Morphological and Physiological Changes Induced in the Date Palm Trees (*Phoenix dactylifera*) Exposed to Atmospheric Fluoride Pollution

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ABSTRACT

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Air quality bio-monitoring using plant leaves has been applied to assess the effects of atmospheric pollution. This study was conducted to evaluate the effects of fluoride (F) on date palm (*Phoenix dactylifera*) trees situated around a phosphate fertilizer-producing factory constituting a major source of pollution. Monthly observations on the southwest side of a phosphate fertilizer plant located in the coastal zone of the Gabès region have been assessed. This study was focused on the impact of F accumulation on the photosynthetic pigment content, cell membrane, and selected osmoprotectants (proline and soluble sugars) of the surveyed trees. Leaf samples were collected at various distances from the phosphate fertilizer factory (three sites at 0.5, 2.5, and 3.5 km and a control site at 35 km). Date palm trees accumulated significant amounts of F in leaves, with no visible lesions but showed a marked reduction in the photosynthetic pigment content, and damage to the cell membranes, as indicated by an increased malondialdehyde (MDA) content. The significant increases in the proline and soluble sugars contents in response to fluoride accumulation may be considered as defense mechanisms induced in response to fluoride stress. Based on photosynthetic pigment content, malondialdehyde (MDA), osmoprotectants levels and fluoride content, the date palm would be classified as a tolerant species.

Keywords: Adaptation, biochemical responses, biomonitoring, date palm, fluoride

Atmospheric pollution constitutes one of the major problems in industrial environments. Fluoride is one of the most important phytotoxic air pollutants (Weinstein and Davison 2003). Fluorides are absorbed through leaf stomata and move by transpiration into the principal sites of accumulation at the tip and leaf margins, where they can cause physiological, biochemical, and structural damage, and even cell death, depending on the concentration in the cell sap (Jacobson et al. 1966). Atmospheric fluoride can also reach the soil and contaminate plants via the roots. Thus,

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plants can also uptake fluoride from polluted soils (Ahmad et al. 2012; Gritsan, 1992; Jha et al. 2008; Wang et al. 2012).

The interactions between plant and different types of pollutants and the influence of environmental pollution on physiological and ultrastructural aspects were investigated by various researchers. In fact, Weinstein and Davison (2003) reported the existence of a relationship between the fluoride ion (F) concentration and damage in tree leaves. Mezghani et al. 2005 showed large differences in F concentrations among different plant species and large variations in the degree of plant tolerance to F pollution.

Fluoride concentrations in the range 1-10 µg F/g dry weight (dw) are considered as the normal background values in plant leaves (Davison et al. 2006, Brougham et al. 2013).

However, Sheldrake et al. (1978) signaled that other plants such as elderberry (*Sambucus nigra*) and camellia (*Camellia sasanqua*) did not show any toxicity symptoms at up to 3600 mg F/g dw.

Some plant species have been suggested for active and passive biomonitoring of airborne fluoride effects (Franzaring et al. 2013; Junior et al. 2008). Biomonitoring of air quality using plants has been widely applied to detect the effects of air pollution (Anicic et al. 2011; Markert et al. 2003) where many plant groups have been used in monitoring pollution pointing out their advantages; while there has been little focus on evergreen trees (Sawidis et al. 2012).

The assessment of air pollution impact includes analyses of visible injury (Ghosh et al. 1998; Oksanen and Holopainen 2001), accumulation of toxic substances and evaluation of biochemical and physiological pollutant-induced changes in parameters related to photosynthesis, respiration, enzyme activities, lipid synthesis, proteins and other metabolites (Bamniya et al. 2012, Elloumi et al. 2014, Herbinger et al. 2002). In fact, pollution may cause a decline in the number of seeds per fruit and in the leaf number and area (Gupta and Ghouse 1986; Wali and al. 2007). Air pollution can also induce qualitative and quantitative changes in the secondary metabolite composition (Kanoun et al. 2001).

In industrial areas, where mosses and lichens are absents, higher plants have gained special importance and are used as valuable biomonitors. Recent researches on the effects of F on the growth and physiological characteristics were focused on F-sensitive plants, such as *Prunus dulcis* (Elloumi et al. 2005) and *Punica granatum* (Ben Abdallah et al. 2006).

However, the olive plant, *Olea europaea*, is considered as F-tolerant plant (Zouari et al. 2016).

In Gabès, the Tunisian Chemical Group specialized in transformation and treatment of phosphate, constitutes the main source of fluoride pollution in the atmosphere. The atmospheric fluorides and sulfur emitted by the factory primarily occur in gaseous forms, such as sulfur dioxide and hydrogen fluoride, and to a lesser extent in inorganic particulate including sulfur trioxide, calcium fluoride, lead fluoride and calcium phosphate fluoride (Azri et al. 2002; Ben Abdallah et Boukhris 1990). Also, Ben Abdallah et al. (2006) and Mezghani et al. (2005) reported that fluoride is the most important phytotoxic air pollutant in the vicinity of the phosphate fertilizer factory.

In Gabès, the date palm (*Phoenix dactylifera*) trees are typical local trees growing over large areas. Even where high levels of pollution exist, they can be seen almost everywhere in the industrial and agricultural areas. Therefore, the aims of this present work were (1) to survey the leaves of local date palm trees growing in Gabès industrialized areas for fluoride accumulation and (2) to study some morphological and physiological parameters of some trees exposed to industrial emissions.

MATERIALS AND METHODS

Study area.

The present study was carried out in the industrial area of Gabès located 376 km south-east of Tunis on the southern side of the Gulf of Gabès (Mediterranean Sea, Gabès city), which has an arid climate with a low average rainfall (from 167 to 176 mm average annual pluviometry) and an average annual temperature from 18.8 to 19.3ºC.

In this study, we selected three oases located relatively close to the factory complex (site 1 [S1] 0.5 km, site 2 [S2] 2.5 km, and site 3 [S3] 3.5 km from the factory), and, as a control oasis with less fluoride exposure, a more distant one (35 km from the factory).

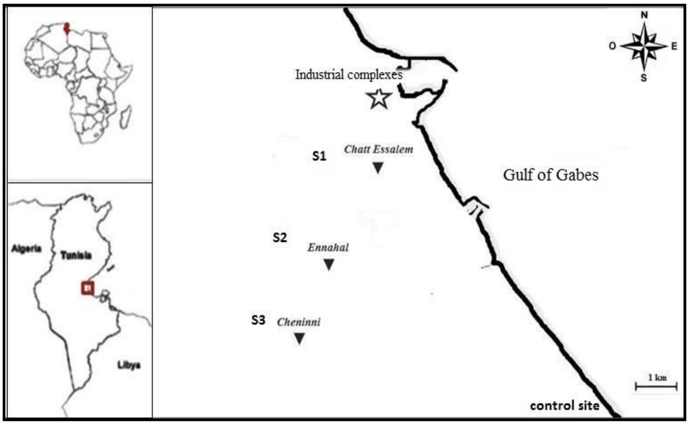

Fig. 1. Map of the study area in south east Tunisia, selected sites are named (S1, S2, S3, and control).

Sample collection.

The sampling of date palm trees,variety 'Bouhattem', was done during April, May and June 2014 to avoid rain washing fluoride.

Date palm leaves were collected with a maximum of 5 m trunk height. Three leaf samples were taken from several branches in different parts of the tree side exposed to the factory fume.

Only leaves occupying the middle of the shoots were taken.

Determination of fluoride content.

Unwashed leaf samples from all trees were dried for 24 h at 105 °C. For each repetition a 2 g powdered sample was heated in an oven at 550 ºC until white ashes were formed.

After extraction of the ash sample into NaOH solution, F concentrations were determined potentiometrically using a fluoride-specific ion electrode and a reference electrode. For measurement of total F (complex and free) in the solution, the NaOH extract was acidified with acetic acid glacial to pH 5.3 and mixed 1:1 with Total Ionic Strength Adjustment tampon buffer (TISAB). The TISAB buffer contained 57 ml acetic acid, 58 g NaCl and 4 g CDTA per litre, adjusted to pH 5.2 with 6 M NaOH and diluted to 1 liter with distilled water (Ben abdallah et al. 2006).

Determination of pigment content.

Chlorophyll a (chl a), chlorophyll b (chl b), and total chlorophyll (chl a+b) determinations were taken from fully expanded leaves of plants. A sample of 0.1 g of leaves was weighed and ground in 5 ml of 80% acetone. After filtration, the extraction was adjusted to 10 ml with 80% acetone, and the content of photosynthetics pigments was determined spectrophotometrically according to Arnon (1949).

Malondialdehyde (MDA) content.

The level of lipid peroxidation in the leaf tissues was measured in terms of malondialdehyde content (MDA a product of lipid peroxidation), determined according to Heath and Packer (1968) with minor modifications as described by Zhang and Kirham (1944).

A 0.25 g leaf sample was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 g for 5 min. Then, 4 ml of 20% TCA containing 0.5% TBA were added to a 1 ml aliquot of the supernatant. The mixture was heated at 95ºC for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of the supernatant was read at 532 nm and the value of the nonspecific absorption at 600 nm was subtracted.

Proline and soluble sugar content.

Proline content was analyzed according to Bates et al. (1973). Soluble sugars were analyzed according to Robyt and White (1987).

Statical analyses.

All statistical analyses were performed with SPSS version 17 software. Duncan’s Multiple Range test was used to determine the significance of differences between treatments, at $P \leq 0.05$.

RESULTS

Visual symptoms.

Examined date palm trees did not show any morphological abnormalities such as chlorosis, leaf curling, or necrosis. Leaves did not show any visible lesions. The under surface of the plant leaves tended to accumulate dust and appeared white. Leaves from plants removed from control area did not show any morphological abnormalities.

Fig. 2. Leaves of date palmcollected at site 1, located at 0.5 km from the Gabès phosphate fertilizer factory.

Compared to leaves removed from control site, leaves from polluted sites showed a considerable reduction of the foliar surfaces even though they have the same age.

Fluoride concentration in leaves.

F concentrations of date palm leaves collected at different distances from the phosphate fertilizers are presented in Fig. 3. The maximal contents of fluorine (95.5 µg/g dw) were recorded in leaves removed from site 1 which is the closest site to the source of pollution. At 35 km from the factory, the F concentration decreased (61.7 µg/g dw).

Regarding the temporal evolution of fluorine concentrations, the highest fluorine contents were recorded in May. This increase coincided with the decrease in precipitation. In June, recorded fluorine contents were lower than those of May.

The F concentrations in sampled date palm leaves, noted in April, May, and June were significantly higher than those of control (Fig. 3).

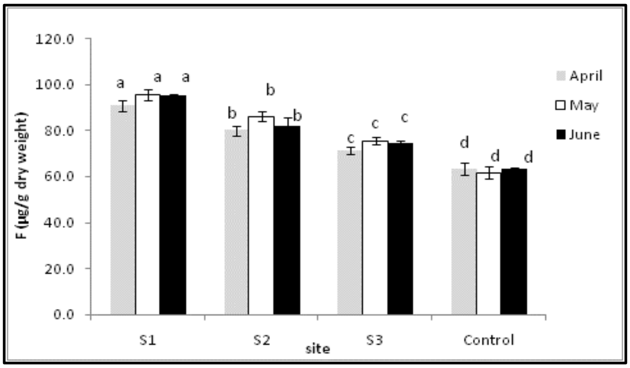

Fig. 3. Temporal variation of fluoride (µg/g dry weight) in date palm leaves at the polluted sites at increasing distances from the phosphate fertilizer factory. S1 at 0.5km, S2 at 2.5 km, S3 at 3.5 km, and the control site at 35 km from the factory. For each month, sites with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

Pigment contents.

The pigment contents of date palm trees are presented in Fig.4. There were no significant differences in leaf chlorophyll content between the sites and months.

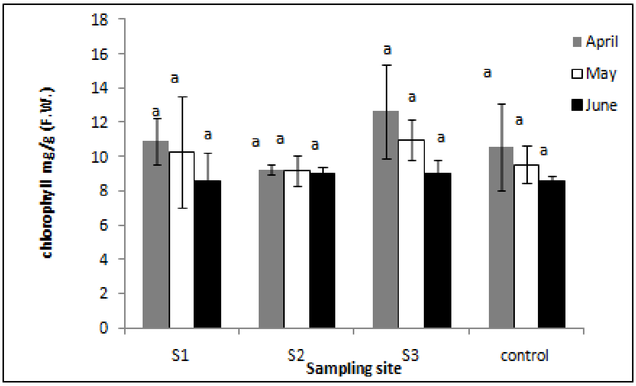

Fig. 4. Total chlorophyll contents (mg/g fresh weight) in the leaves of date palm trees at increasing distances from the phosphate fertilizer factory. S1 at 0.5km, S2 at 2.5 km, S3 at 3.5 km, and the control site at 35 km from the factory. For each month, sites with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

Chlorophyll a (Chl a), Chlorophyll b (Chl b) and total chlorophyll (Chl (a+b) contents were measured at S1, the highest polluted site. Total chlorophylls showed no significant changes in response to fluoride accumulation. However, Chl b showed increase in June. Chl a decrease significantly; it was more sensitive to F stress. Chl *a* content decreased about 65% after three months of exposure to fluoride (Fig.5).

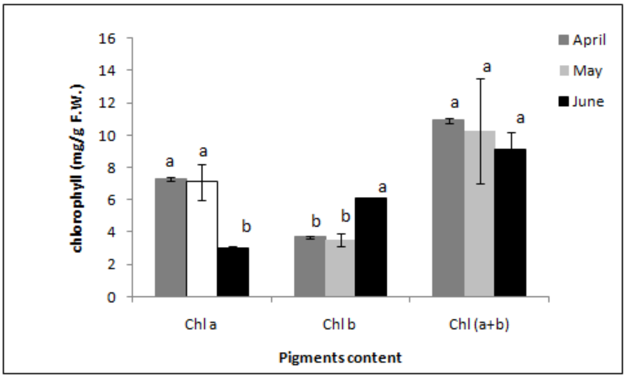

Fig. 5. Chlorophyll a (chl a), Chlorophyll b (chl b), and total chlorophyll (chl a+b) contents (mg/g fresh weight in the leaves of date palm at S1 (at 0.5 km the factory). For each pigment, means with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

Malondialdehyde (MDA) content.

As an indicator of oxidative stress due to fluoride toxicity, an enhanced formation of malondialdehyde (MDA) was observed (Fig.6).

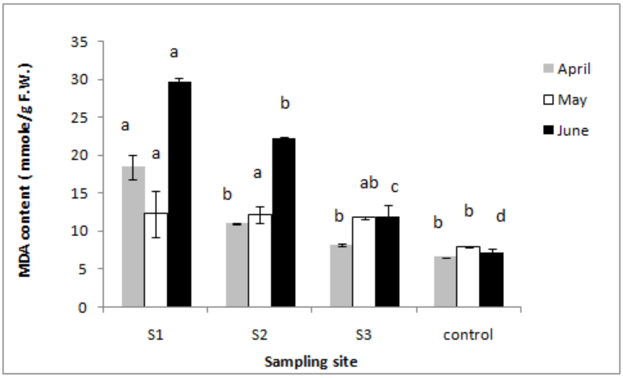

Fig. 6. MDA content (mmol/g fresh weight) in the leaves of date palm trees at increasing distances from the phosphate fertilizer factory. S1 at 0.5km, S2 at 2.5 km, S3 at 3.5 km, and the control site at 35 km from the factory. For each month, sites with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$

The MDA content rose markedly at the polluted sites indicating enhanced lipid peroxidation. The MDA content in leaves of date palm increased with fluoride contamination. The highest MDA levels were detected in the S1 (29.5 mmole/g fresh weight fw) where they were 4 times higher than those recorded in leaves removed from the control site (7.1 mmole/g fw) .

Large increases in the foliar MDA accumulation during the exposure periods were detected each month at all the sites. MDA content was highest at S1 in June (29.5 mmole/g fw), decreasing at the S2 (22.1 mmole/g fw) and S3 (11.9 mmole/g fw) sites.

Proline and soluble sugar content.

The impact of fluoride accumulation on some selected osmoprotectants (proline and soluble sugars) was determined. Compared to the control site, the proline content at S1 was increased in June (Fig.7). Proline levels increased from 20.8 µmol/g fw in April to 29.8 µmol/g fw in June.

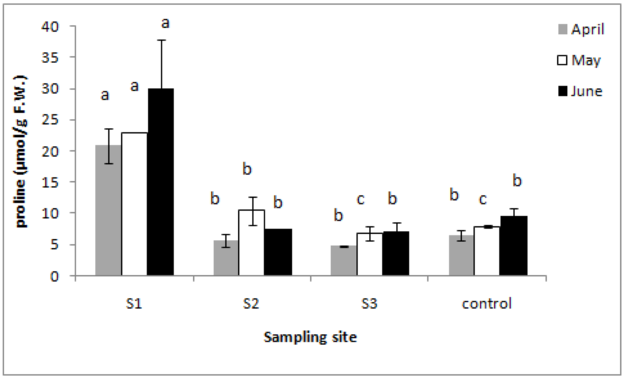

Fig. 7. Proline content (µmol/g fresh weight) in the leaves of date palm trees at increasing distances from the phosphate fertilizer factory. S1 at 0.5 km, S2 at 2.5 km, S3 at 3.5 km, and the control site at 35 km from the factory. For each month, sites with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

The fluoride content also had a great influence on the leaf soluble sugars concentration (Fig. 8). In fact, at all sites, soluble sugar increased from April to June. As compared to the control site, the soluble sugar content was significantly increased in June in all sites and more importantly in S1 (226.33 mg/g dw).

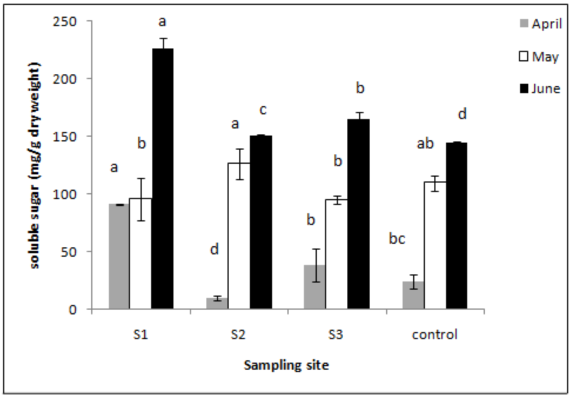

Fig. 8. Soluble sugars content (mg/g dry weight) in the leaves of date palm trees at increasing distances from the phosphate fertilizer factory. S1 at 0.5 km, S2 at 2.5 km, S3 at 3.5 km, and the control site at 35 km from the factory. For each month, sites with different letters are significantly different according to Duncan's Multiple Range test at $P \leq 0.05$.

DISCUSSION

Leaves of date palm were monitored for the F contamination. The F accumulation in the study area was high in polluted sites. Date palm possesses an important accumulative capacity of fluoride. This fluoride accumulation in leaves differed significantly depending on sampling sites and times. The high fluoride values were noted in sites close to the phosphate fertilizer-producing factory suggesting that this factory constitutes an important source of pollution. The highest concentrations of F were found in leaves sampled in May.

Thus, the dry period is an important factor that strongly influences air pollutant concentrations. In fact, in polluted areas, the dry season accelerates pollutant deposition. Therefore, pollutant availability for plant absorption is greater during drier periods. In arid regions, although high levels of solar radiation, evapotranspiration is high due to the low humidity in these areas (Allen et al. 2006). The decrease in the F contents in June, compared to the values in April and May, coincided with a marked precipitation registered for June (117 mm) (Elloumi et al. 2016). Date palm was found able to accumulate fluoride without exhibiting any symptoms of F toxicity with F concentrations up to 95 µg/g dry weight. As date palm is an evergreen species, air pollution biomonitoring can be done all the yearlong.

The chlorophyll content decreased with the increase of F concentrations. This reduction in the chlorophyll content in sampled leaves, as compared to controls, may be attributed to the high emission and deposition of dust on leaves, which adversely affects the metabolic activity of the plant (Ben abdallah et al. 2006). The reduction in chlorophyll content in the fluoride-stressed date palm trees could be due to structural alterations in the chloroplasts such as disorganization of the thylakoid system and damage to the stroma chloroplasts (Li et al. 2001; Singh et al. 2010). Moreover, according to Elloumi et al. (2015), the decrease of photosynthesis, stomatal conductance, and transpiration rates observed in almond plants grown in a fluoride-polluted zones could be attributed to abnormalities of the stomata, such as less stomatal density and stomatal closure. The reduction of the photosynthetic performance in the F pollution of date palm trees could be considered as an adaptive mechanism.

In the present study, high MDA levels were detected. MDA is an end product of membrane lipid peroxidation and high MDA levels in plants are used as an indicator of oxidative stress (Niedworok and Bielaszka 2007). Some organic solutes in plants (such as proline and soluble sugars) act osmoprotectants in adaptation to environmental stress such as drought, heavy metals and increased salinity. Sugar metabolism is adversely affected in plants growing under stressful conditions. In many plant species, the accumulation of soluble sugars has been observed in response to various environmental stresses. The accumulation of proline and soluble sugars in stressed date palm plant tissues can be used as endpoints to assess fluoride tolerance (Chakrabarti et al. 2015; Maitra et al. 2013; Ram et al. 2014). Proline accumulation can serve as a selection criterion for the tolerance of most species to stressed conditions (Ashraf and Foolad 2007). The capacity for osmotic adjustment observed in the fluoride stressed date palm trees via the accumulation of proline and soluble sugars could be considered as an adaptive mechanism.

The results of the present work showed that exposure of date palm to fluoride air pollution increased proline and sugar contents. The increase in proline contents is an important factor for providing higher tolerance to fluoride.

The increased proline content is referred to as a protective mechanism due to the generation of reactive oxygen species by fluoride. The accumulation of proline under the effect of stress provides energy for the growth and survival of the plant and helps it to tolerate stress. Thus, date palm could be considered a tolerant species; it is very well adapted to the atmospheric fluoride pollution in Gabès area, with absence of visible damages.

RESUME

Ben Amor A., Elloumi N., Chaira N. et Nagaz K. 2018. Les réponses morphologiques et physiologiques induites chez le palmier dattier (*Phoenix dactylifera*) exposé à la pollution fluorée atmosphérique. Tunisian Journal of Plant Protection 13 (si): 11-22.

La bio-surveillance végétale est un bio-indicateur de l'évaluation de la qualité de l'air et de l'environnement. Cette étude consiste à évaluer les effets des fluorures (F) sur le palmier dattier (*Phoenix dactylifera*) au voisinage des unités de traitement et de production des engrais phosphatés, constituant la principale source de la pollution atmosphérique dans la région de Gabès. Des observations mensuelles sur les teneurs en chlorophylle, la membrane cellulaire et quelques osmoprotecteurs tels que la proline et les sucres solubles ont été effectuées. Trois sites ont été choisis en fonction de leur distance par rapport à la source polluante (à 0,5, 2,5, et 3,5 km, le site témoin 35 km). Les feuilles du palmier dattier ont accumulé les valeurs significatives du fluor, avec l'absence des lésions visibles. Une réduction remarquable des teneurs de pigment photosynthétique a été enregistrée. L'augmentation significative de proline et de sucres solubles en réponse à l'accumulation de fluorure peut être considérée comme une stratégie de défense. En se basant sur les teneurs de pigment photosynthétique, le malondialdehyde (MDA), le contenu de fluorure, les teneurs de sucres solubles et de proline, le palmier dattier pourrait être classé comme une espèce tolérante.

Mots clés: Adaptation, bio-surveillance, palmier dattier, réponses biochimiques

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ملخص

بن عمر، عفاف وندى اللومي ونزار شعيرة وكمال الدين نقاز. 2018. الاستجابات المورفولوجية والفيزيولوجية المستحثة لدى نخيل التمر المعرض للتلوث الفلوري للغلاف الجوي.

Tunisian Journal of Plant Protection 13 (si): 11-22.

تعتبر المراقبة البيولوجية استنادا إلى النباتات مؤشرا بيولوجيا فعالا لتقييم نوعية الهواء والبيئة. تهدف هذه الدراسة إلى تقييم تأثير التلوث بالفلوريدات الناتج عن وحدات صنع الأسمدة الفوسفاتية بمنطقة قابس على نخيل التمر المجاور. تم اختيار 3 مسافات مختلفة انطلاقا من المصنع 0.5 و 2.5 و 3.5 كم، أما منطقة الشاهد فتم اختيارها على بعد 35 كم. سجلت أوراق النخيل ارتفاعا نسبيا للفلور دون ظهور أعراض بينما سجل مستوى اليخضور انخفاضا واضحا. ويعتبر ارتفاع مستوى البرولين والسكريات القابلة للذوبان استراتيجيات دفاع عند أشجار نخيل التمر. باعتماد بعض المؤشرات البيوكيميائية (مستوى اليخضور والمالونديلدهيد والفلوريدات والسكريات القابلة للذوبان والبرولين)، يمكن تصنيف نخيل التمر كنوع نباتي متحمل للتلوث الهوائي.

كلمات مفتاحية: استجابة بيوكيميائية، تأقلم، مراقبة بيولوجية، نخيل التمر

LITERATURE CITED

Ahmad, N., Vandenberg, L.J., Shah, H.U., Masood, T., Büker, P., Emberson, L., and Ashmore, M. 2012. Hydrogen fluoride damage to vegetation for peri-urban brick kilns in Asia: a growing but unrecognized problem. Environmental Pollution 162: 319–324.

Allen, R.G., Pereira, L.S., Raes, D., and Smith, M. 2006. Meteorogical data. Evaporación del cultivo. Guías para la determinacion de los requerimientos de agua de los cultivos. Pages 29-64. In : Food and Agriculture Organization of the United Nations, Rome, Italy.

Anicic, M., Spasic, T., Tomasevic, M., Rajsic, S., Tasic, and M. 2011. Trace elements accumulation and temporal trends in leaves of urban deciduous trees (*Aesculus hippocastanum* and *Tilia* spp.). Ecological Indicators 11: 824-830

Ashraf, M., and Foolad, M.R. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance Environmental and Experimental Botany 59: 206-216.

Azri, C., Tili, A., Serbaji, M., and Methioub, K. 2002. Étude des résidus de combustion des fuels liquide et solide et de traitement chimique du phosphate brut dans la ville de Sfax (Tunisie). Atmospheric Pollution 174: 297-307.

Bamniya, B.R., Kapoor, C.S., Kapoor, K., and Kapasya, V. 2012. Harmful effects of air pollution on physiological activities of *Pongamia pinnata* (L.) Pierre. Clean Technologies and Environmental 14: 115-124.

Bates, L.S., Waldren, R.P., and Teari, D. 1973. Rapid determination of free proline for water stress studies. Plant and Soil 39 : 205–207.

Ben Abdallah, F., and Boukhris, M. 1990. Action des polluants atmosphériques sur la végétation de la région de Sfax (Tunisie). Atmospheric Pollution 127: 292-297

Ben Abdallah, F., Elloumi, N., Mezghani, I., Boukhris, M., Garrec, G.P. 2006. Survival strategies of pomegranate and almond trees in a fluoride polluted area. Comptes Rendus Biologies 329: 200-207.

Brougham, K.M., Roberts, S.R., Davison, A.W., and Port, G.R. 2013. The impact of aluminium smelter shut down on the concentration of fluoride in vegetation and soils. Environmental Pollution 178: 89-96.

Chakrabarti, S., and Patra, P.K. 2015. Biochemical and antioxidant responses of paddy (*Oryza sativa* L.) to fluoride stress. Fluoride 48: 56-61.

Davison, A.W., and Weinstein, L.H. 2006. Some problems relating to fluorides in the environment: effects on plants and animals. Pages 98-251. In: Tressaud Alain, editor. Fluorine and the environment: atmospheric chemistry, emissions and lithosphere, Oxford.

Elloumi, N., Ben Abdallah, F., Mezghani, I., Rhouma, A., and Boukhris, M. 2005. Effect of fluoride on almond seedlings inculture solution. Fluoride 38: 193-198.

Elloumi, N., Zouari, M., Chaari, L., Jomni, C., Marzouk, B., and Ben Abdallah, F. 2014. Effects of cadmium on lipids of almond seedlings *(Prunus dulcis).* Botanical Studies 55: 61. doi:10.1186/s40529-014-0061-7.

Elloumi, N., Zouari M., Chaari L., Jomni C., Ben Rouina, B., Ben Abdallah, F., and Kallel, M. 2015. Morphological and physiological changes induced in *Olea europaea* and *Prunus dulcis* exposed to air fluoride pollution. Brazilian Journal of Botany 38: 99-106.

Elloumi, N ., Ben Amor, A., Zouari, M., Belhaj, D., Ben Abdallah, F., and Kallel M. 2016. Adaptive biochemical responses of *Punica granatum* to atmospheric fluoride pollution. Fluoride 49: 357-365.

Franzaring, J., Klumpp, A., and Fangmeier, A. 2007. Active biomonitoring of airborne fluoride near an HF producing factory using standardized grass cultures. Atmospheric Environment 41: 4828-4840.

Ghosh, S., Skelly, J.M., Innes, J.L., and Skelly, L. 1998. Temporal development of visual ozone injury on the foliage of *Prunus serotina*-astatistical evaluation. Environmental Pollution 102: 287-300.

Gritsan, N.P.1992. Phytotoxic effects of gaseous fluorides on grain crops in the southeast Ukraine. Fluoride 25: 115-122.

Heath, RL., and Packer, L.1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 125: 189-198.

Herbinger, K., Tausz, M., Wonisch, A., Soja, G., Sorger, A., and Grill, D. 2002. Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. Plant Physiology and Biochemistry 40: 691-696

Jacobson, J.S., Weinstein, L.H., McCune, D.C., and Hitchcock, A.E. 1966. The accumulation of fluoride by plants. Journal of the Air Pollution Control Association 16: 412-417.

Jha, S.K., Nayak, A.K., and Sharma, Y.K. 2008. Response of spinach (*Spinacia oleracea*) to the added fluoride in an alkaline soil. Food and Chemical Toxicology 46: 2968-2971.

Junior, A.M.D., Oliva, M.A., and Ferreira, F.A. 2008. Dispersal pattern of airborne emissions from an aluminium smelter in Ouro Preto, Brazil, as expressed by foliar fluoride accumulation in eight plant species. Ecological indicators 8: 454-461.

Kanoun, M., Goulas, M.J.P., and Biolley, J.P. 2001. Effect of a chronic and moderate ozone pollution on the phenolic pattern of bean leaves (*Phaseolus vulgaris* L. cv. *Nerina*): relations with visible injury and biomass production. Biochemical Systematics and Ecology 29: 443-457.

Li, C., Zheng, Y., Zhou, J., Xu, J., and Ni D. 2001. Changes of leaf antioxidant system, photosynthesis and ultrastructure in tea plant under the stress of fluorine. Biologia Plantarum 55: 563-566.

Maitra, A., Datta, J.K., and Mondal, N.K. 2013. Amelioration of fluoride toxicity with the use of indigenous inputs. Journal of Stress Physiology and Biochemistry 9: 207-219.

Markert, B.A., Breure, A.M., and Zechmeister, H.G. 2003. Bioindicators and biomonitors: Elsevier, Amsterdam,1014 pp.

Mezghani, I., Elloumi, N., Ben Abdallah, F., Chaieb, M., and Boukhris, M. 2005. Fluoride accumulation by vegetation in the vicinity of a phosphate fertiliser plant in Tunisia. Fluoride 38: 69-75.

Niedworok, E., and Bielaszka, A. 2007. Comparison of the Effect of Vitamins A and E on Aging Processes of Edible Vegetable Oils. Polish Journal of Environmental Studies. 16: 861-865.

Oksanen, E., and Holopainen, T . 2001. Responses of two birch (*Betula pendula Roth*) clones to different ozone profiles with similar AOT40 exposure. Atmospheric Environment 35: 5245-5254.

Ram, A., Verma, P., and Gadi, B.R. 2014. Effect of fluoride and salicylic acid on seedling growth and biochemical parameters of watermelon (*Citrullus lanatus*). Fluoride. 47: 49-55.

Robyt , J.F., and White, B.J. 1987.Biochemical techniques: theory and practice. Brooks/Cole Publishing, USA, 407 pp.

Sawidis, T., Krystallidis, P., Veros, D., and Chettri, M . 2012 . A study of air pollution with heavy metals in Athens city and Attica basin using evergreen trees as biological indicators. Biological Trace Element Research. 148: 396-408.

Sheldrake, R., Goss, G.E., L.E. St. John., and Lisk, G.J. 1978. Lime and charcoal amendments reduce fluoride absorption by plants cultured in a perlite-peat medium. Journal of the American Society for Horticultural Science. 103: 268-270.

Singh-Rawal, P., Jajoo, A., and Bharti, S. 2010. Fluoride affects distribution of absorbed excitation energy more in favour of photosystem 1. Biologia Plantarum 54: 556-560.

Wang, C., Yang, Z., Chen, L., Yuan, X., Liao, Q., and Ji, J. 2012 . The transfer of fluorine in the soil-wheat system and the principal source of fluorine in wheat under actual field conditions. Field Crops Research 137: 163-169.

Weinstein, L.H., and Davison, A.W. 2003. Native plant species suitable as bioindicators and biomonitors for airborne fluoride. Environmental Pollution 125: 3-11.

Zhang, J.X., and Kirham, M.B. 1994. Drought stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. Plant and Cell Physiology 35: 785-791.

Zouari, M., Ben Ahmed, C., Elloumi, N., Ben Rouina, B., Labrousse, P., and Ben Abdallah, F. 2016 .Effect of irrigation water fluoride on relative water content, photosynthetic activity, and proline accumulation in young olive trees (*Olea europea* L. cv. Chemlali) in arid zone. Research Report. Fluoride 49: 303-309.
