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FLUORIDE BIOMONITORING AROUND AN INDUSTRIAL PHOSPHATE FACTORY USING BARK AND LEAVES FROM DIFFERENT TREE SPECIES IN ARID SOUTHERN TUNISIA

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ABSTRACT: Bark and leaves of *Eucalyptus occidentalis*, *Acacia salicina,* and *Tamarix aphylla* were evaluated as a possible biomonitor of air and soil fluoride contamination in arid southern Tunisia. Plant and soil samples were collected at four sites various distances from a phosphate fertilizer factory: site 1: close to the industrial areas; site 2: located in residential areas, 16 km from the industrial areas; site 3: located 30 km from the industrial areas; and site 4: a background control site outside Gabès City 50 km from any major industrial activities. Site-dependent variations were found with the highest concentration level measured close to the industrial phosphate factory and the lowest concentration level in the control site. Fluoride accumulated in the bark and leaves of the three tested trees. A higher accumulation was found in bark compared to leaves. All the tree species used in this study are suitable for inexpensive biomonitoring. *Eucalyptus occidentalis*, especially its bark, accumulated more fluoride than the other species at an equal distance from the emission source and, thus, may be more useful for monitoring fluoride pollution in arid southern Tunisia.

Keywords: *A. salicina*; Arid southern Tunisia; Bark; *E. occidentalis;* Fluoride biomonitoring; Leaves; *T. aphylla*.

INTRODUCTION

Fluoride pollution constitutes one of the major problems in urban environments.[1] Fluoride compounds are released to the environment by several industrial processes like aluminum smelters and phosphate fertilizer factories.[2] Fluoride uptake by plants growing in the vicinity of the emission sources is a potential hazard to human health due to transmission in the food chain.[3]

Biomonitoring is an effective tool for detecting potential health risks to animals and humans.[2,4,5] Several studies have highlighted the relevant role of trees as biomonitors for air and soil pollution and their bark, rings, and leaves, have been used to detect the deposition, the accumulation, and the distribution of pollutants.[6-9] The use of trees, rather than lower plants, for biomonitoring has become increasingly more important worldwide due to their excellent availability and low sampling costs.[4] Davison et al.[10] reported that the background fluoride concentration in most plant species is less than 10 mg/kg. However, tree species, have the capacity to accumulate hundreds, even thousands, of parts per million even when the atmospheric and soil available fluoride concentrations are at background levels.[1]

Along the East-coast of Tunisia, for several years, a set of phosphoric acid production factories have produced phosphogypsum in large great quantities (approximately 10 million tons per year). Currently, the phosphogypsum is stored in piles in the vicinity of the factory, and the storage causes the pollution of the water table and the soils by acid and heavy metal infiltrations.[11] Moreover, during the

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phosphate attack by sulphuric and phosphoric acids, fluoride compounds such as HF, H_2SiF_6, and CaF_2 are released by the factory chimneys.[12] These fluoride compounds are much more toxic than most other air pollutants.[13] Nevertheless, biomonitoring fluoride pollution in the Gabès region has received little attention. Most of investigations were unpublished or confined to the biochemical responses of some fruit trees to atmospheric fluoride pollution[14] or the study of fluoride transfer to the arial parts of steppic native species vegetation in order to select species for *in situ* phytoremediation.[15] Thereby, information is scarce on fluoride accumulation in forest trees growing almost everywhere in the study region, mostly on roadsides in both industrial and residential areas. This could be used as an efficient biomonitoring tool in these polluted environments.

Therefore, the aims of the present study were to: (i) evaluate tree leaves and bark as a possible biomonitor of fluoride levels across a wide profile of locations, and (ii) select suitable tree species which can be used as biomonitor plants.

MATERIAL AND METHODS

Study area: The study area is located in the south region of Tunisia in Gabès city (Figure 1) and it is subject to an arid climate with an average annual pluviometry from 167 to 176 mm and an average annual temperature from 18.8 to 19.3ºC. There are medium-sized industrial phosphate activities in the eastern part of the city, which have a local effect on the urban environment pollution. The Gabès region presently is considered one of the most polluted areas in the Mediterranean Basin, according to a recent study carried out by the Facility for Euro-Mediterranean Investment and Partnership (FEMIP) and the World Bank.[16] Moreover, phosphogypsum (PG) coming from the Tunisian Chemical Group (TCG), used in the treatment of the Tunisian phosphates, represents one of the major sources of environment contamination in the central part of Gulf of Gabès[17] and is, therefore, considered among the possible major dangers threatening the vulnerable ecosystems in this area.[16] The natural vegetation in the study area is characterized by small shrubs typical of chamaephytic steppes.[18] The city has been forested with tree species like *Eucalyptus occidentalis*, *Acacia salicina*, and *Tamarix aphylla*. These species were growing everywhere over large areas, even where a high level of urban development existed.

Plant sample collection: Plant samples were collected from different areas during February - March 2016. Fifteen samples were taken from each of the following types of sites (Figure 1): site 1: close to the industrial areas (Ghannouche); site 2: located in residential areas, 16 km from the industrial areas (Teboulbou); site 3: located 30 km from the industrial areas (Kattena); and site 4: a background control area outside Gabès City (Mareth), 50 km from any major industrial activities.

The leaf samples were selected from the middle section of the main leafy area of the tree. Bark samples were taken from the stem, at a height 1.5 m above the ground from all sides of the tree, by using a stainless-steel knife. The thickness of the bark samples was about 3 mm. All samples were oven-dried at 80ºC for 24 hr, ground, and then homogenized by sieving them through a 0.2 mm sieve. For fluoride analysis, plant samples were processed as described by Elloumi et al.[19] A 0.5 g sample of powdered plant tissue was ashed for 1 hr at 550ºC with 4 g of a sodium-potassium carbonate mixture, after which the temperature was raised to 950ºC for 30 min. After cooling, the ashed material was dissolved in 20 mL 1M HCl and filtered, made up to 50 mL with demineralized water, and stored in plastic flasks until analyzed. Fluoride concentrations were determined by potentiometry.

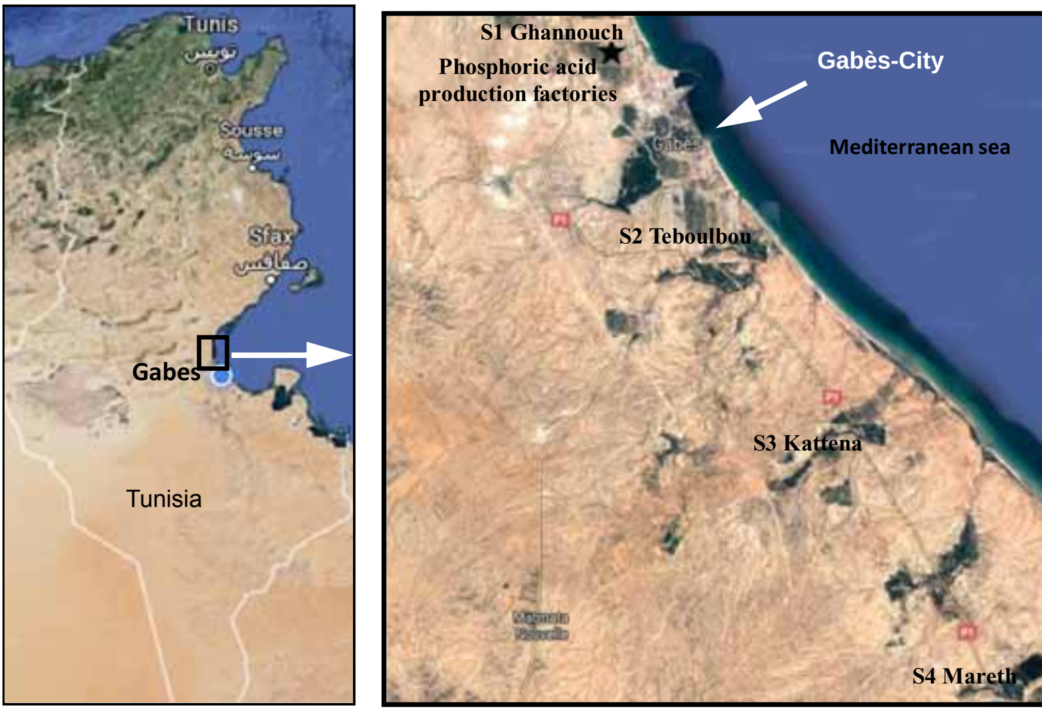

Figure 1. Map of Gabès-City localization in Tunisia indicating the study sites: Ghannouch (S1), Teboulbou (S2), Kattena (S3), and Mareth (S4).

Soil sample collection: Topsoil (0–15 cm) samples were collected from the area near the sampling trees. Each sample came from a mix of 4 random points around the selected tree. Fluoride content was determined using the potentiometric technique as described by Mezghani et al.[20] Soil samples were sieved through a 2 mm mesh. A 5 g sample of soil were mixed with HCl:H_2O (1:1) for 90 min. Then, the HCl extract was mixed with total ionic strength-adjustment buffer and analyzed using a fluoride-specific ion electrode (inlab/Model WTW) coupled to a pH-meter (pH ION R503). Soil pH and electrical conductivity (EC) were determined (saturated paw method[21]) by a pH meter and a conductivity meter, respectively. Soil organic matter (SOM) was determined by the Walkley–Black method.[22]

Data analysis: In order to investigate the relationship between the fluoride content of the soils and corresponding trees, the transfer factor (TF), defined as the ratio of fluoride concentration in trees to that in soil, was applied to assess the plants' capability to absorb fluoride from the soil.[23] A TF>1 suggests the possibility of fluoride accumulation.

$$\text{Transfer factor (TF)} = \frac{\text{Concentration of fluoride in plant tissues at the site}}{\text{Concentration of fluoride in the corresponding soil}}$$

All data were analysed using SPSS (16.0) for Windows. Two-way analysis of variance (ANOVA) procedures were used to compare the differences among the sites. A difference at $p<0.05$ level was considered as statistically significant.

RESULTS

Soil characterization: The mean fluoride concentrations, the soil organic matter (SOM), pH, and EC measured in soils are shown in Table 1.

Table 1. Concentrations of fluoride (ppm), soil organic matter (SOM, %), pH, and electrical conductivity (EC, ms/cm) in the soils of the four study sites

Site	F (ppm)	SOM (%)	pH	EC (ms/cm)
Ghannouch (S1)	1344	0.62	7.48	6.7
Teboulbou (S2)	875	0.51	7.59	6.2
Kattena (S3)	502	0.46	8.24	5.75
Mareth (S4)	120	0.38	8.64	5.49
p	0.001	0.04	NS	NS

p-values from two-way ANOVA are given; NS: non significant

Among the four locations, the values of fluoride revealed a gradient of fluoride pollution, with the highest levels in S1 with an average of 1344 ppm and the lowest in S4 with only 120 ppm. In the present study, soil organic matter content was positively correlated with the fluoride content in the surface soils. Indeed, soils at the polluted site had a significantly higher organic matter content compared with soils more distant from the factory. The pH measurements showed a relatively equal distribution in the area and the lowest were found in the samples collected from S1 and S2. High electrical conductivity values were found in the vicinity of S1, while the lowest values were found in S4. However, there were no significant differences among all the study sites (Table 1).

Fluoride accumulation in tree bark and leaves: Fluoride accumulation in tree bark and leaves from each species and sampling area are shown in Figure 2. As can be seen from this Figure, all fluoride concentrations in bark were found at high levels in samples collected from the industrial site (S1) and ranged significantly from 420 ppm in *Eucalyptus* to 42 ppm in *Acacia* ($p<0.003$). Compared to the trees growing at S1, the mean fluoride concentration in the bark of the trees growing at S2, S3 and S4 was decreased by 15%, 37%, and 73%, respectively. For all the tree species, the fluoride concentrations in the leaves were significantly less than the fluoride concentrations in the bark ($p<0.001$) and ranged between 250 ppm (S1) and 28 ppm (S4) for *Eucalyptus* and *Acacia*, respectively.

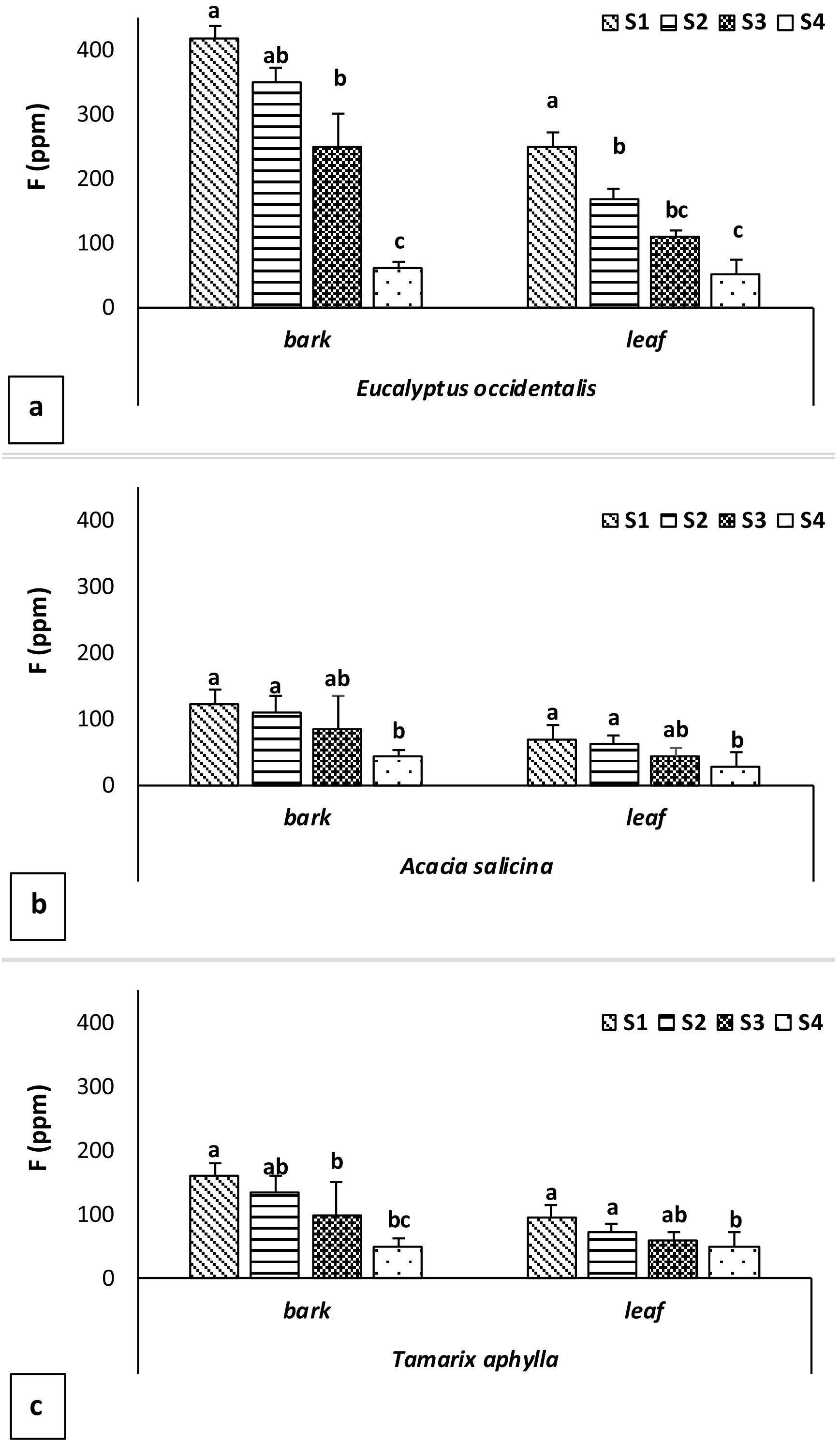

Figure 2. Fluoride concentrations (ppm) in bark and leaves of *E. occidentalis* (a), *A. salicina* (b), and *T. aphylla* (c) within all investigated sites (S1: close to the industrial areas, S2: located in residential areas16 km from the industrial areas, S3: located 30 km from the industrial areas, and a background control site, S4: outside Gabès City 50 km far from any major industrial activities). Different letters denote significant differences between study sites (Tukey's HSD test at $p<0.05$).

Overall, all tree species showed a sharp pollution gradient in relation to the distance from the industrial site (Figure 3).

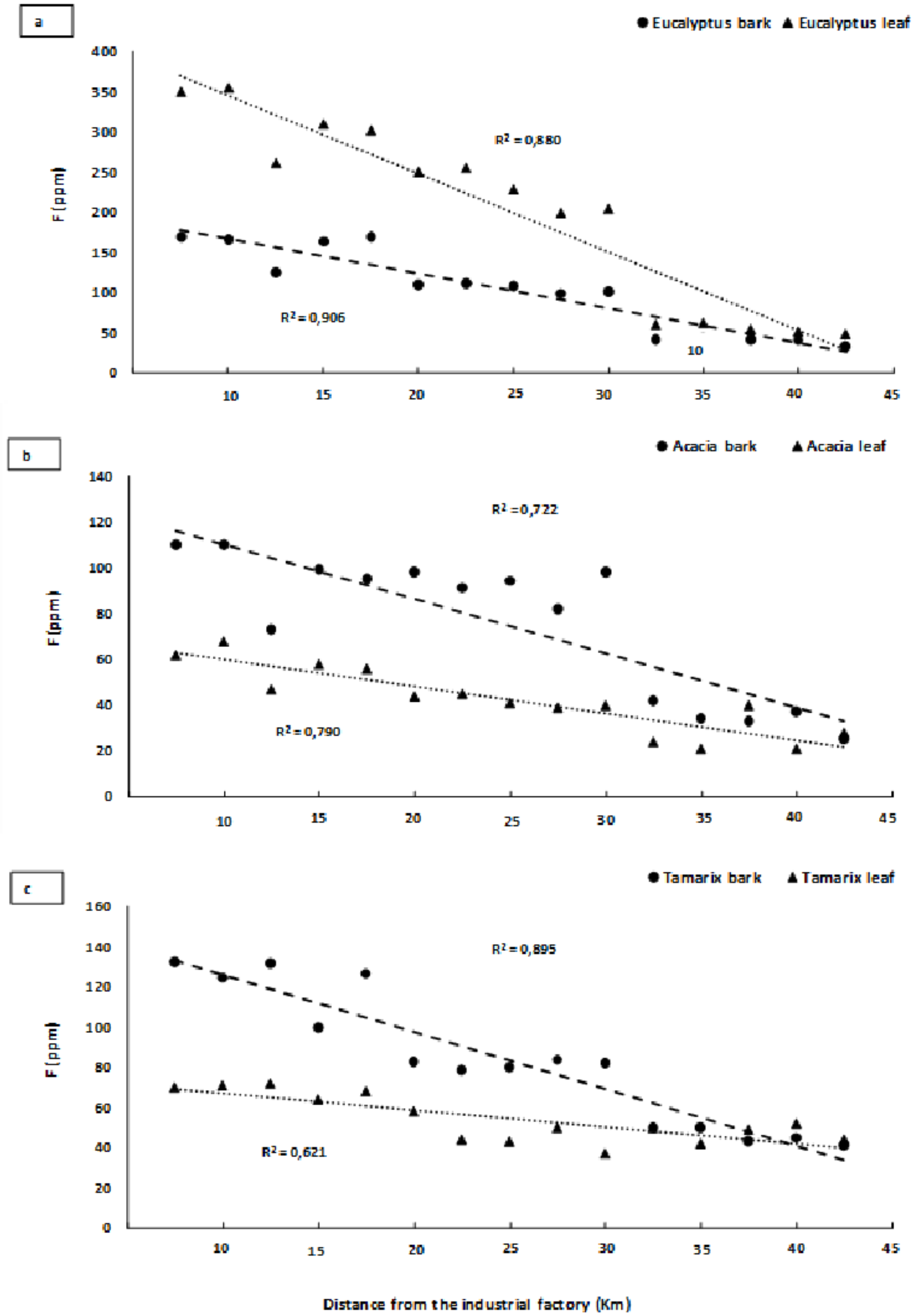

Figure 3. Fluoride pollution gradient in the study areas expressed as fluoride concentration (ppm) in bark and leaves of *E. occidentalis* (a), *A. salicina* (b), and *T. aphylla* (c) in relation to the distance from the emission source.

Transfer factor: Transfer factors from soil to the tree bark and leaves in all the study sites and for all the tree species were low i.e. <1 (Table 2).

Table 2. Transfer factors (TF) from soil to the bark and leaves of *E. occidentalis*, *A. salicina,* and *T. aphylla* in all the study sites

Site	*E. occidentalis*			*A. salicina*			*T. aphylla*		
	Bark	Leaf	*p*	Bark	Leaf	*p*	Bark	Leaf	*p*
Ghannouch (S1)	0.313	0.186	*0.05*	0.092	0.052	*NS*	0.119	0.082	*0.04*
Teboulbou (S2)	0.400	0.194	*0.02*	0.126	0.071	*0.04*	0.152	0.08	*0.01*
Kattena (S3)	0.498	0.219	*0.01*	0.165	0.088	*0.02*	0.195	0.116	*NS*
Mareth (S4)	0.500	0.442	*NS*	0.350	0.233	*NS*	0.417	0.417	*NS*

p-values from two-way ANOVA are given; *NS:* non significant

TF from soil to tree bark were significantly higher than TF from soil to tree leaves ($p<0.05$). Fluoride TF from soil to tree bark was the highest for *Eucalyptus* and the lowest for *Tamarix* in S4 and S1, respectively. Similarly, the highest TF from soil to tree leaves was found for *Eucalyptus*, while the lowest was found for *Acacia* in S4 and S1, respectively.

DISCUSSION

The results of our study showed that values of fluoride content of 0–15 cm surface soil revealed a gradient of fluoride pollution, with the highest levels in S1 (Ghannouche) and the lowest in S4 (Mareth). The high total fluoride content in the industrial site (1344 mg kg^{-1}) may be mostly due to the air borne fluoride emission from the industrial phosphate factory, which is considered as one of the major sources of fluoride contamination[1,15] and to the storage of phosphogypsum in the vicinity of the factory.[12] Kabata-Pendias[24] noted that fluoride concentrations exceeding 1000 mg kg^{-1} can occur in soils affected by anthropogenic inputs such as phosphate fertilizers. A wide variation of total fluoride concentration of soils has been reported by various authors.[25,26,15] Jha et al.[25] registered an average of 450 mg kg^{-1} of total fluoride soil content in the vicinity of brick fields in the suburb of Lucknow, in India. Brougham et al.[26] noted that soils from sites located directly downwind from the emission-source at Anglesey Aluminium had a mean fluoride concentration exceeding 1000 mg F kg^{-1}.

In the present study, it was found that increase in fluoride content causes an accumulation of organic matter content in the surface soil. Similar results were reported by Arnesen [27] who noted that the presence of fluoride in the soil decreases the growth and activity of micro-organisms resulting in a greater accumulation of organic matter. Contrary to this parameter, the lowest pH values, which revealed neutral to slightly alkaline soils in all study sites, were found in the vicinity of the industrial phosphate factory. This can explain the availability of fluoride in this site which increases with decreasing soil pH.[24] The high values of salinity in all sites can be attributed to the high rate of dry deposition in the country's climate, which is classified as an arid area.[28]

The analysis of F concentration in the studied tree bark and leaves confirmed a significant pollution gradient with the highest fluoride content in samples collected in the vicinity of the emission source. This is consistent with results elsewhere.[2,14] Kabata-Pendias and Pendias[1] considered a level of 900 ppm as an excessive or toxic level for trees. This concentration was almost two orders of magnitude higher than the highest concentration that has been measured in the present work (420 ppm in *Eucalyptus* bark). According to the literature, the fluoride values found in this study are much lower than values that have been described elsewhere. Indeed, Brougham et al.[26] reported maximum values of 2387 ppm F in leaves of coniferous tree species close to the Anglesey Aluminium. Rodriguez et al.[2] reported values >3500 ppm in deciduous tree species in the vicinity of an aluminium production plant in Argentina.

Tree bark, which accumulates atmospheric pollutants through wet and dry deposition, has been found to be a useful bioindicator for airborne pollution monitoring.[29] In the current study, the bark presents a good bioindicator of urban pollution, since the fluoride content of tree bark in the vicinity of the emission source is higher than the fluoride content of tree bark more distant from the factory. Moreover, the F content of bark is higher than that of leaves. The air pollutant content of bark reflects more consistently an accumulation over a long period of time,[30] as in contrast to leaves which fall periodically, bark persists for longer. It has been shown in previous studies that the rougher the structure of the bark, the greater the accumulation of air particles because of its greater ability to trap and accumulate the particles more effectively.[4]

Comparing the plant parts from the three assessed tree species, it is obvious that *Eucalyptus* parts are more effective in accumulating the fluoride when compared to *Tamarix* or *Acacia* tree parts. These results suggest that species specific characteristics determine the accumulation of air pollutants. Comparable findings were reported by Sawidis et al.[4] and Boukhris et al.[15] which supported other studies showing the factors which determine the accumulation of air pollutants on plant surfaces. Rodriguez et al.[2] reported that specific differences in growth habit and leaf properties between *P. radiata*, *E. rostrata*, and *P. hybridus*, determine the uptake and leaf accumulation of fluoride. These authors explained the higher fluoride accumulation efficiency of *Eucalyptus* by the hypothesis that the excretion of large amounts of extracellular fluids within leaf tissues may favour the adsorption of deposited dusts.

The accumulation of pollutants from soil to plant parts did not follow any particular pattern and varied with respect to pollutants, species, and plant parts.[31] The TF in our study generally showed an unimportant movement of fluoride from the soil to the bark and leaves of the assessed tree species (TF<1). Kabata Pendias[24] noted that the availability of F to plants is usually not closely related to the total or soluble fluoride content of a soil. This suggests that although the distribution of pollutants is quite high in the contaminated soils, the movement of these elements from soil to plant is very limited because of some physical factors like pH and OM and because of some inherent resistive mechanism within the plant body.[23] Boukhris et al.,[15] with similar conditions of pollution in the study region (Gabès), reported that for plants growing in the vicinity of a source of atmospheric fluoride, foliar fluoride concentrations will be dominated by direct uptake from the air and the contribution from the soil will be minimal.

CONCLUSION

In this work, the fluoride concentration in soil confirmed a significant pollution gradient with the highest fluoride content in samples collected in the vicinity of the emission source. All the tree species used in this study are suitable for inexpensive biomonitoring, but *Tamarix aphylla* and *Acacia salicina* should only be used in absence of *Eucalyptus occidentalis.* This species showed the highest capacity to accumulate fluoride in its bark and leaves and therefore it is a good biomonitor of fluoride contamination in areas subjected to industrial pollution. Further studies in relation to the fluoride uptake and tolerance mechanisms should be carried out. Trees can be used as effective biomonitors to detect low concentrations of fluoride both from soil and atmospheric sources, although it is sometimes difficult to distinguish between the amount of fluoride taken up from the soil and that deposited on leaves and bark.

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