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EFFECTS OF FLUORIDE POLLUTION ON REPRODUCTION PHENOLOGY OF TWO MEDITERRANEAN PLANT SPECIES

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ABSTRACT: A field study was conducted along a fluorine gradient of soil pollution in Tunisia over one year to survey reproduction phenology of *Erodium glaucophyllum* and *Rantherium suaveolens*. The aim of this study was to better understand fluoride (F) pollution effects on plant development cycles of the two plant species in an arid fluoride-polluted area in the Gulf of Gabes. Three sites were monitored: (i) Gabes, the most polluted site, (ii) Skhira, a less polluted site, and (iii) Smara, a reference non-polluted site. Variations of fluoride concentrations were detected in the three soils with levels of 1792, 178, and 53 mg kg^{-1} of F in the soils of Gabes, Skhira, and Smara, respectively. Furthermore, F concentrations in the aerial above-ground parts of the two native plant species in Gabes were above the usual background concentrations and up to 360 mg kg^{-1} and 310 mg kg^{-1} in *R. suaveolens* and *E. glaucophyllum,* respectively. *E. glaucophyllum* and *R. suaveolens* were affected differently in their reproductive growth by F. *E. glaucophyllum* showed earlier flowering in the most polluted site of Gabes than in the control site (Smara) and its elusive strategy as a hemicryptophyte to enhance its chance of colonizing the F-enriched soils involved the earlier achievement of its reproduction stage. In contrast, *R. suaveolens* had later fructification and seed dispersal phenophases in the most polluted site, compared to the control site, and its colonization strategy (CS) involved favouring vegetative biomass rather than reproduction.

Keywords**:** Delayed fructification; Earlier flowering; *Erodium gaucophyllum*; *Rantherium suaveolens*.

INTRODUCTION

Environmental pollution with fluoride compounds is currently one of the world's most important problems because of its harmful effect on ecosystems.[1] Fertilizers are produced in the phosphate industry and their use is widespread in the world.[2-4] By-product discharges from this industry and gaseous and particulate emissions, especially fluorine, constitute one of the most important phytotoxic air pollutants[5] and cause severe environmental pollution in the area surrounding phosphate industry factories. There are growing concerns about fluoride accumulation in plant organs and considerable research has considered the potential toxicity of fluoride,[6-10] with special attention given recently to the morphological, anatomical, and biochemical reactions of plants growing under stressful conditions.[11-15] However, long-term (life cycle) studies are less numerous[16] and only a few have provided information on plant fitness.[17-19]

Fluoride affects plant growth phenology, especially flowering which is the most sensitive phenological stage affected by pollution.[20] As has been recently demonstrated, this phenophase has a considerable impact on total seed production and natural seeding in areas under heavy-metal pollution.[21] Plant phenophases are

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important life-history traits that may be used as biomarkers of the effects of pollution.[22,23] Flowering delays have been commonly reported as plant responses under polluted environments.[24,21,25] In addition, according to Donker et al.[26] and Huang et al.[21] pollution toxicity has been identified as a powerful selective force.

Rhanterium suaveolens Desf. (*Asteraceae*) and *Erodium glaucophyllum* L. (L'Hér.) (*Geraniaceae*), the two studied plant species, had been selected as fluoride tolerant plant species in polluted steppes in SE Tunisia in an earlier study[27] Aiming to assess long term effect of fluoride on phenology of these two native plant species, fluorine concentrations in both soil and plant samples were followed-up at two polluted sites compared to the control. Simultaneously, one-year monitoring survey of the phenological cycle of the two studied species was also carried out.

MATERIALS AND METHODS

Study area: Both polluted study sites, Gabes and Skhira, are located in the southeast of Tunisia, on the coast and near the industrial phosphate factories of the gulf of Gabes. The reference site of Smara (60 km south of Sfax city) is far away from any source of pollution. As reported in previous studies,[27,11] a gradient of fluoride pollution was detected in these sites: Gabes was the most polluted site followed by Skhira and Smara. This latter was used as a reference site. Subject to an arid bioclimate,[28] over the 30 last years.[29,16,30], these three sites have been exposed to low average rainfall (159 mm) and high average temperatures (21ºC). The soils of the three sites are calcic-magnesic soils containing gypsum and limestone.[31]

Plant material and phenological patterns: Rhanterium suaveolens Def., (chamaephyte species) and *Erodium glaucophyllum* (L.) L'Hér., (hemicryptophyte species), which are abundant at the three sites, are fluoride-tolerant species.[27] The phenological stages of both species were monitored in 2011–2012, and a comparison was made following the gradient of fluoride pollution of the sites. Observations in triplicate were undertaken in May 2011, June 2011, January 2012, March 2012 and May 2012. For each species at each site, three mature individuals were randomly selected and monitored during the phenological survey where frequencies of phenophase occurrence at each sampling date were monitored. Plant phenophases were scored when the phenophase was clearly present in the marked plants and was identical to the phenophase of the 10 neighbouring individuals from the same plant species, following the method described in Montserrat-Marti and Perez-Rontome.[32] Phenophases were expressed as percentages of flowering (Fl), fructification (Fr), and dissemination (Diss), characterized by the percentage of flowers, fruits, and dispersed seeds of each individual, respectively.

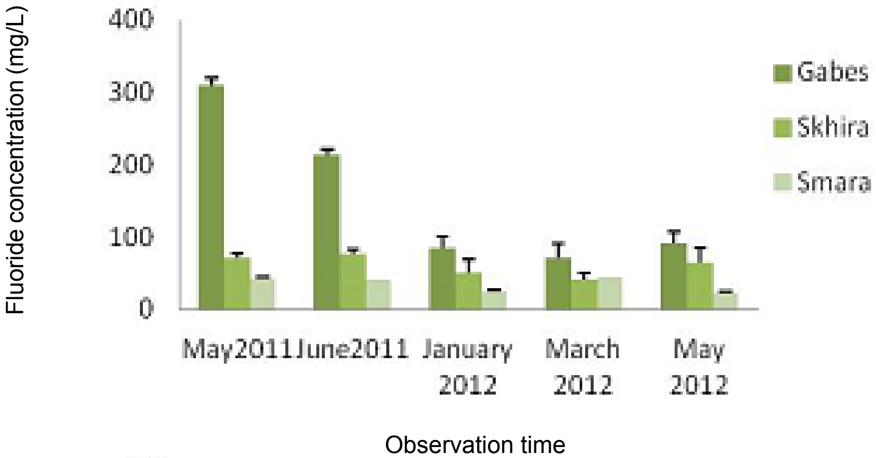
Soil and plant fluoride analysis: During the study period, three soil samples were collected near each individual (0–20 cm depth) per species per site. Likewise, leaves and stems of the middle of the shoots of three plants per species were also collected. Samples were stored in bags, and then dried at room temperature until analysis. Fluoride analyses were performed on each sample at each of the five observation times.

Soil samples were sieved (2 mm mesh), then HCl extraction was performed from 5 g of soil sample and mixed with total ionic strength adjustment buffer (TISAB) for analysis using a fluoride-specific ion electrode (Inlab/Model WTW) coupled to a pH-meter (pH ION R503) at ambient temperature.[9] In addition, dried ground (<1.0 mm) plant samples were mixed with potassium carbonate and sodium carbonate and heated in an electric oven at 700ºC. Then, hydrochloric acid wet process mineralization was performed for all samples followed by filtration and adjustment with distilled water for determination of F content by potentiometry as described by Mezghani et al.[33]

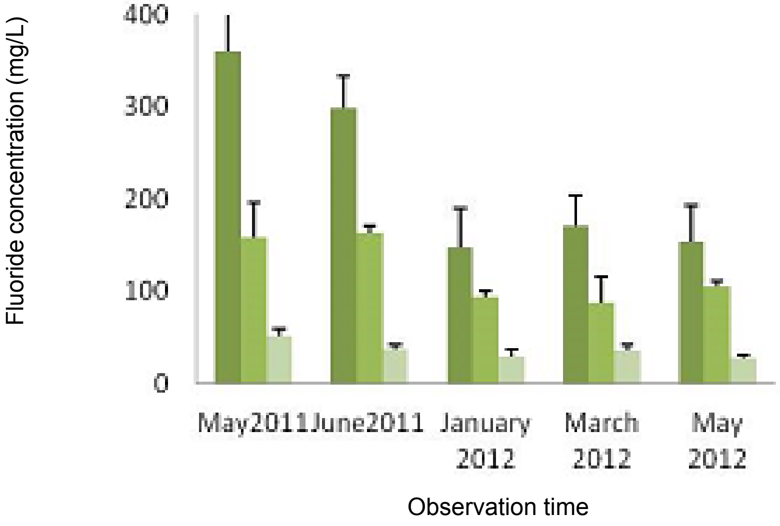
Statistical analysis: All data were analysed using SPSS (16.0) for Windows. One-way analysis of variance (ANOVA) procedure was used to compare the differences in the soil and plant variables among the different sites. Tukey's HSD test was used to perform pairwise comparisons. Difference at $p<0.05$ level was considered as statistically significant.

RESULTS

Fluoride content in soils and plants: The mean fluoride concentrations in soils and the aboveground part from each species and sampling site are shown in Figures 1A–1D.



1A



1B

Figures 1A and 1B. Average fluoride content (mg F/kg dry weight of plant) in the aboveground parts of plants of *E. glaucophyllum* and *R. suaveolens* at the three sites during the observation period (May 2011–May 2012). Values are means±SD. n = 3 for plants. 1A: Fluoride concentration in *E. glaucophyllum;* 1B: Fluoride concentration in *R. suaveolens.*

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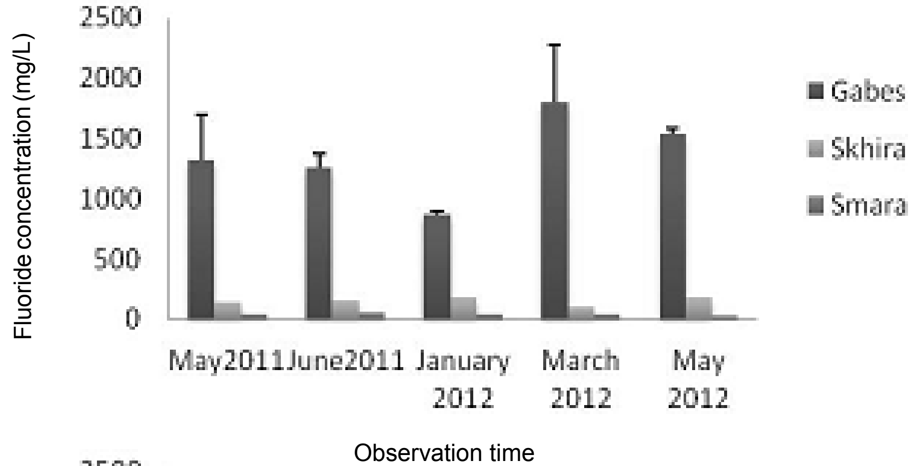
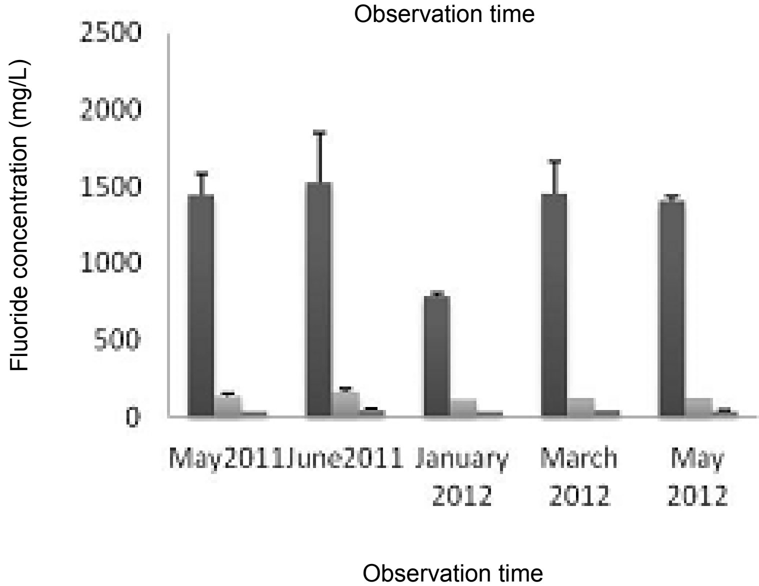


1C



1D

Figures 1C and 1D. Average fluoride content (mg F/kg dry weight) in the soil at the three sites during the observation period (May 2011–May 2012). Values are means±SD. n = 3 for plants. 1C: Fluoride concentration in the soil in which *E. glaucophyllum* was grown; 1D: Fluoride concentration in the soil in which *R. suaveolens* was grown.

Among the three sites, the values of soil fluoride concentrations revealed a significant gradient of fluoride pollution (Table 1), with the highest levels in Gabes with an average of 1330 mg kg^{-1} and the lowest in Smara with only 40 mg kg^{-1}.

The gradient of soil fluoride pollution, Gabes>Skhira>Smara, was significantly confirmed along the year of monitoring.

Regarding plant fluoride concentrations, the different ANOVA tests applied showed high significant differences between species, sites, and sampling dates (Table 2). However, interaction between these three factors was not significant.

For both species, all F concentrations were found at high levels in samples collected from Gabes (the most polluted site) and ranged from 300 to 70 mg kg^{-1} for *E. glaucophyllum* and from 350 to 150 mg kg^{-1} for *R. suaveolens*. *R. suaveolens*, a chamaephyte species, accumulated significantly greater amounts of fluoride during the year of monitoring in the two polluted sites compared to *E. glaucophyllum*. The average F concentrations in the *R. suaveolens*'s plant parts were almost 226 mg kg^{-1} and 122 mg kg^{-1} from Gabes and Skhira, respectively, compared to almost 154 mg kg^{-1} and 60 mg kg^{-1} in the *E. glaucophyllum* plant parts from Gabes and Skhira, respectively.

Table 1. Results of analysis of variance for the soil fluoride concentrations, the two different species, the three different sites, the various sampling dates, and their interaction (F=soil fluoride concentration, df=degrees of freedom)

Factor	F	df	p
Species	0.387	1	0.536
Sites	850.62	2	0.000
Sampling dates	9.645	4	0.000
Species x sites x sampling dates	1.455	8	0.193

Table 2. Results of analysis of variance for the plant fluoride concentrations, the two different species, the three different sites, the various sampling dates, and their interaction (F=plant fluoride concentration, df=degrees of freedom)

Factor	F	df	p
Species	47.984	1	0.000
Sites	194.84	2	0.000
Sampling dates	35.796	4	0.000
Species x sites x sampling dates	637.83	8	0.712

Reproductive growth patterns: E. glaucophyllum was in the fructification stage between May and June (Figure 2). During the dry period (July to September 2011), the aboveground part is totally dried. The breaking of vegetative rest, i.e., rosette morphogenesis, of this species started earlier in Gabes and Skhira (the two polluted sites) than in Smara (the reference site),. The beginning of flowering occurred after March 2012 for Smara and Skhira and started earlier in Gabes. Fructification and dissemination of seeds were earlier in Gabes in May 2012 than in Skhira and Smara. However, dissemination phenophase was reached by 70% of individuals in May 2012 in Gabes compared to only 25% at the same period in 2011, corresponding to higher F concentrations in plants in May 2011 than in May 2012 (Figures 1 and 2).

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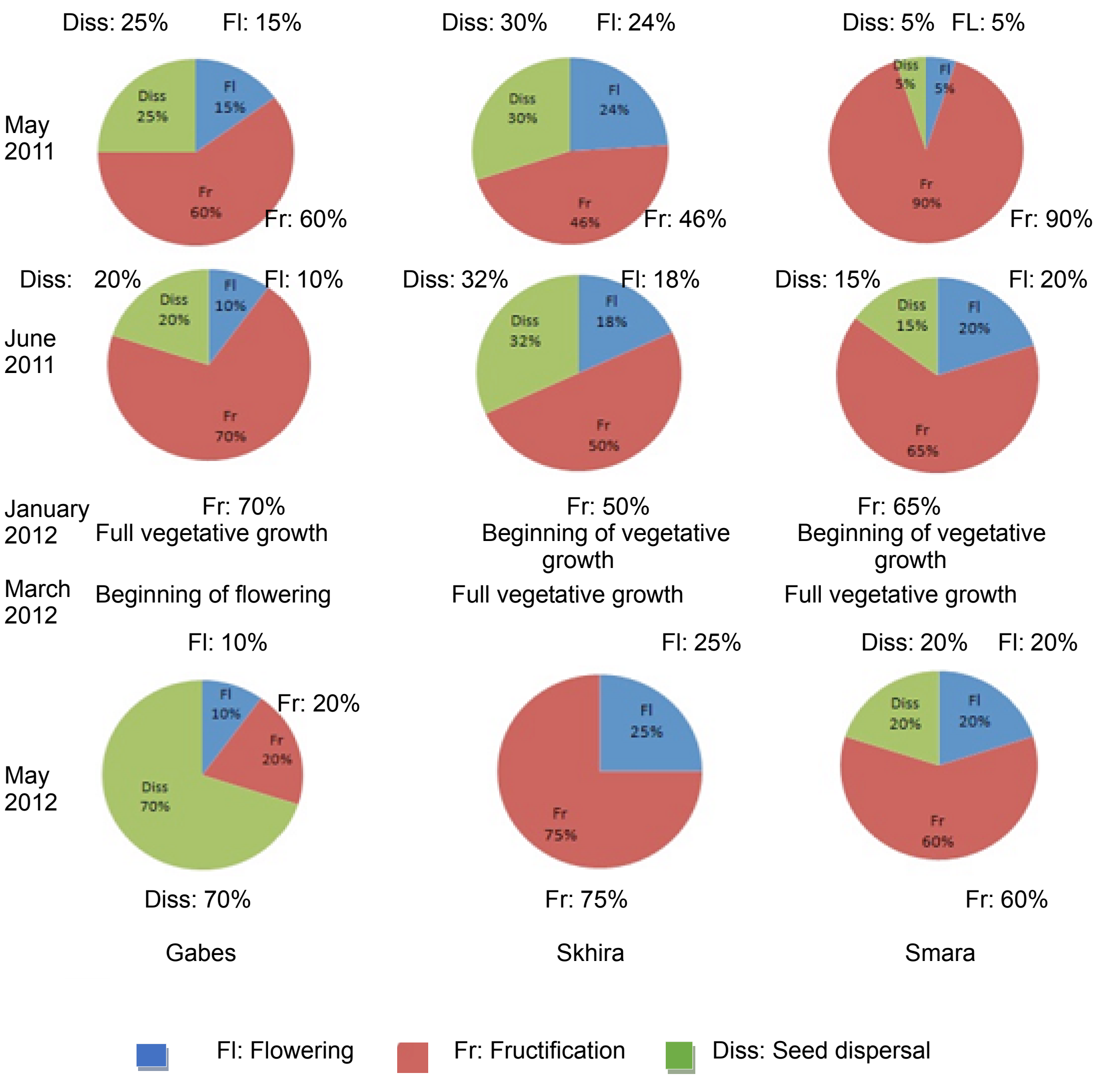

Figure 2. Patterns of change in reproductive phenophases of *E. glaucophyllum* in Gabes, Skhira and Smara during the 5 observation times from May 2011 to May 2012. (Fl: Flowering; Fr: Fructification; Diss: seed dispersal).

Leaf flushing and flowering was observed in *R. suaveolens* at all sites in May 2011 (Figure 3). However, although individuals were in full flowering in Gabes, fructification had also begun in Skhira and fructification and seed dispersal in Smara, the control site. These phenophases were completed in June 2011 in Gabes, just before vegetative dormancy during the dry period and vegetative growth at the beginning of the year 2012. Flowering only began in May 2012. The flowering phenophase was delayed in Gabes and, for the two other studied sites, fructification had already occurred, similarly to May 2011.

Thus, different phenological patterns were observed in both plant species in Gabes (the most polluted site) compared to the control (Smara site).

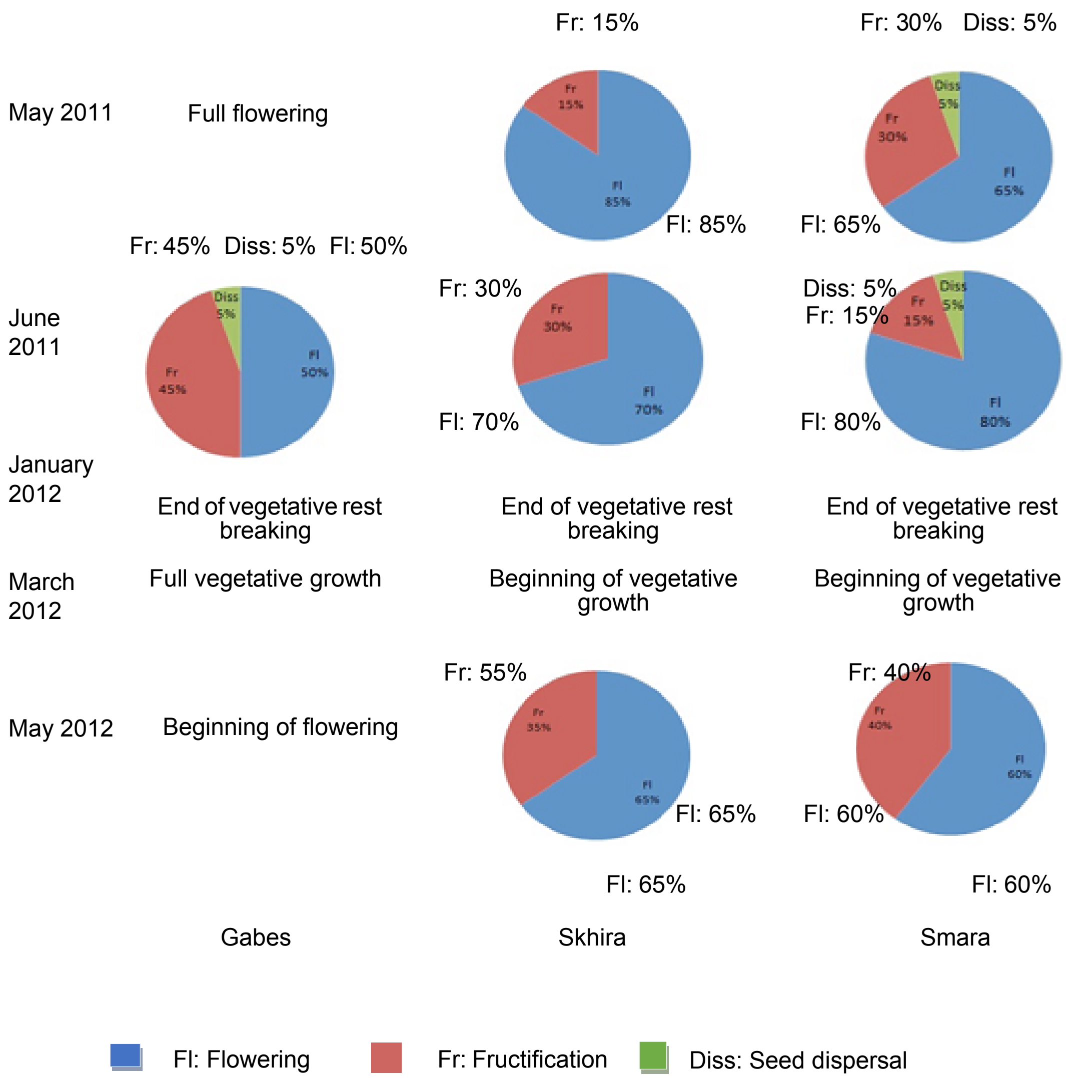

Figure 3. Patterns of change in reproductive phenophases of *R. suaveolens* in Gabes, Skhira and Smara during the 5 observation times from May 2011 to May 2012. (Fl: Flowering; Fr: Fructification; Diss: seed dispersal).

DISCUSSION

Fluoride content in soils and plants: The results of our study showed that values of F content of 0–20 cm surface soil revealed a gradient of fluoride pollution, with the highest levels in Gabes and the lowest in Smara with only 51 mg kg^{-1} at most. This was used as the reference site since its fluoride content can be considered as the geochemical background level according to Davison and Weinstein.[17] The gradient of fluoride concentrations in soils were maintained throughout the monitoring year. Fluoride is tightly retained by the soil, and only a small amount can be removed by plant leaching process.[34]

F concentrations in the aboveground parts of both studied plant species were above the background contents of F in plants described according to Davison and Weinstein[17] of <10 mg kg^{-1}, and ranged from 23 to 355 mg kg^{-1}.

Significant difference in fluoride contents between *E. glaucophyllum* and *R. suaveolens* along the year of monitoring could also be explained by differences between the life forms of the two plants and their exposure to atmospheric pollution.[35] Indeed for *E.glaucophyllum*, as a hemicryptophyte species, even if its leaves in rosette are more exposed to polluted particles from soil, the shorter period of life spent by its renewed aboveground part can explain its lower fluoride accumulation along the year compared to the chamaephyte *R. suaveolens* with a persistent aboveground part.

Phenological fitness of plants under fluoride pollution: Sexual reproduction is often initiated in response to unfavorable conditions in the environment.[36] In our study case, under an arid fluoride polluted bioclimate, results showed that *E. glaucophyllum* and *R. suaveolens* were affected in their reproductive growth. However, the two species are known as stress tolerant species with the first being more adapted to disturbed areas and the second being more competitive, following Grime's theory.[37] *E. glaucophyllum,* showed earlier reproductive growth in both polluted sites (Skhira and Gabes) than in the control site (Smara). A faster life cycle according to the elusive strategy of this plant species in the face of arid conditions can also shorten its exposure to F pollution. These results fit very well with the *E. glaucophyllum*'s life-trait as an arid hemicryptophyte. Indeed, Zvereva et al.[38] have concluded that Raunkiaer's classification of life forms appeared to be the best predictor of species' responses to pollution. Furthermore, in metalliferous soils, the population of *Rumex dentatus* had a short life cycle, with large reproductive effort maintained at the expense of speedy vegetative development.[21] Our survey of reproductive growth cycle has shown that early flowering of *E. glaucophyllum* was detected in the most polluted site of Gabes. In the same line, according to Grime,[39] it is commonly assumed that weedy or ruderal species flower earlier in response to stress. Early flowering and fructification of *E. glaucophyllum* in the two fluoride polluted sites, may also be predictive of its genotypic adaptation under fluoride pollution conditions, based on the fact that genetic adaptation of many plant species under heavy metal contamination have been reported to be associated with earlier flowering behavior.[21]

Unlike the first species, *R. suaveolens* had a delayed fructification and seed dispersal phenophases in the most polluted site of Gabes. For this chamaephyte, our hypothesis is that its adaptive strategy seems to favour vegetative biomass vs reproduction facing fluorine pollution, which is in agreement with its colonization strategy (CS). Furthermore, in the arid bioclimate, a late dissemination can, in some cases, also be a strategy for species to delay and minimize the exposure of seeds to predation.[40] Delayed flowering and fructification were also detected in many plant species under different conditions of heavy metal pollution[14,20,24,41] and can increase the chance of the plant not being able to complete their reproduction stages.[21]

According to Brun et al.[41] plants with different life cycles might respond differently to abiotic stresses. In our short-term monitoring survey case study, we found that *E. glaucophyllum* seems to be more adapted to an arid fluoride polluted area in the Gulf of Gabes than *R. suaveolens*. Using its elusive strategy, *E. glaucophyllum* achieves the reproduction stage earlier which increases its colonization chances in the F-enriched soils of the contaminated areas. These results complement the study of Jeddi and Chaieb[18] with the same plant species and confirm the efficiency of *E. glaucophyllum* as a native Mediterranean species for *in-situ* phytoremediation programs on traffic metal-polluted soils in Gabes region.

CONCLUSION

A gradient of fluoride pollution was confirmed in our three studied sites with Gabes being the most polluted site and followed by Skhira and Smara. *E. glaucophyllum* and *R. suaveolens*, accumulate an important amount of fluoride along the year following the same gradient and show two different adaptive strategies in the fluoride polluted arid bioclimate. The modified flowering and fructification times (earlier or delayed) detected in this work, represent an important life-history trait which has a large effect on the total reproductive output and the potential fitness of the plant species. Further studies of the long-term effects of fluoride exposure in plants, including plant sustainability, in future communities and ecosystems should be carried out.

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