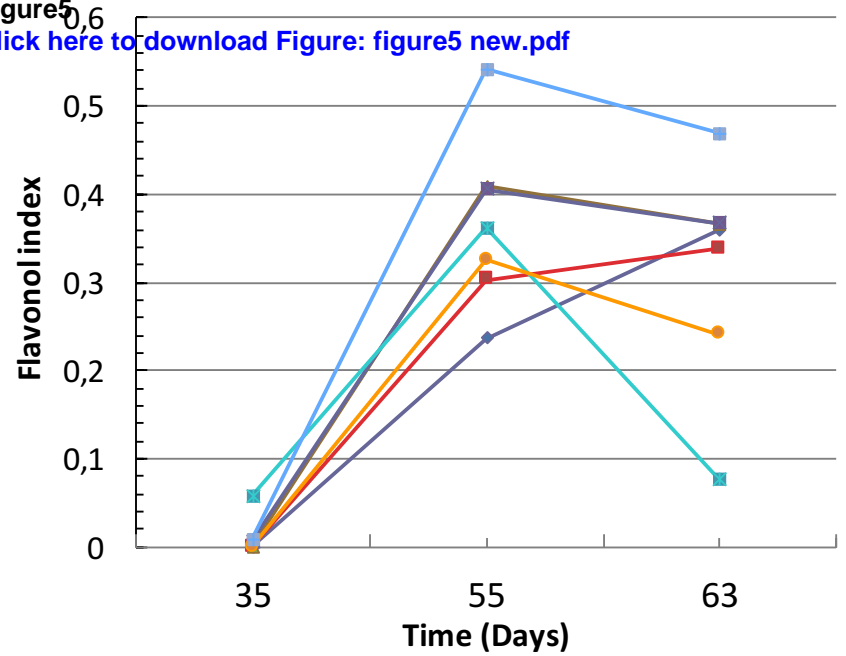
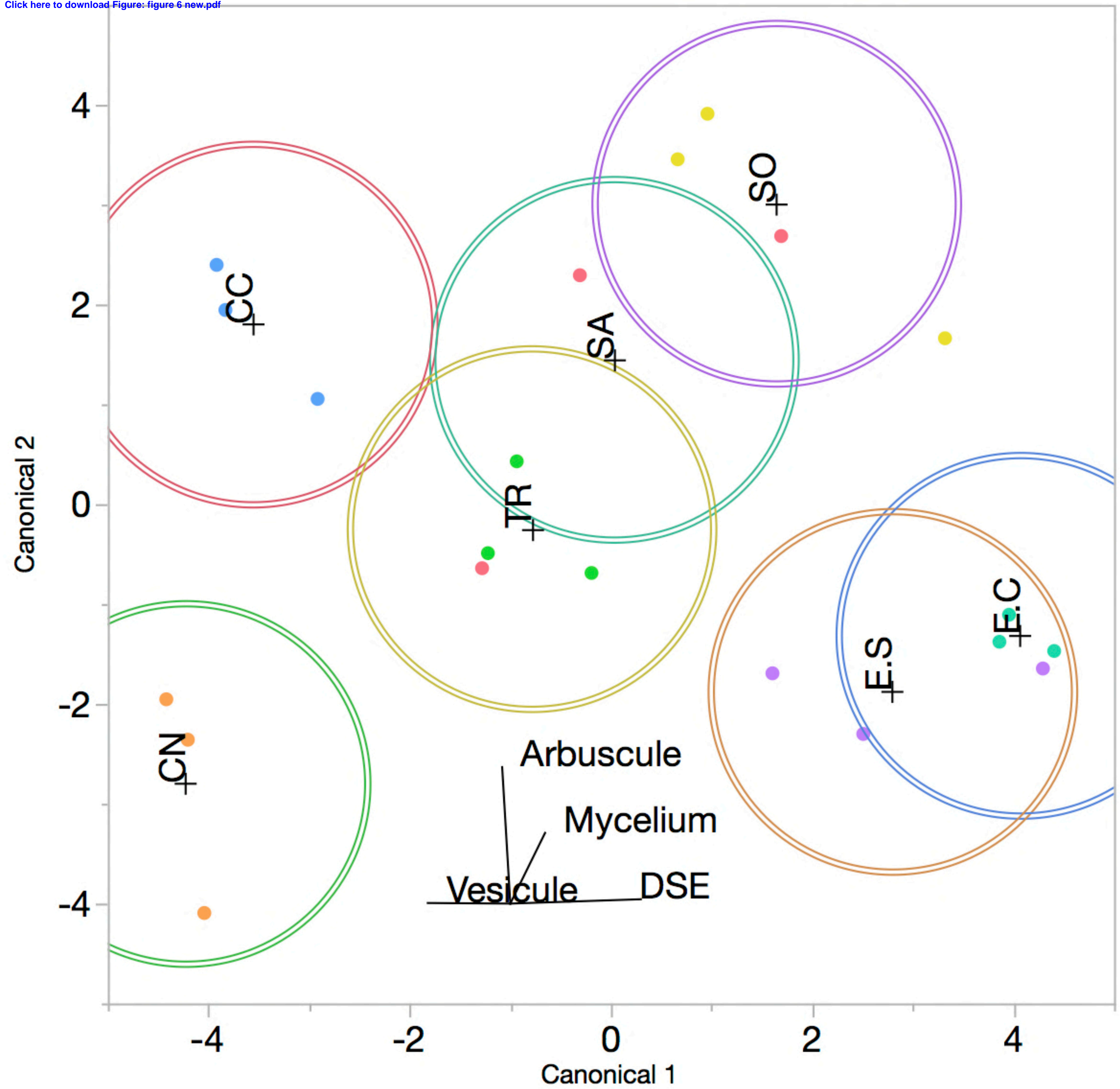


Figure 5,6
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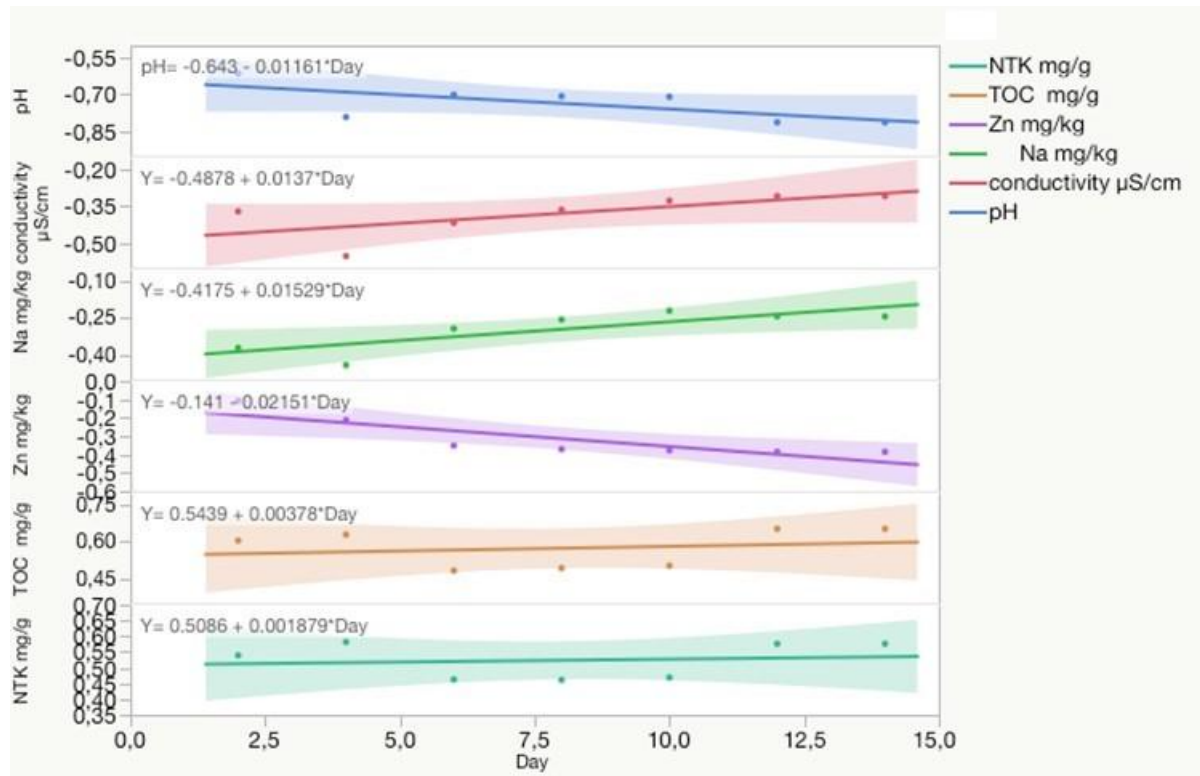




Supplementary data 2: Average metal and metalloid concentrations (mg/kg of dry weight) in soil samples from each soil type.

Soil origin	Element concentrations (mean \pm SE, mg.kg ⁻¹)								
	As	Cu	Fe	K	Mn	Na	Pb	Sb	Zn
Control (CN)	10 \pm 1b	12 \pm 1c	1491 \pm 14d	1303 \pm 13d	143 \pm 2d	737 \pm 11bc	70 \pm 3c	6 \pm 1c	35 \pm 1d
Calanque de Saména (SA)	301 \pm 139b	27 \pm 3c	33860 \pm 6535 b	5440 \pm 704b	540 \pm 108b	2486 \pm 804a	263 \pm 63c	4 \pm 1c	355 \pm 134cd
Calanque des Trous (TR)	13 \pm 4c	28 \pm 8c	11566 \pm 1788c	4918 \pm 1328bc	192 \pm 44cd	2084 \pm 566ab	628 \pm 283c	4 \pm 1c	528 \pm 200c
Cap croisette (CC)	8 \pm 3c	18 \pm 5c	10600 \pm 1654c	4978 \pm 588bc	271 \pm 35cd	867 \pm 108bc	45 \pm 21c	4 \pm 1c	88 \pm 26d
E. Chimney (E.C)	2470 \pm 58a	110 \pm 1b	9270 \pm 231c	2499 \pm 82c	370 \pm 6bc	1202 \pm 17ab	28800 \pm 808a	2520 \pm 58a	7670 \pm 173b
E. Slagheap (E.S)	120 \pm 3bc	2100 \pm 58a	47500 \pm 1328a	2565 \pm 64c	760 \pm 17a	846 \pm 1bc	16200 \pm 462b	1720 \pm 46b	15200 \pm 289a
Sormiou (SO)	41 \pm 1c	20 \pm 1c	25993 \pm 264b	9174 \pm 29a	550 \pm 3ab	515 \pm 14c	210 \pm 3c	13 \pm 1c	126 \pm 1cd

Different letters following means \pm standard error (SE) in a column indicate significant difference (n=3, p \leq 0.05, Wilcoxon test).



Supplementary data 3: Evolution of Spearman correlations between germination percentages and soil parameters up to 15 days after imbibition ($n= 10, p \leq 0.05$). $\rho_{X,Y}$: Spearman correlation, $X=G$: germination percentage, $Y=pH, EC, TOC, (Na): Na$ concentration, $(Zn) Zn$ concentration. The Line of Fit element shows a linear regression with confidence intervals in shady colors.

Supplementary data 4: Bioaccumulation factor (BCF) and translocation factor (TF) values for each metal and metalloid in *A. halimus* growing on the different soil types.

Soil origin	Elements								
	As	Cu	Fe	K	Mn BCF	Na	Pb	Sb	Zn
Control (CN)	0.25±0.02 c	1.22±0.05b	0.37±0.01 e	9.11±0.58a	0.48±0.01c	1.74±0.08e	0.17±0.03b	0.16±0.01b	0.78±0.03bc
Calanque de Saména (SA)	0.77±0.12 b	1.03±0.06bc	0.53±0.04 d	3.52±0.19d	0.47±0.04c	3.75±0.77bc d	0.51±0.05b	4.0±0.4a	0.8±0.1bc
Calanque des Trous (TR)	0.120±0.003c	0.03±0.00e	0.24±0.00f	5.45±0.07c	0.26±0.01d	5.79±0.52a	0.02±0.00b	0.01±0.00b	0.02±0.00e
Cap croisette (CC)	1.2±0.1a	1.72±0.15a	0.85±0.05 b	3.31±0.16d	1.09±0.05a	4.81±0.57ab c	0.91±0.14b	0.25±0.02b	1.07±0.10b
E. Chimney (E.C)	1.39±0.09 a	0.88±0.03c	1.16±0.04 a	7.25±0.96b	0.59±0.04b	3.58±0.37cd	13.84±3.43a	0.2±0.08b	1.43±0.23a
E. Slagheap (E.S)	0.95±0.03 b	0.39±0.03d	0.65±0.01 c	9.33±0.08a	0.310±0.002d	2.56±0.21de	0.44±0.02b	0.21±0.01b	0.40±0.03d
Sormiou (SO)	0.26±0.02 c	1.61±0.07a	0.55±0.02 d	1.86±0.12e	0.60±0.02b	4.92±0.37ab c	0.32±0.03b	0.07±0.00b	0.75±0.05c
	TF								
Control (CN)	0.62±0.05 a	0.58±0.09b	0.12±0.02 a	2.36±0.18e	2.25±0.05a	10.79±0.57a	0.44±0.1a	1±0a	2.58±0.18ab
Calanque de Saména (SA)	0.03±0.00 c	0.32±0.02c	0.03±0.00 ef	2.5±0.11de	0.11±0.01d	6.89±1.13bc	0.05±0b	0.06±0.00b	0.41±0.02e

Calanque des Trous (TR)	0.28±0.07 b	0.29±0.02c	0.08±0.02 bc	3.24±0.17cd	0.24±0.02bc	10.56±0.94a	0.07±0.01b	0.06±0.00b	0.68±0.02d
Cap croisette (CC)	0.17±0.00 b	0.42±0.01bc	0.04±0.00 de	4.18±0.15ab	0.23±0.00bc	8.41±0.79ab	0.15±0.01b	1±0a	1.10±0.03c
E. Chimney (E.C)	0.03±0.00 c	0.38±0.04c	0.1±0.01c d	4.45±0.63a	0.14±0.01cd	4.88±0.49c	0.04±0.01b	0.05±0.01c	0.23±0.02ef
E. Slagheap (E.S)	0.02±0.00 c	0.04±0.00d	0±0f	3.46±0.09bc	0.11±0.01d	5.62±0.52bc	0±0b	0.02±0.00d	0.10±0.01f
Sormiou (SO)	0.14±0.01 b	0.89±0.04a	0.07±0cd	3.33±0.22c	0.31±0.01b	4.67±0.36c	0.39±0.05a	1±0a	1.91±0.11b

Different letters following means ± standard error (SE) in a column indicate significant difference (n=3, p≤0.05, Wilcoxon test).

Supplementary data 5: Na/K ratio in different soil types, and in root and shoot of *A. halimus* growing on these different soils.

Soil origin	Soil Na/K	Root Na/K	Shoot Na/K
Control (CN)	0,5656±0,0008a	0,109±0,004c	0,51±0,03b
Calanque de Saména (SA)	0,42±0,02c	0,42±0,05a	1,03±0,04a
Calanque des Trous (TR)	0,33±0,01d	0,35±0,05a	1,09±0,08a
Cap croisette (CC)	0,1741±0,0003e	0,25±0,02b	0,470±0,003b
E. Chimney (E.C)	0,481±0,003b	0,25±0,01b	0,280±0,004c
E. Slagheap (E.S)	0,329±0,002d	0,091±0,008c	0,139±0,001d
Sormiou (SO)	0,056±0,0004f	0,16±0,02b	0,207±0,003d

Different letters following means ± standard error (SE) in a column indicate significant difference (n=3, p≤0.05, Wilcoxon test).

support data 1

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