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Influence of fluoride and trace metal element pollution on leaf properties of date palm (*Phoenix dactylifera* L.)

Abstract:
The effect of air pollution on soil contamination and anatomical features of date palm leaves (stomatal density and length of stomatal pore) was reported. The soil and leaves of date palm plants from industrial zone of Sfax (Tunisia) or rural area were analyzed. In fact, soil and date palm leaves from the industrial area showed relatively higher levels of several toxic elements such as fluoride, lead and cadmium. Besides, leaves of plants from the polluted site showed a decrease in stomata size and an increase in their density. These anatomical symptoms denoting a plant response to trace metal element toxicity suggests that date palm plants are suitable species for green belts given that their resistance and adaptation to pollutions in megacities.
Key words: Air pollution, fluoride, trace metal elements, stomata, date palm plants.

Introduction:

Over the years, there has been a continuous increase in human population, road transportation and industries, which has resulted in further increase in gaseous and particulate pollutant concentration. These pollutants may cause adverse negative effects on human health, affect plant life and impact the global environment by changing the earth atmosphere (Delmail *et al*., 2013; Murtaugh *et al*., 2017).

Plants should be considered as an integral part of any comprehensive plan aimed to preserve a green landscape in the area threatened by the increase of industrial activities. However, these plants may be severely affected by exposure to pollution. Air pollution can directly affect plant *via* leaves or indirectly *via* roots. Most plants showed physiological and biological changes before exhibiting visible damage to leaves when exposed to air pollutants (Elloumi *et al*., 2015). The pollutants can cause leaf injury (necrosis, chlorosis and stomata damage), reduce growth, and yield in plants (Dos Anjos *et al*., 2018). Foliage from trees near air pollution sources can even be 'coated' with particulates, which may cause stomatal occlusion, thus, leading to reduced photosynthesis (Moradi *et al*. 2017).

Date palm (*Phoenix dactylifera* L.) plants are widely planted in parks and along streets in southern Tunisia to improve the microclimate and green areas. The purpose of this study was to determine the effect of air pollution on soil pollutant contents in the industrial zone of Sfax (Tunisia) and to investigate the functional properties of leaves under these conditions.

Materials and Methods:

Study area, plant and soil collection

The studied date palm plants (*Phoenix dactylifera* L.) were chosen from two experimental stations located along the Sfax coast (southern Tunisia): the polluted site (PS) at a distance of 1 km from the lead smelter and phosphate fertilizer industries (34°70'N, 10°72'E) and the control site (CS) at western, at a distance of 20 km from the industries (34°54'N, 10°58'E). The main air pollutants emitted by these factories were fluoride (F), cadmium (Cd) and lead (Pb) (Ben Abdallah *et al*., 1990; Mezghani *et al*., 1999; Elloumi *et al*., 2003, 2015, 2017). Referring to Azri *et al.* (2000), dust emissions by the phosphate fertilizer industries are estimated annually to 1610 tons. The Triple Superphosphate unit of the phosphate fertilizer industries discharges into the atmosphere very high heavy metal flows (e.g. 653 kg Cd/year) and the particles of cadmium oxide (Ø <1

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µm) falls out up to 5 km from the fertilizer industry. On the contrary, no pollution sources were present in the CS, which commonly used as an unpolluted site.

The adult date palm plants from the two sites (PS and CS) were similar in age (approximately 30 years old) and they were planted on a sandy soil. Polluted and control sites were submitted to an arid climate and they were presented very similar geochemical, ecological and climatic conditions. The region is characterized by a semi-arid to arid climate. Recorded annual precipitations, over 60 year's period, give an average rainfall of about 237.8 mm. The prevailing winds are south or south-easterly (Béjaoui *et al.*, 2016).

For each site, three plants were selected. Leaflets of the date palm were collected from middle-aged leaves, using stainless steel secateurs. Sampling was carried out in June 2012.

For mineral elements analyses, soils samples were collected to a depth of 20 cm. It was air dried, sieved at 2 mm. In parallel, collected leaves were thoroughly washed with distilled water to remove any dust and then dried at 105°C prior to crushing to a powder.

Fluoride analysis

Fluoride content in soil and leaves of date palm plants was determined using the potentiometric technique as described by Zouari *et al*. (2014). A specific fluoride electrode (WTW pH Ion 735 Inolab, Germany) was used for the fluoride-assay.

Trace elemental analysis

The analytical methods for Cd and Pb in soil and date palm leaves were described in detail in previous studies (Bankaji *et al.*, 2015; Yu *et al.*, 2016). In leaves samples, dry leaf (1 g) were placed in an oven at 250°C for 3 h and then digested with 10 ml of HNO_3 (1M). In soil samples, 1 g of each soil sample was digested in 5 ml of HNO_3 and $HClO_4$ (4:1, v:v) at 120°C. All of the digested samples were adjusted to a constant volume using distilled water. After filtration, the contents of Cd and Pb in the extract were determined using atomic absorption spectrophotometry (AAnalyst 300, Perkin Elmer, USA).

Leaf anatomy

Stomatal density and length of stomatal pore were determined as previously described by Chaari Rkhis (2010). A thin layer of nail polish was applied to the leaf abaxial and adaxial side. Once dried, the polish layer was carefully peeled-off with adhesive tape, and then fixed on a microscope slide. The slide of stomatal was examined under a light microscope (Leitz Dialux 22 EB, Germany) and the observations were recorded using a Windias software with the enlargement of 250 times.

Statistical analysis

A one-way analysis of variance (SPSS software, 17.0) was performed. Duncan test ($p \leq 0.05$) was used to compare averages of all measured parameters.

Results and Discussion:

The soil content in F, Pb and Cd from the two sites were, respectively 16.57, 3.33 and 0.35 ppm in control site (CS) and 1176, 64.71 and 9.89 ppm in polluted site (PS) (Figure 1). Indeed, F, Pb and Cd contents in soil of PS were respectively 22, 19 and 28 times higher than those of CS.

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Results showed that the richness of polluted soil in the analyzed pollutants was in the following order F>Pb>Cd. This result indicated the pollution of the studied soil by fluoride and heavy metals. The low pollutants concentrations observed in the unpolluted site was due to low anthropogenic activities and limited human population.

The mean concentrations of pollutants measured in leaves of date palm plants were presented in Figure 2. The content in F, Pb and Cd, were respectively 2.35, 0.95 and 0.07 ppm in leaves of CS and 211.53, 29 and 3.36 ppm in leaves of PS.
As it can be seen from this figure, all of the elements were found at high levels in samples collected from the industrial site. Similar result was recorded by Al Jabary *et al*. (2016), who reported relatively high Pb and Cd concentrations (39.05 and 35.7 ppm) in *Phoenix dactylifera* leaves collected from Al-Qurna District (southern of Iraq). In this study, the high pollutants levels in leaves of polluted date palm plants can be explained by (i) the leaves air filtration and (ii) the roots uptake from the soil. According to Elloumi *et al.* (2017), foliar uptake through the stomata may be the principal route for pollutants accumulation in leaves of olive plants growing around air polluted areas.

To find out if exposure to the polluted environment affected functional leaf properties, stomatal density and stomatal pore length were determined (Table 1). In the polluted site and in comparison, to control plants, the stomatal pore length was reduced by 25 and 9% in abaxial and adaxial side respectively. This reduction in stomata size could be considered as a favorable adaptation as it might help in reducing the gaseous pollutants absorption and then reduced the pollution stress. Such response was suggested to be a common mechanism adopted by the plants to cope with the pollution load. Furthermore, the decrease of stomatal size may be an avoidance mechanism against the inhibitory effect of pollutants on physiological activities such as photosynthesis (Verma *et al*. 2006). Referring to Punwong *et al.* (2017), the reduction in stomatal size could be a result of soil contamination, which may create conditions of drought stress in plants. Reducing the stomatal index is probably a mechanism to reduce and/or inhibit water loss during transpiration, which can lead to reduced transpiration, gas exchange and photosynthesis (Anderson and Hess, 2012).

The obtained results also showed that stomatal density in polluted date palm plants increased by 27 and 23% in abaxial and adaxial side respectively. Gostin *et al.* (2009) studied changes in *Trifolium montanum* anatomy growing in a polluted urban area, where larger stomatal density was observed that corroborated with the results of the present study. According to Ogunkunle *et al.* (2013) dust that coats plant leaves can increase leaf temperature, block light, and obstruct stomata, leading to less efficient gas exchange and photosynthesis. The observed high stomatal density of date palm plants from the polluted site might suggest that it needs to transpire faster than normal plants to carry out biochemical activities, due to the presence of dust clogging some of the stomatal pores. According to Kapitonova (2002), high stomatal density in leaves of plants from polluted environments is due to the plants response to the loss of mature and healthy stomata through a degradation process caused by air pollution. Thus, the increased stomatal density of polluted date palm plants could be a strategy to compensate the decrease in stomatal pores.

Conclusion:
The present study revealed a serious fluoride and metallic contamination of soil collected from an industrial site of Sfax that indicating the vulnerability of this area. The obtained results suggest that special attention must be given to such fragile area. The growth and fruiting of date palm plants despite of the high content of F, Pb and Cd in the soil and leaves suggest the tolerance of this species. Leaf epidermal modifications evaluated by an increase of stomatal density and a decrease in stomatal size could be an adaptive feature of date palm plants to tolerate the high pollution of this area. Further analysis concerning pollutant in date fruit should be released to check their quality.

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Table 1. Effect of fluoride and heavy-metals pollution on stomatal density and stomatal pore length of *Phoenix dactylifera* grown in control and polluted sites.

	Control site		Polluted site	
	Upper surface of leaf	Lower surface of leaf	Upper surface of leaf	Lower surface of leaf
Stomatal density (stomata mm^{-2})	207.37 ± 12.11	235.31 ± 11.57	263.86 ± 15.37	291.75 ± 8.35
Stomatal pore length (µm)	16.56 ± 0.48	16.16 ± 1	12.42 ± 1.47	14.69 ± 0.53

Values are means of five samples (n = 5) ± standard deviations. [a,b] Different letters indicate significant differences ($p \leq 0.05$, Duncan test) between plants grown in CS and PS.

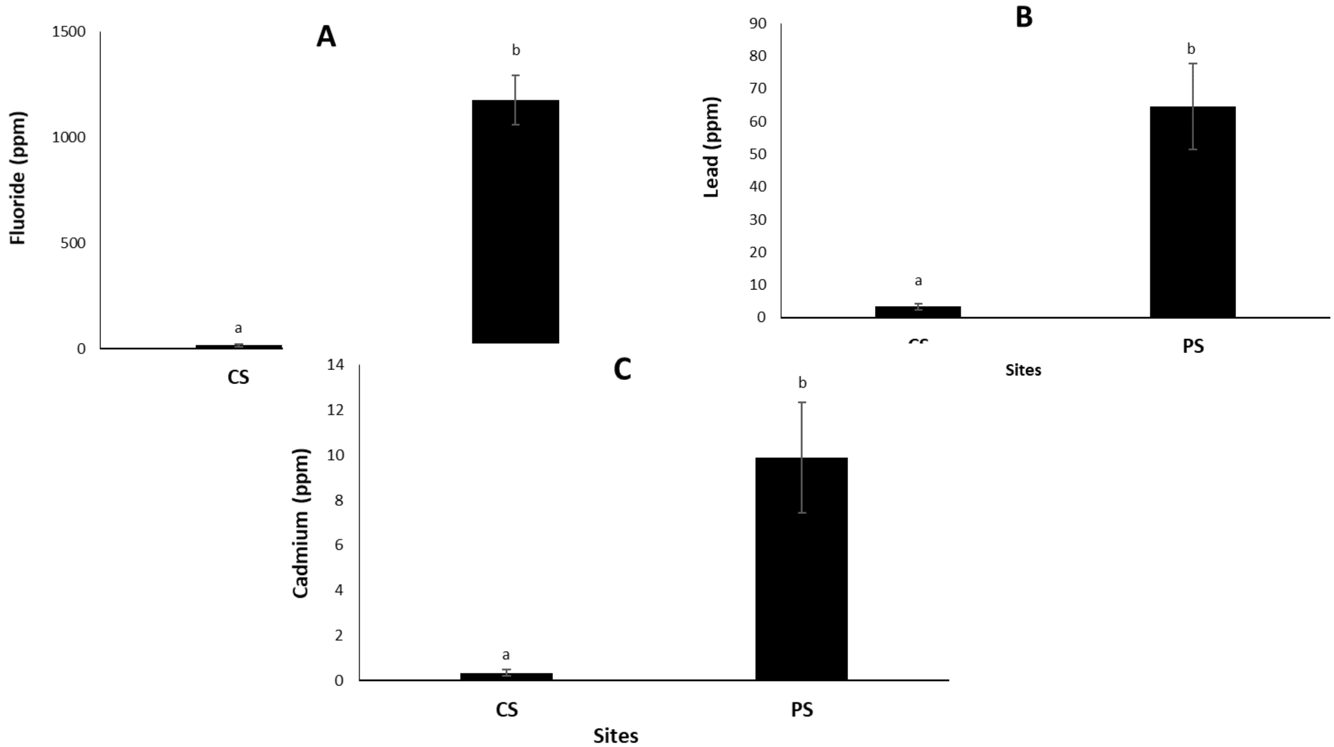

Figure 1. Fluoride (A), lead (B) and cadmium (C) content in soil of control (CS) and polluted sites (PS). Values are means of five samples (n = 5) ± standard deviations. [a,b] Different letters indicate significant differences ($p \leq 0.05$, Duncan test) between plants grown in CS and PS.

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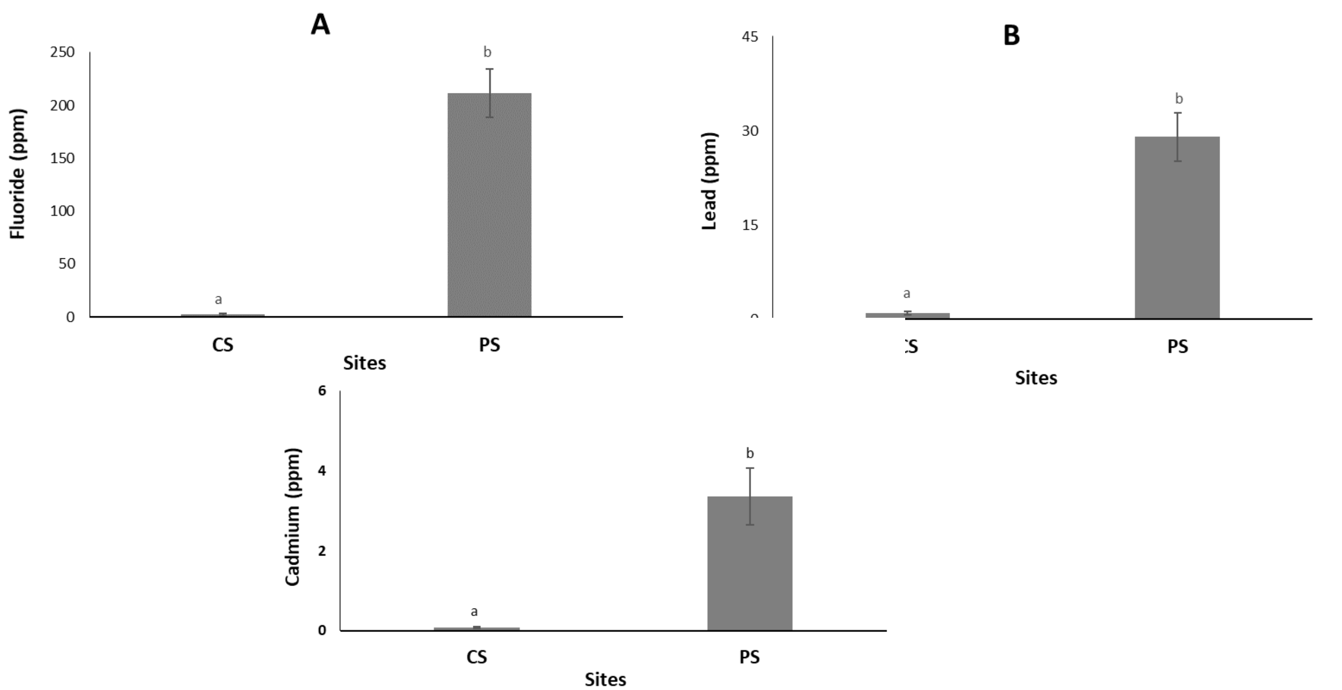

Figure 2. Fluoride (A), lead (B) and cadmium (C) content in leaves of date palm plants grown in control (CS) and polluted sites (PS). Values are means of five samples (n = 5) ± standard deviations. [a,b] Different letters indicate significant differences ($p \leq 0.05$, Duncan test) between plants grown in CS and PS.

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