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1 **Tolerance strategies of two Mediterranean native xerophytes under fluoride pollution in**
2 **Tunisia**

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27

28 **Abstract**

29 A field study was conducted along a fluorine gradient of soil pollution in Tunisia from Gabes,
30 the most polluted site, to Smara, the reference site. Variations of fluoride (F) concentrations in
31 soils were detected over one year in Gabes, Skhira and Smara. F concentrations in the aerial
32 part of two native plant species, i.e. *Erodium glaucophyllum* and *Rantherium suaveolens*, were
33 above the usual background concentrations. Bioaccumulation factors ranged from 0.08 to 1.3.
34 With F concentrations in aerial parts up to 355 mg kg⁻¹, both species may be described as F
35 accumulators. Both species showed an earlier vegetative growth in Gabes than in Smara.
36 However, some difference between their strategies could be observed i.e. *E. glaucophyllum*
37 shortening the period of its vegetative growth with an escape strategy and *R. suaveolens*
38 decreasing its ratio of alive:dead parts potentially lowering the F toxicity by storage in dead
39 cells. However, at a tissue level, mechanisms of tolerance were similar. Leaf section
40 micrographs of both species showed a higher calcium accumulation in leaf midveins at Gabes
41 than at Smara, confirming the role of calcium in plant F tolerance strategies.

42
43 **Keywords:** Calcium accumulation, *Erodium gaucophyllum*, *Rantherium suaveolens*,
44 vegetative growth, alive:dead part ratio, escape effect.

1. Introduction

Despite the global importance of the phosphate industry and its high economic value for many countries (Haneklaus et al. 2015; Tayibi et al. 2009), by-product discharge and gaseous and particulate emissions, especially fluorides (F), cause severe environmental pollution where phosphate plants are located. The impact of F on the environment, and notably on the health of the flora and fauna, have been previously detailed (Davison and Weinstein 2006; Fornasiero 2001; Weinstein and Davison 2003). However, most of the previous studies on the effects of F on plant species have dealt with F accumulation in plant organs to determine potential toxicity (Álvarez-Ayuso et al. 2011; Ben Abdallah et al. 2006a, 2006b; Pack and Sulzbach 1976; Vike and Håbjør 1995). Among previous studies, long-term assays are less numerous (Domingos et al. 2003; Fornasiero 2003) and only a few have provided information on plant health and reproduction (Davison and Weinstein 2006; Kozlov et al. 2007). However, the effects of F on plant seasonal development, corresponding to a sensitive indicator usually affected by pollution (Kozlov et al. 2007; Ryser and Sauder 2006; Zvereva et al. 2010), need to be evaluated especially in the field, most of the studies on this topic being conducting under controlled conditions.

Retarded growth and leaf trait damages have been reported as general and well-known plant responses to F emissions from industrial activities (Franzaring et al. 2007; Mesquita et al. 2011). Ionomics, dealing with mineral nutrients, may be helpful to better understand stress tolerance mechanisms in plants (Singh et al. 2016). In a previous study, we found variations in Ca and Mg leaf content in *Atractylis serratulooides* due to fluoride pollution, both elements are involved in fluoride tolerance of this plant species (Boukhris et al. 2015b).

Rhanterium suaveolens Desf. (*Asteraceae*) and *Erodium glaucophyllum* L. (L'Hér.) (*Geraniaceae*) have been also identified as F tolerant plant species in polluted ecosystems in SE Tunisia (Boukhris et al. 2015a). Our hypothesis is that all the gypsophytes spontaneously

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71 growing in the surroundings of industrial sites emitting F share a common mechanism of
72 enhancing F tolerance using Ca and Mg. A one-year monitoring survey of plant vegetative
73 growth of these two native plant species along a gradient of F pollution in the Gulf of Gabes
74 (East of Tunisia) was therefore carried out. Moreover, scanning electron microscopy (SEM)
75 coupled to X microanalysis (EDAX) was used to determine whether F pollution impacts major
76 nutrient distribution in leaf and root of both plant species.

77 78 **2. Materials and methods**

79 *2.1. Study area*

80 The study area is located along a F gradient on the south-east coast of Tunisia from Gabes to
81 Smara (60 km south of Sfax city, Fig. 1). The two sites of Gabes and Skhira are located close
82 to phosphate fertilizer factories and, Smara, distant from any industrial activity, was used as a
83 reference site, as previously reported by [Boukhris et al. \(2015a 2015b\)](#). The soils of the three
84 sites are calcic-magnesian soils containing gypsum and limestone ([Mtimet 2001](#)).

85 Located in the Mediterranean arid bioclimate ([Emberger 1954](#)), these three sites are exposed to
86 low rainfall and elevated annual average temperatures, ca. 159 mm and 21°C, respectively, over
87 the 30 last years ([Chaieb and Boukhris 1998](#); [Floret and Pontanier 1978](#); [Zahran 2010](#)). In 2011,
88 annual average temperature was 21 °C, ranging from 10 °C in January to 28 °C in from July to
89 September. This same year, rainfall ranged from 0 to 88 mm between July and October (Online
90 Resource).

91 92 *2.2. Plant material and growth patterns*

93 *Rhanterium suaveolens* Def., a chamaephyte, and *Erodium glaucophyllum* L. (L'Hér.), a
94 hemicryptophyte, were selected since both plant species were identified as fluoride-tolerant
95 species and are abundant at the three sites ([Boukhris et al. 2015a](#)). Seasonal vegetative growth

1 96 monitoring was based on estimations of percentages of green tissues (photosynthetically active
2 97 sensu [Smith et al. 1997](#)). Concerning the aboveground phytomass of each tuft, the ratio of
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4 98 alive:dead shoots was visually estimated as described by [Bokhorst et al \(2011\)](#). A shoot was
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7 99 considered dead when all its leaves had died and considered as alive when at least one green
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10 100 leaf was still present. This ratio was monitored for both species during one year (2011–2012)
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12 101 and a comparison was made following the gradient of F pollution of the different sites.
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14 102 Observations in triplicate were undertaken in May 2011, June 2011, January 2012, March 2012
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17 103 and May 2012. For each species at each site, three mature individuals were randomly selected
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19 104 and monitored during the growth survey. In June, the phytovolumes (PV) of aboveground parts
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22 105 of five individuals of *E. glaucophyllum* and *R. suaveolens* per site were determined following
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24 106 the equation $PV = 2/3 \pi (1/2 (1/2D_1 + 1/2D_2))^2)^{1/2}H$ as described in [Mighri et al \(2011\)](#) for *Artemisia*
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26 107 *herba-alba* with D_1 and D_2 the two orthogonal diameters of the crown, and H the height in cm.
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29 108 Since the vegetation physiognomy present in the three areas was sparse, containing plant
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32 109 patches and bare ground areas, we also did a density monitoring in a 1-hectare surface area per
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34 110 site for each site using the method of point quadrat ([Gounot 1969](#)) in June. A total of twenty
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36 111 quadrats were randomly placed in each 3 sites. Within each quadrat, we numbered individuals
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39 112 of *E. glaucophyllum* and *R. suaveolens* and expressed the results as density of tuft per m² for
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41 113 each species.

42 43 44 114 45 46 115 *2.3. Soil and plant fluoride analyses*

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48 116 During the period of the study and simultaneously with the ratio of alive:dead shoot monitoring,
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51 117 soil and plant samples were collected for F analyses. Three soil samples (0-20 cm depth) near
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53 118 each individual per species per site were collected and air-dried. Likewise, leaves and stems of
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56 119 the middle of the shoots of three plants per species were also collected. Plant samples were
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58 120 washed with tap water several times and subsequently dipped into 0.01 M HCl for 5 min
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121 followed by thorough washing with distilled water. Then the collected samples were dried at
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2 122 80 °C during 72 h in an oven (Memmert) and stored until analysis. Fluoride analyses were
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5 123 performed on each soil and shoot sample at each of the five monitoring times.

6
7 124 Soil and plant samples were analyzed in triplicate. Soil samples were sieved (2 mm mesh), then
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10 125 an extraction was performed using 5 g of soil sample mixed with 20 ml HCl (1N) during 90
11
12 126 min and mixed with total ionic strength adjustment buffer (TISAB) for analysis using a fluoride-
13
14 127 specific ion electrode (inlab/Model WTW) coupled to a pH-meter (pH ION R503) at
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17 128 ambient temperature (Mezghani et al. 2005). In addition, dried ground (<1.0 mm) plant samples
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19 129 (0.5g) were mixed with potassium carbonate (4g) and sodium carbonate (4g) and heated in an
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22 130 electric oven at 700 °C during 3 h. Then hydrochloric acid wet process mineralization was
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24 131 performed for all samples followed by filtration and adjustment with distilled water for
25
26 132 determination of F content by potentiometry as described by Mezghani et al. (2005).

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29 133 Bioaccumulation factors (BAF), i.e. F concentration in aerial part/F concentration in
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31 134 surrounding soil, were calculated for each soil/plant pair and monthly average BAF were given
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34 135 for each site.

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37 38 39 137 *2.4. Scanning electron microscopy and elemental analysis*

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41 138 According to the results of F analyses, we selected plant samples with the highest F
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44 139 concentrations (i.e. in June 2011) for microanalysis X. Fresh samples of leaves, stems and roots
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46 140 of both plant species were prepared, as described by Rabier et al. (2008).

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49 141 30 µm thick transversal sections were cut at -25 °C using a cryomicrotome (Cryo-cut II
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51 142 microtome Reichert-Jung), then immediately placed on SEM specimen holders and carbon
52
53 143 metallized (10–15 nm) for observation under an ESEM (Environmental Scanning Electron
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56 144 Microscope) Philips XL 30 microscope with detector EDAX sdd apollo 10. X-ray mapping was
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58 145 performed for 20 min to give the elemental distribution for each selected element (Si, Mg, F,

146 Ca, S, Cu, Cl, Fe, K, Na, Mn, P, Ni, Al and P). In all cases, the voltage was 20 kV. SEM images
1 were obtained with back-scattered electron (BSE) or secondary electron (SE) imaging. BSE
2 147 were obtained with back-scattered electron (BSE) or secondary electron (SE) imaging. BSE
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4 148 imaging was used to study the micromorphology of the plant organs. To help identification of
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7 149 the different tissues of the analyzed organs, transversal histological sections were stained with
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10 150 carmino-green staining and observed with an optical microscope.

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14 152 *2.5. Statistical analysis*

16 153 Statistical analyses were performed for all data using JMP 10 statistical software (SAS Institute,
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19 154 Cary, North Carolina, USA). Fluoride concentrations in the aerial parts and in soils were
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22 155 compared over the monitoring period for each plant species at $p \leq 0.05$. Densities of plants and
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24 156 phytovolumes were compared between sites in June for each plant species at $p \leq 0.05$. The non-
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27 157 parametric tests Dunn All Pairs for Joint Ranks or Wilcoxon Each Pair were used due to non-
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29 158 normal distributions of data.

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34 160 **3. Results**

37 161 *3.1. Fluoride contamination in soils and plants*

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39 162 The gradient of F soil pollution i.e. Gabes > Skhira > Smara is maintained over time (Table 1).
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42 163 The site at Gabes was the most polluted, with an average concentration of F ca. 1300 mg kg⁻¹,
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44 164 Skhira was ca.10 times less polluted, with an average of 140 mg kg⁻¹ and the Smara site
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47 165 considered as reference had only ca. 40 mg kg⁻¹. A lower concentration in F in soils from Gabes
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49 166 was observed in January 2012 compared to March and May 2012, but cannot be attributed to a
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52 167 dilution effect after a period of rainfall and less particulate F deposit (Online Resource).
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54 168 Average F concentrations in plant aerial parts followed the same gradient as soil contamination,
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57 169 with ca. 190, 91 and 36 mg kg⁻¹ F in Gabes, Skhira and Smara, respectively. F concentrations
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59 170 in plants and BAF in Skhira were constant throughout the observation period although they

171 were higher in May 2011 in Gabes and Smara compared to January 2012. However, BAF were
172 < 1 at all sites and in all periods except in the reference site of Smara in May 2011.

173

174 3.2 Plant traits

175 *E. glaucophyllum* showed a maximal ratio of alive:dead shoots between January and March
176 2012 (Fig. 2A) representing the period of favourable climatic conditions for vegetative growth
177 of this Mediterranean species. However, a lag time before the maximal level of this ratio was
178 observed between the sites i.e. this maximal occurred in January in Gabes and later in the other
179 sites, particularly in the control (Smara). Full foliage (ratio = 90) of this species was
180 concomitant with flowering phenophases (March 2012). During the dry period (July to
181 September 2011), the aboveground parts of each tuft were totally dried.

182 In contrast, for *R. suaveolens*, the ratio of alive:dead shoots appeared proportionally reduced
183 following the pollution gradient (Gabes>Skhira>Smara), from May 2011 to June 2011.
184 Moreover, the maximal level of this ratio for this perennial was observed in Gabes since March
185 although it was the highest only in May in Smara and Skhira (Fig. 2B), this lag time mostly not
186 being linked with high rainfall (Online Resource).

187 Thus, different ratio of alive:dead shoots patterns were observed in both plant species in Gabes
188 compared to Smara.

189 The phytovolumes of both species decreased following the increasing F gradient in June (Table
190 2). The chamaephyte *R. suaveolens* had significantly higher phytovolumes than the
191 hemicryptophyte *E. glaucophyllum*.

192 In June, the density of individuals forming tufts was significantly higher in Gabes than in Smara
193 whatever the plant species (Table 2). The density of *E. glaucophyllum* was also higher in Gabes
194 than in Skhira illustrating a gradient of density of this species following the gradient of F

195 concentration in soils. For *R. suaveolens*, the density was low and no significant different in
196 density was observed between Gabes and Skhira sites.

197

198 3.3. Nutrient localization in organ sections

199 EDAX cartography was performed on leaf, stem and root sections of *E. glaucophyllum* and *R.*
200 *suaveolens* for all the detected peaks of the X-ray spectra to determine element localization in
201 plant tissues. Only maps of Si, Mg, F, Ca, S in leaf and root sections (Fig. 3, 4, 5 and 6) are
202 shown since all elements in stem maps as well as Cu, Cl, Fe, K, Na, and P maps in roots and
203 leaves had identical distribution patterns between the Gabes and Smara samples. Even with
204 high total F content in the organs from Gabes, F concentrations in both species were below the
205 threshold of detection of the equipment ($\geq 1\%$) at the level of a 30 μm thick transversal section
206 mapping with the selected electron acceleration voltage.

207 Concerning *E. glaucophyllum*, more Ca and less S were accumulated in the midvein of leaf
208 sections and in the cortex of the root sections from Gabes than from Smara (Fig. 3g, h, i, k and
209 4g, h, i, k). For *R. suaveolens*, more Ca was deposited in the leaf midvein and in the cortex of
210 the root in Gabes compared to Smara (Fig. 5g, h, i, k and 6g, h, i, k).

211 Neither structural difference nor alterations were detected comparing SEM micrographs of
212 plant sections (leaf and root) from Gabes to Smara for both species (Fig. 3a, b ; 4a, b ; 5a, b and
213 6a, b).

214 *R. suaveolens* leaf lamina was thinner (Fig. 5) than *E. glaucophyllum* leaf lamina (Fig. 3), with
215 a thick-walled epidermis and thick cuticle.

216 Bright crystals in the midvein were clearly visible in BSE observations of *E. glaucophyllum*
217 leaf section (Fig. 3a) and point analysis of these crystals indicated high Ca contents.

218

219 4. Discussion

220 4.1. Fluoride soil contamination and bioaccumulation of the two native plant species

221 Fluoride concentrations ranged from 33 to 1683 mg kg⁻¹ in the analyzed soils from the Gulf of
222 Gabes. However, the soil F concentrations in Smara, representing a maximum of 51 mg kg⁻¹ F,
223 can be considered as geochemical background level compared to F average concentration in
224 many soils of the world ranging from 100 to 600 mg kg⁻¹ F, as reviewed by Davison and
225 Wenstein (2006). Variations of F concentrations in soils were detected throughout the year in
226 Gabes and Smara. In Gabes, the lowest concentration in January 2012 was concomitant with a
227 period of rainfall. However, as reviewed by Kabata-Pendias and Szteke (2015), a high
228 percentage of F input is firmly retained by the soil, mainly as CaF₂ in alkaline soils, and leaching
229 removes only a small amount of F from soils. Moreover, in Skhira, no seasonal variation of F
230 in soils was observed. A possible link between these variations and a reduction of the industrial
231 superphosphate production in Gabes during the "Jasmine revolution" may be borne in mind
232 (Taib 2013). As previously described, the same three soils have a pH ca 7.5 with a conductivity
233 ranging from 1.5 to 2.3 mS cm⁻¹ along with Cr concentrations ranging from 41 to 120 mg kg⁻¹
234 (Boukhris et al. 2015a). It has been demonstrated that Cr may increase F toxicity in plants due
235 to enhanced F absorption when F and Cr concentrations are high (Zhou and Sun 2002). In these
236 author's study, F and Cr concentrations in soils ranges from 219 to 823 mg kg⁻¹ and 73 to 88
237 mg kg⁻¹, respectively. Soil concentrations up to 1683 and 120 mg kg⁻¹, for F and Cr respectively,
238 were previously reported in the area of our study (Boukhris et al. 2015a) with significantly
239 higher concentrations in Gabes than in Skhira. Accordingly, a higher phytotoxicity of F in
240 Gabes than in Skhira is observed.

241 F concentrations in the aboveground part of both plant species were above the background
242 contents of F in plants, usually lower than 10 mg kg⁻¹ (Davison and Wenstein 2006).
243 Bioaccumulation factors (BAF) ranged from 0.08 to 1.3. Due to F contents in aerial parts
244 ranging from 26 to 355 mg kg⁻¹, *E. glaucophyllum* and *R. suaveolens* may be described as F

245 accumulators. Similar results were previously obtained for *Atractylis serratuloides*, another
246 native plant species growing in the steppes of SE Tunisia (Boukhris et al. 2015b). Previous
247 papers described plant species able to accumulate up to thousand mg kg⁻¹ of F in their aerial
248 parts (Saini et al. 2013; Singh et al. 2018) but most of these studies were conducted in laboratory
249 conditions and some of them under hydroponic conditions far away from realistic conditions.
250 However, our results may not drive to a phytoextraction strategy, biomass and F accumulation
251 of *E. glaucophyllum* and *R. suaveolens* being together not efficient. However, from an
252 ecological point of view, the occurrence of these plant species nearby sources of F pollution
253 may play a role that need to be reinforced by limiting wind and water erosion processes.

254 Both species have an important ecological interest in plant communities of Tunisian arid
255 ecosystems (Le Houérou 2008). They are characteristic of the two most dominant soil types of
256 southern Tunisia. *R. suaveolens* is the main species of the Jeffara sandy coastal plain, and its
257 natural distribution is from central Tunisia to North of Tripolitania in Libya. The species *E.*
258 *glaucophyllum*, a gypsophyte, is characteristic of gypsum substrate of south Tunisia, which
259 constitute a great ecological originality of the region (Floret 1981). Floret and Pontanier (1982)
260 estimated the density of *R. suaveolens* around 27.000 tufts ha⁻¹, corresponding to ca. 2-3 tufts
261 m⁻². Their observations are consistent with the current results of our field observation (Table 2)
262 indicating that the density of plants is a stable criterion in such abiotic conditions.

263

264 4.2. Plant tolerance strategies under fluoride pollution

265 Vegetative growth of *E. glaucophyllum* and *R. suaveolens* were affected by F pollution. *E.*
266 *glaucophyllum* and *R. suaveolens* are both known as stress tolerant species but the first one is
267 known as more adapted to disturbed areas and the latter, more competitive following Grime's
268 theory (Tarhouni et al. 2010). *E. glaucophyllum*, showed an earlier leaf flushing, corresponding

269 to a maximal ratio of alive:dead shoots, in Skhira and Gabes than in Smara. Consequently, the
270 faster life cycle may shorten the period of exposure to F. One hypothesis may be that this plant
271 species has an escape strategy (that differs from the excluder strategy preventing F absorption)
272 following [Franks \(2011\)](#). This is in agreement with some life-traits of arid hemicryptophytes
273 described as ‘drought-escapers’, sensu [Shantz \(1927\)](#), or ‘drought-evaders’, sensu [Small
\(1973\)](#). Accordingly, [Zvereva et al. \(2010\)](#) have concluded that Raunkiaer’s classification of
275 life forms appeared to be the best predictor of species’ responses to pollution.

276 *R. suaveolens* had a higher ratio of alive:dead shoots in Gabes than at the other sites during
277 favourable period and a lower during dry season. Due to a vegetative growth period limited by
278 the drought episodes in the year, it also suggests a faster vegetative growth of this species under
279 F pollution and may be part of the plant response to F pollution as it was demonstrated by
280 [Zvereva et al. \(2010\)](#). However, this decreasing ratio of alive:dead shoots in June was linked to
281 a lower phytovolume leading to a bioconcentration effect of F in plant tissues. It has been
282 proven that dead cells could sorb metals in the cell wall but also potentially retain metals within
283 the cells themselves ([Ebbs et al. 2016](#)). In a same way but for a non-metal element, the
284 hypothesis of a high F tolerance of this plant species due to a F storage in the dead parts is
285 emitted.

286 These different growth patterns under F pollution between the two observed plant species may
287 reflect their different biological types i.e. hemicryptophyte (*E. glaucophyllum*) and
288 chamaephyte (*R. suaveolens*) ; and provide information regarding the long-term effect of F
289 exposure on their ecosystems since vegetative traits are the key characteristics for predicting
290 the response of future communities and ecosystems ([Jablonski et al. 2002](#)).

291 To reduce a long-term impact on their plant community, it is necessary to better understand the
292 mechanisms of their fluoride tolerance.

293 4.3. Corroborating the role of calcium in fluoride tolerance of plants

294 Leaf section micrographs of both species showed a thick cuticle, especially in *R. suaveolens*,
295 with narrow lamina and sclerenchymatous cell bundle, revealing their xerophytic characteristics
296 enabling reduction of leaf tissue pollutant diffusion and water loss through stomata, as
297 previously described in industrialized arid lands (Haworth and McElwain 2008; Thévenard et
298 al. 2005). Neither necrosis nor leaf injuries were observed on spontaneous populations of both
299 species in the field, even in Gabes. In both species, Ca distribution in leaf midveins was higher
300 in Gabes than in Smara, and this may be considered as a physiological response to F. The role
301 of Ca is primordial in lowering F phytotoxicity. The complex CaF_2 reduces F translocation and
302 its phytotoxic effects on the aboveground parts of plants (Álvarez-Ayuso et al. 2011; Ben
303 Abdallah et al. 2006b; Weinstein and Davison 2003). Moreover, in leaf sections from both plant
304 species from Gabes, the Ca distribution pattern in maps was superimposable with the detected
305 bright crystals in the mesophyll and the midvein, that suggests the formation of Ca crystals.
306 Calcium crystals are involved in plant detoxification and defence according to Nakata and Mc
307 Conn (2000). This is also in agreement with the important source of Ca in the calci-magnesian
308 soils from the Gulf of Gabes (Boukhris et al. 2015b). This highlights the involvement of calcium
309 ion in *E. glaucophyllum* and *R. suaveolens* F tolerance strategy, as was recently reported in our
310 previous work on *Atractylis serratuloides* under the same ecological conditions (Boukhris et al.
311 2015b). These results are in agreement with the recognized role of calcium in plant abiotic-
312 stress resistance (Song et al. 2008).

313 5. Conclusion

314 *E. glaucophyllum* and *R. suaveolens*, two native gypsophytes spontaneously growing nearby
315 superphosphate factories in Tunisia, may be considered as F accumulators with F contents in
316 their aerial parts following the gradient of F soil pollution as following: Gabes>Skhira>Smara.
317 Higher accumulation of Ca at a tissue level in the aerial parts of both species in Gabes suggests

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318 a protection mechanism of Ca against F phytotoxicity potentially related to Ca-F interactions
319 as previously demonstrated in other gypsophytes. Phenological adaptations of these species that
320 may lower their F exposure in the most F polluted sites were also observed. However,
321 shortening the vegetative growth period or reducing the alive aerial plants may raise questions
322 regarding the long-term impact of this pollution on the sustainability of the plant cover and its
323 potential consequence on F dispersion in the environment with a reduced vegetation barrier.

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325 **Reference**

- 1
2 326 Álvarez-Ayuso E, Giménez A, Ballesteros JC (2011) Fluoride accumulation by plants grown
3
4
5 327 in acid soils amended with flue gas desulphurisation gypsum. *Journal of Hazardous*
6
7 328 *Materials* 192:1659-1666
- 8
9 329 Ben Abdallah F, Elloumi N, Mezghani I, Boukhris M, Garrec J-P (2006a) Survival strategies
10
11
12 330 of pomegranate and almond trees in a fluoride polluted area. *Comptes Rendus Biologies*
13
14 331 329:200-207
- 15
16 332 Ben Abdallah F, Elloumi N, Mezghani I, Garrec J-P, Boukhris M (2006b) Industrial fluoride
17
18
19 333 pollution of Jerbi grape leaves and the distribution of F, Ca, Mg, and P in them. *Fluoride*
20
21 334 39:43-48
- 22
23
24 335 Bokhorst S, Bjerckew JW, Streetz LE, Callaghan TV, Phoenix GK (2011) Impacts of multiple
25
26
27 336 extreme winter warming events on sub-Arctic heathland: phenology, reproduction,
28
29 337 growth, and CO₂ flux responses. *Global Change Biology* 17:2817–2830
- 30
31 338 Boukhris A, Laffont-Schwob I, Mezghani I, El Kadri L, Prudent P, Pricop A, Tatoni T, Chaieb
32
33
34 339 M (2015a) Screening biological traits and fluoride contents of native vegetations in arid
35
36 340 environments to select efficiently fluoride-tolerant native plant species for *in-situ*
37
38
39 341 phytoremediation. *Chemosphere* 119:217-223
- 40
41 342 Boukhris A, Laffont-Schwob I, Rabier J, Salducci M-D, El Kadri L, Tonetto A, Tatoni T,
42
43
44 343 Chaieb M (2015b) Changes in mesophyll element distribution and phytometabolite
45
46 344 contents involved in fluoride tolerance of the arid gypsum-tolerant plant species
47
48 345 *Atractylis serratuloides* Sieber ex Cass. (Asteraceae). *Environmental Science and*
49
50
51 346 *Pollution Research* 22 (10):7918-7929
- 52
53 347 Chaieb M, Boukhris M (1998) *Flore Des Zones Arides Et Sahariennes De Tunisie*, Ed. L'Or
54
55
56 348 du Temps, Tunis 290 p (In French)
- 57
58
59
60
61
62
63
64
65

- 349 Davison AW, Weinstein LH (2006) Some Problems Relating to Fluorides in the Environment:
1
2 350 Effects on Plants and Animals. In: Tressaud A. (ed), *Fluorine and the Environment*,
3
4 351 *Atmospheric chemistry, Emissions and lithosphere* Vol 1, Elsevier, pp. 251-298
5
6
7 352 Domingos M, Klumpp A, Rinaldi MCS, Modesto IF, Klumpp G, Delitti WBC (2003)
8
9 353 Combined effects of air and soil pollution by fluoride emissions on *Tibouchina pulchra*
10
11 354 Cogn., at Cubatão, SE Brazil, and their relations with aluminium. *Plant and Soil* 249:
12
13 355 297-308
14
15
16
17 356 Ebbs SD, Bradfield SJ, Kumar P, White JC, Musante C, Ma X (2016) Accumulation of zinc,
18
19 357 copper, or cerium in carrot (*Daucus carota*) exposed to metal oxide nanoparticles and
20
21 358 metal ions. *Environmental science. Nano.* 3(1): 114-126
22
23
24 359 Emberger L (1954) Une Classification Biogéographique Des Climats. *Rec. Trav. Lab. Bot.*
25
26 360 *Géol. Zool. Univ. Montpellier, Sér. Bot.* 7:3–43 (in French)
27
28
29 361 Floret C (1981) The effects of protection on steppic vegetation in the Mediterranean arid zone
30
31 362 of Southern Tunisia. *Vegetatio*: 46 (1) 117–129
32
33
34 363 Floret C, Pontanier R (1982) L'aridité en Tunisie présaharienne : Climat – sol – végétation et
35
36 364 aménagement. *Trav. et Doc. ORSTOM*, n° 150 – Paris, 544 p (in French)
37
38
39 365 Floret C, Pontanier R (1978) Relations climat-sol-végétation dans quelques formations
40
41 366 végétales spontanées du sud Tunisien (production végétale et bilan hydrique des sols).
42
43 367 *Inst. Rég. Arides– Médenine, Dir. Ress. Eau et Sols Tunis, CEPE/CNRS Montpellier et*
44
45 368 *Orstom - Paris, 96 p (in French)*
46
47
48
49 369 Fornasiero RB (2001) Phytotoxic effects of fluorides. *Plant Science* 161:979-985
50
51 370 Fornasiero RB (2003) Fluorides effects on *Hypericum perforatum* plants: first field
52
53 371 observations. *Plant Science* 165, 507-513
54
55
56 372 Franks SJ (2011) Plasticity and evolution in drought avoidance and escape in the annual plant
57
58 373 *Brassica rapa*. *New Phytologist* 190: 249–257
59
60
61
62
63
64
65

- 374 Franzaring J, Klumpp A, Fangmeier A (2007) Active biomonitoring of airborne fluoride near
1
2 375 an HF producing factory using standardised grass cultures, *Atmospheric Environment*
3
4 376 41:4828–4840
5
6
7 377 Gounot M (1969) Méthodes d'étude quantitative de la végétation. Masson, Paris. 314 p
8
9
10 378 Haneklaus N, Schnug E, Tulsidas H, Tyobeka B (2015) Using high temperature gas-cooled
11
12 379 reactors for greenhouse gas reduction and energy neutral production of phosphate
13
14 380 fertilizers. *Annals of Nuclear Energy* 75:275-282
15
16
17 381 Haworth M, McElwain J (2008) Hot, dry, wet, cold or toxic? Revisiting the ecological
18
19 382 significance of leaf and cuticular micromorphology. *Palaeoecology* 262:79-90
20
21
22 383 Jablonski LM, Wang X, Curtis PS (2002) Plant reproduction under elevated CO₂ conditions: a
23
24 384 meta-analysis of reports on 79 crop and wild species. *New Phytologist* 156 (1):9–26
25
26
27 385 Kabata-Pendias A, Szteke B (2015) Trace elements in abiotic and biotic environments, chapter
28
29 386 15: Fluorine, CRC Press, pp109-118
30
31
32 387 Kozlov MV, Eränen JK, Zverev VE (2007) Budburst phenology of white birch in industrially
33
34 388 polluted areas. *Environmental Pollution* 148:125-131
35
36
37 389 Le Houérou HN (2008) Bioclimatology and Biogeography of Africa. Springer-Verlag
38
39 390 Heidelber. 240 p.
40
41
42 391 Mesquita GL, Tanaka FAO, Cantarella H, Mattos Jr D (2011) Atmospheric absorption of
43
44 392 fluoride by cultivated species. Leaf structural changes and plant growth. *Water Air Soil*
45
46 393 *Pollut* 219, 143–156
47
48
49 394 Mezghani I, Elloumi N, Ben Abdallah F, Chaieb M, Boukhris M (2005) Fluoride accumulation
50
51 395 by vegetation in the vicinity of a phosphate fertiliser plant. *Fluoride* 38:69-75
52
53
54 396 Mighri H, Akrouit A, Neffati M (2011) Assessment of essential oil yield of *Artemisia herba-*
55
56 397 *alba* cultivated in Tunisian arid zone. *Journal of Medicinal Plants Research* 5 (21):
57
58 398 5296-5300
59
60
61
62
63
64
65

- 399 Mtimet A (2001) Soils of Tunisia. In: Zdruli P, Steduto P, Lacirignola C, Montanarella L (eds)
1
2 400 Soil Resources of Southern and Eastern Mediterranean Countries CIHEAM, Bari pp.
3
4 401 243–262
5
6
7 402 Nakata PA, McConn MM (2000) Isolation of *Medicago trunculata* mutants defective in
8
9 403 calcium oxalate formation. *Plant Physiology* 124:1097-110.
10
11
12 404 Pack MR, Sulzbach CW (1976) Response of plant fruiting to hydrogen fluoride fumigation.
13
14 405 *Atmospheric Environment* 10:73-81
15
16
17 406 Rabier J, Laffont-Schwob I, Notonier R, Fogliani B, Bouraïma-Madjebi S (2008) Anatomical
18
19 407 element localization by EDXS in *Grevillea exul* var. *exul* under nickel stress.
20
21 408 *Environmental Pollution* 156:1156-116
22
23
24 409 Ryser P, Sauder WR (2006) Effects of heavy-metal-contaminated soil on growth, phenology
25
26 410 and biomass turnover of *Hieracium piloselloides*. *Environmental Pollution* 140:52–61
27
28
29 411 Shantz HL (1927) Drought resistance and soil moisture. *Ecology* 8(2):145-157
30
31
32 412 Saini P, Khan S, Baunthiyal M, Sharma V (2013) Effects of fluoride on germination, early
33
34 413 growth and antioxidant enzyme activities of legume plant species *Prosopis juliflora*,
35
36 414 *Journal of Environmental Biology* 34:205-209
37
38
39 415 Singh S, Parihar P, Singh R, Singh VP, Prasad SM (2016) Heavy Metal Tolerance in Plants:
40
41 416 Role of Transcriptomics, Proteomics, Metabolomics, and Ionomics, *Frontiers in plant*
42
43 417 *science* 6, article 1143:1-36
44
45
46 418 Singh G, Kumari B, Sinam G, Kumar KN, Shekhar M (2018) Fluoride distribution and
47
48 419 contamination in the water, soil and plants continuum and its remedial technologies, an
49
50
51 420 Indian perspective – a review, *Environmental Pollution* 239:95-108
52
53
54 421 Small E (1973) Xeromorphy in plants as a possible basis for migration between arid and
55
56 422 nutritionally-deficient environments. *Botaniska Notiser* 126:534-539
57
58
59
60
61
62
63
64
65

- 423 Smith SD, Monson RK, Anderson JE (1997) Physiological Ecology of North American Desert
1
2 424 Plants. Springer-Verlag, Berlin 286 p
3
4 425 Song W-Y, Zhang Z-B, Shao H-B, Guo X-L, Cao H-X, Zhao H-B, Fu Z-Y, Hu X-J (2008)
5
6 426 Relationship between calcium decoding elements and plant abiotic-stress resistance,
7
8
9 427 *International Journal of Biological Sciences* 4(2):116-125
10
11 428 Taib M (2013) The Mineral Industry of Tunisia. In: USGS (eds), *United States Geological*
12
13 429 *Survey* 2011 Minerals Yearbook pp. 41.1-41.7
14
15
16 430 Tarhouni M, Ben Salem F, Ouled Belgacem A, Neffati M (2010) Acceptability of plant species
17
18 431 along grazing gradients around watering points in Tunisian arid zone. *Flora* 205:454-
19
20 432 461
21
22 433 Tayibi H, Choura M, López FA, Alguacil FJ, López-Delgado A (2009) Environmental impact
23
24 434 and management of phosphogypsum. *Journal of Environmental Management* 90:2377-
25
26 435 2386
27
28
29 436 Thévenard F, Gomez B, Daviero-Gomez V (2005) Xeromorphic adaptations of some Mesozoic
30
31 437 gymnosperms. A review with palaeoclimatological implications. *Comptes Rendus*
32
33 438 *Palevol* 4:67-77
34
35
36 439 Weinstein LH, Davison A (2003) Native plant species suitable as bioindicators and biomonitors
37
38 440 for airborne fluoride. *Environmental Pollution*, 125:3-11
39
40
41 441 Vike E, Håbjørg A (1995) Variation in fluoride content and leaf injury on plants associated with
42
43 442 three aluminium smelters in Norway. *Science of the Total Environment* 163:25-34
44
45
46 443 Zahran MA (2010) Climate vegetation. Afro-Asian Mediterranean and Red Sea coastal lands.
47
48 444 Plant and Vegetation. London New York: Springer Dordrecht Heidelberg 344 p
49
50
51 445 Zvereva EL, Roitto M, Kozlov MV (2010) Growth and reproduction of vascular plants in
52
53 446 polluted environments: a synthesis of existing knowledge. *Environmental Reviews*
54
55 447 18:355–367
56
57
58
59
60
61
62
63
64
65

448 Zhou Q, Sun T (2002) Effects of Chromium (VI) on Extractability and Plant Uptake of Fluorine

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
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449 in Agricultural Soils of Zhejiang Province, China. Water, Air, & Soil Pollution 133: 145

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452 Figures

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2 453 **Figure 1** Map of the three studied sites in the gulf of Gabes (Tunisia), Gabes and Skhira sites
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5 454 being nearby superphosphates plants and Smara, being the reference site (Base-map source:
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7 455 Google Earth)

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10 456 **Figure 2** Ratio of alive:dead shoot of *E. glaucophyllum* (A) and *R. suaveolens* (B) in Gabes,
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12 457 Skhira and Smara during the 5 monitoring times from May 2011 to May 2012

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14 458 **Figure 3** SEM micrographs of *E. glaucophyllum* leaf transversal sections (a, for Gabes and b,
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17 459 for Smara) and distribution maps of major element deposition from Gabes (c, e, g, i, k) and
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19 460 Smara (d, f, h, j, l). Elements (Si, Mg, F, Ca, S) are indicated at the bottom of each picture

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22 461 **Figure 4** SEM micrographs of *E. glaucophyllum* root transversal sections (a, for Gabes and b,
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24 462 for Smara) and distribution maps of major element deposition from Gabes (c, e, g, i, k) and
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26 463 Smara (d, f, h, j, l). Elements (Si, Mg, F, Ca, S) are indicated at the bottom of each picture

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29 464 **Figure 5** SEM micrographs of *R. suaveolens* leaf transversal sections (a, for Gabes and b, for
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32 465 Smara) and distribution maps of major element deposition from Gabes (c, e, g, i, k) and Smara
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34 466 (d, f, h, j, l). Elements (Si, Mg, F, Ca, S) are indicated at the bottom of each picture

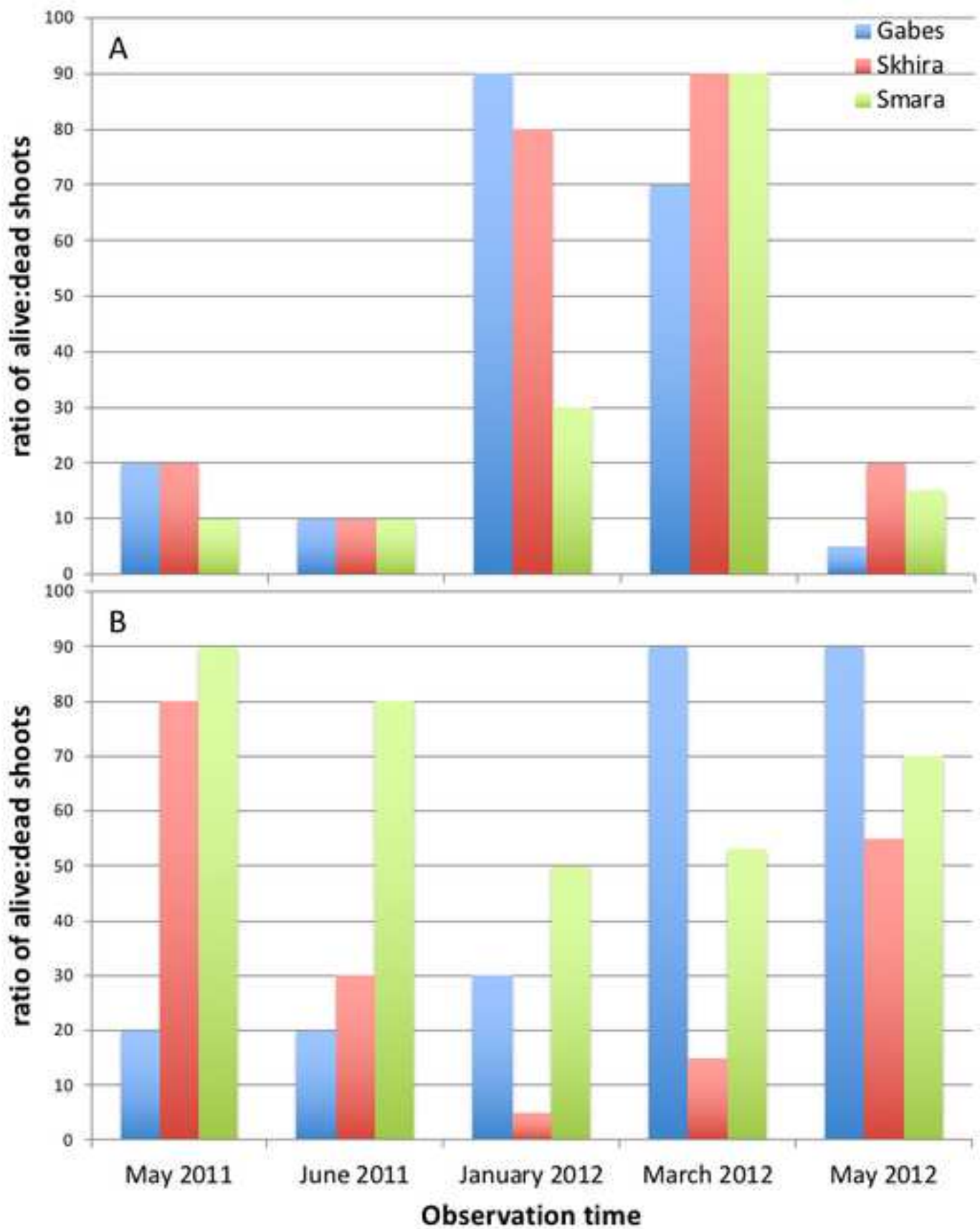
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36 467 **Figure 6** SEM micrographs of *R. suaveolens* root transversal sections (a, for Gabes and b, for
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39 468 Smara) and distribution maps of major element deposition from Gabes (c, e, g, i, k) and Smara
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41 469 (d, f, h, j, l). Elements (Si, Mg, F, Ca, S) are indicated at the bottom of each picture

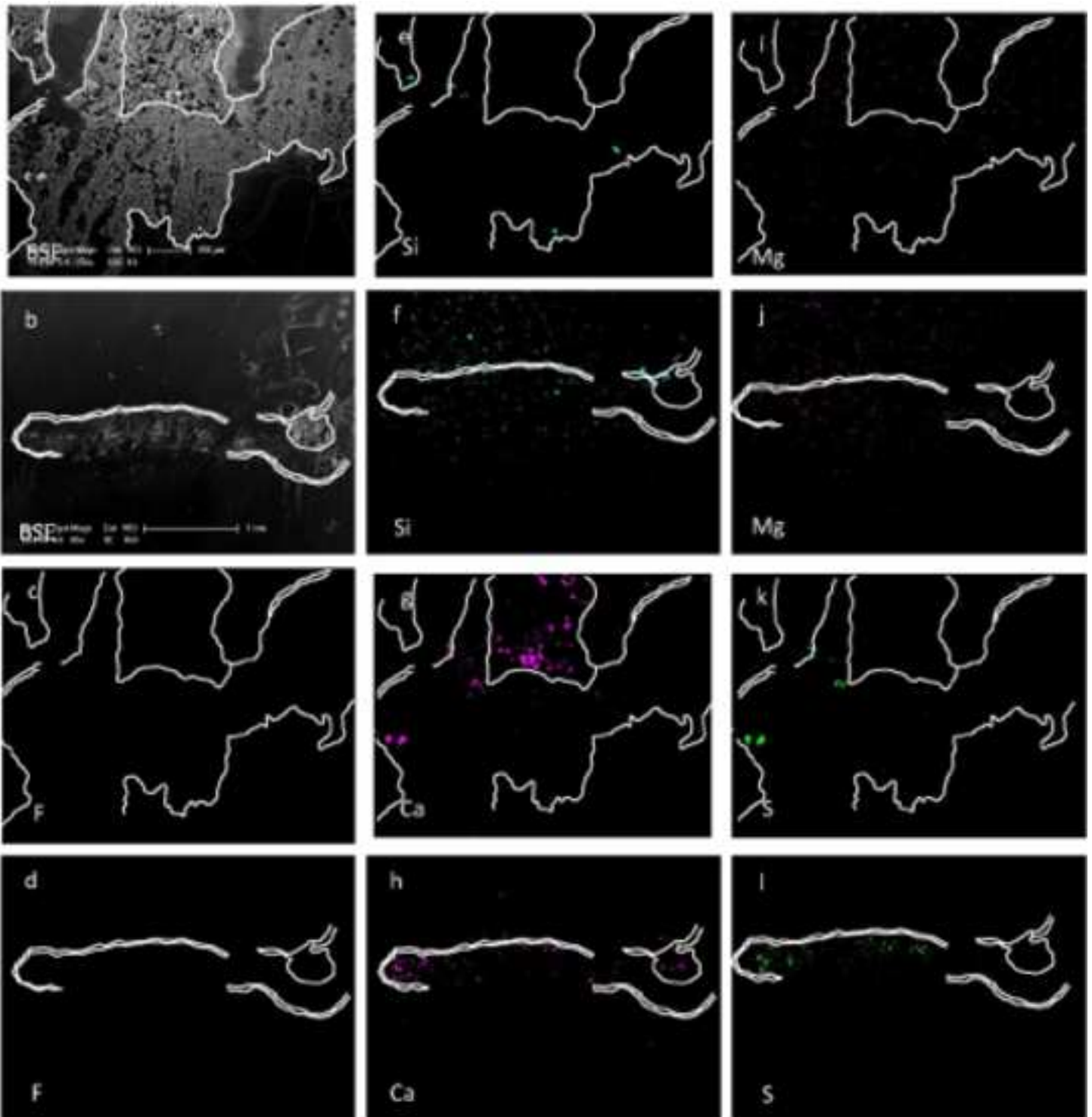
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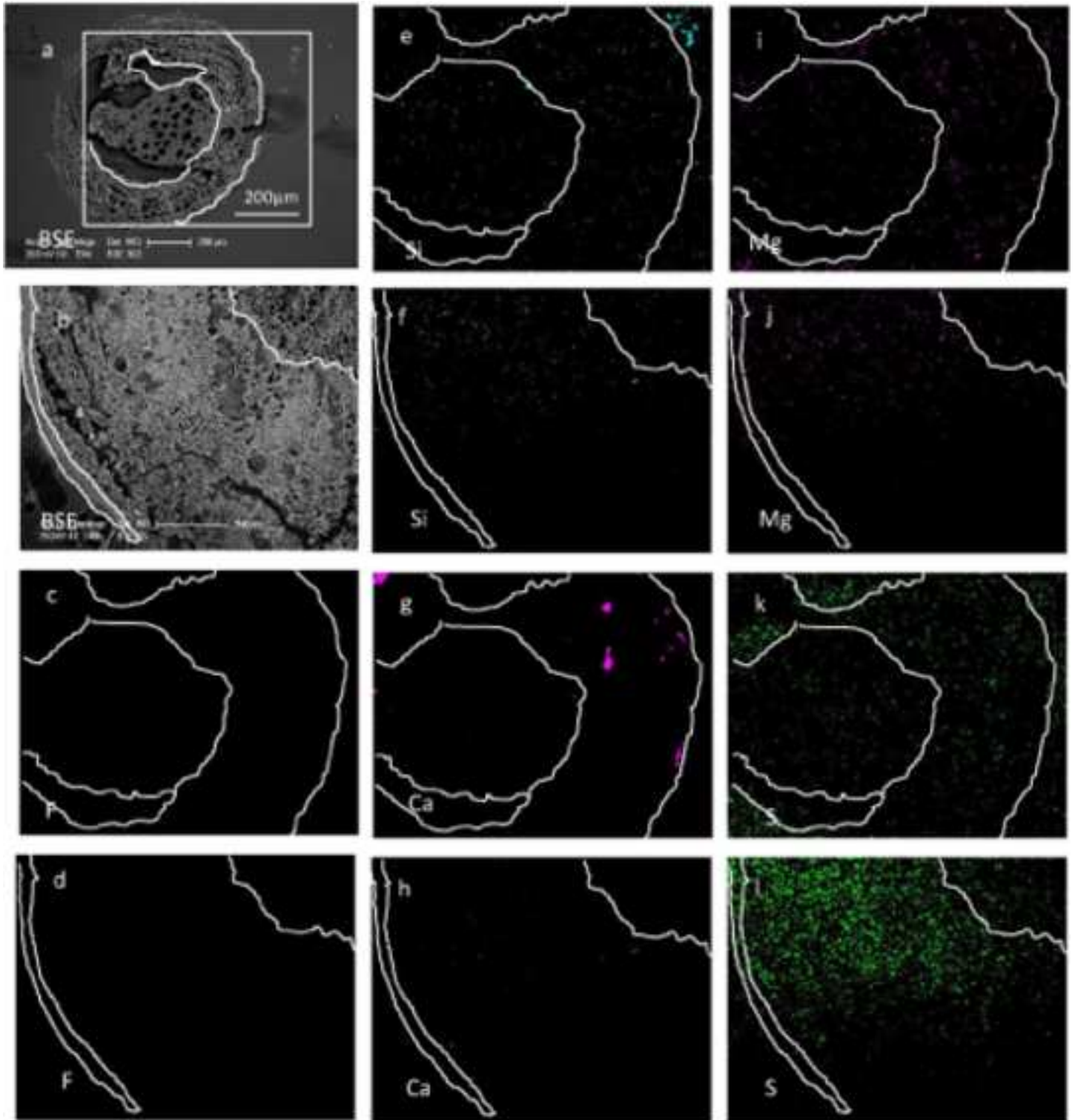
471 **Tables**

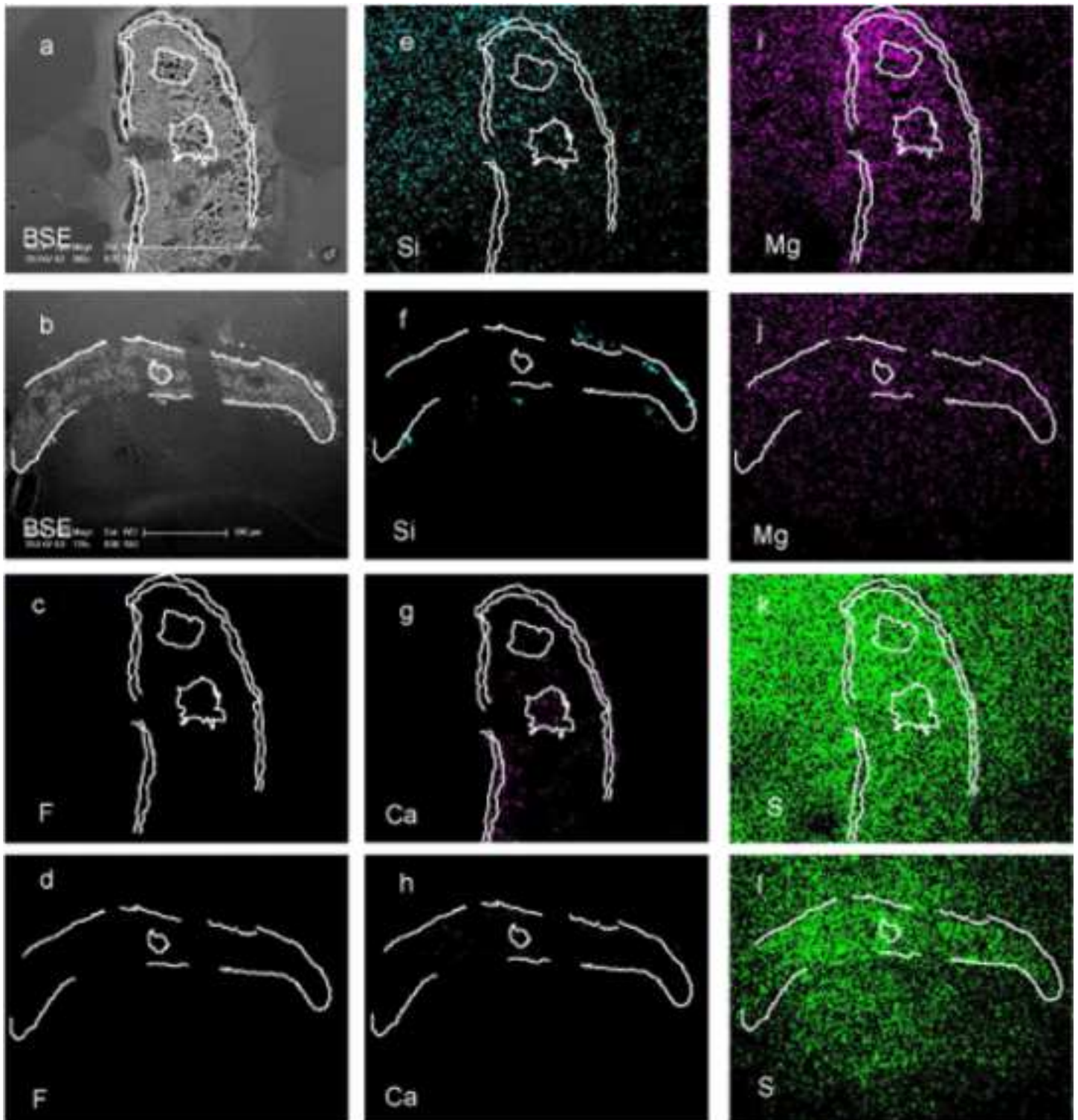
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2 472 **Table 1** Changes in the average fluoride content in soils and in *E. glaucophyllum* and *R.*
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5 473 *suaveolens* at the three sites during the monitoring period (May 2011- May 2012)

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7 474 **Table 2** Phytovolume (dm³) and density of tuft per m² for *E. glaucophyllum* and *R. suaveolens*
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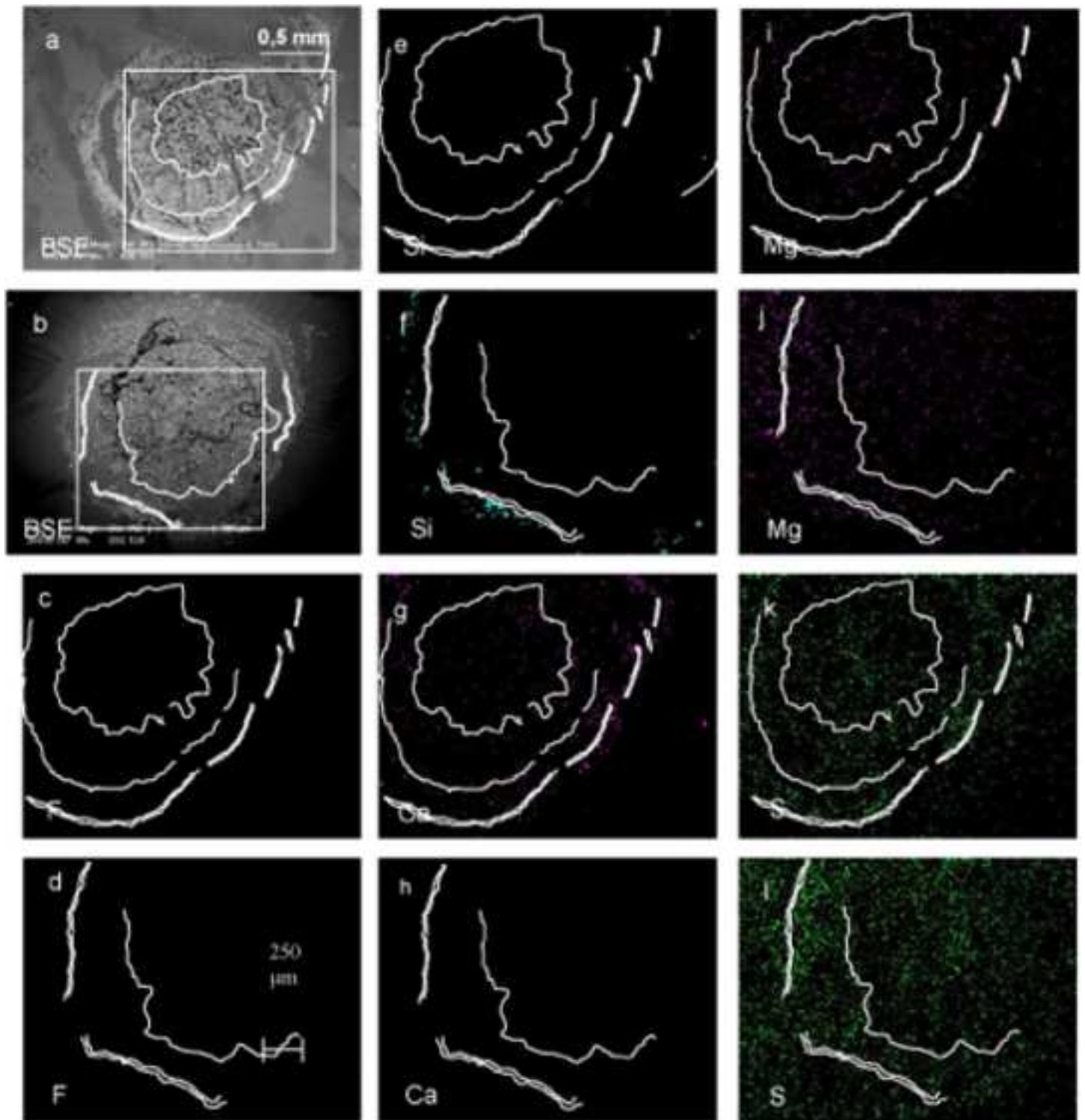


Table1

Fluoride concentration									
Site	Gabes			Skhira			Smara		
Observation time	Soil (mg kg ⁻¹) ¹⁾	Plant (mg kg ⁻¹)	BAF	Soil (mg kg ⁻¹) ¹⁾	Plant (mg kg ⁻¹) ¹⁾	BAF	Soil (mg kg ⁻¹)	Plant (mg kg ⁻¹)	BAF
May 2011	1386±257ab	335 ±88a	0.25±0.08a	141±18a	114±54a	0.8±0.4a	36±5b	47±9a	1.3±0.4a
June 2011	1394±260ab	256±52ab	0.18±0.03ab	161±23a	119±48a	0.7±0.3a	51±8a	38±4ab	0.8±0.1ab
January 2012	827± 50b	115±45b	0.14±0.06ab	143±33a	73±27a	0.6±0.3a	39±9ab	27±6b	0.7±0.2b
March 2012	1625± 377a	121±60b	0.08±0.04b	114±10a	65±31a	0.6±0.2a	43±7ab	40±15ab	0.9±0.2ab
May 2012	1469±82a	122±43b	0.08±0.03b	150±31a	84±26a	0.6±0.3a	33±7ab	26±4b	0.8±0.1ab

Means ± standard deviation (n=6 for plants, n=6 for soils) followed by different letters in a same column are significantly different at p≤0.05

(Dunn test). BAF: Bioaccumulation factor. Soil and plant F concentrations expressed in mg kg⁻¹ dry weight

Table 2 Phytovolume (dm³) and density of tuft per m² for *E. glaucophyllum* and *R. suaveolens*

Site	Phytovolume (dm ³)		Density of tuft m ⁻²	
	<i>E. glaucophyllum</i>	<i>R. suaveolens</i>	<i>E. glaucophyllum</i>	<i>R. suaveolens</i>
Gabes	0.04±0.01c	4.9±0.6c	12±2a	2.1 ±0.2a
Skhira	0.31±0.05b	32.3±2.7b	8 ±1b	1.8±0.2a
Smara	4.73±0.63a	238.9±32.0a	5±1c	1.1±0.1b

Means±SE in a same column followed by a different letter are significantly different at $p \leq 0.05$

(Wilcoxon test, n=5 for phytovolume and n=20 for density)