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Urban Tree Leaf as Bio-indicator for Air Pollution around Superphosphate Fertilizers Plant, Upper Egypt

Ahmed A. El-Khatib [1,*], *A. A. El-Shanawany* [2] *and E. M. El-Amery* [2]

[1]Botany Department, Faculty of Science, 82524 Sohag, Sohag University, Egypt.
[2]Botany Department, Faculty of Science, Al-Azhar University, Assiut, Egypt.

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Abstract: For the purpose of this study, two common tree species namely: *Ficus nitida* and *Eucalyptus golobulus* were selected in the areas surrounding Asyut Superphosphate Fertilizer Plant, Upper Egypt. Physiological and anatomical responses of tree leaf due to airborne particles deposition were studied. Leaves exhibited reduction in their surface area, dry weight, chlorophyll contents, and an increase of carotenoids content. Anatomical features exhibited highly significant variations between control and polluted sites. All the measured parameters showed seasonal and plant species effects, where *F. nitida* appeared to be more sensitive than *E. globulus*. Correlation between leaf characteristics and the mass of captured leaf deposit reflects the existence of an association in between and the drastic conditions caused by the prevailing atmospheric pollution, recommend their using as biomarkers.

Keywords: chlorophyll, carotenoids, leaf anatomy, superphosphate, fertilizers, biomarkers.

1 Introduction

According to UNEP [1], atmospheric pollutants emitted by the fertilizer industry can include CO2, NH3, NOx , N2O, HF, SOx, fertilizer dust, acid mists, and radiation (from phosphogypsum). In addition, it contributed to air heavy metal pollutants, especially cadmium (Cd). However, the phosphate fertilizer industry shares the responsibility to the production of many precursors for secondary pollutants [2]. Although Phosphate fertilizer industry is limited in Egypt (construction capacity up to 1.2 million tons, measured in 15.5% phosphorus pentoxide), it considered one of the stationary sources for production of fugitive particles onto the atmosphere. It contribute to the presence of single or normal, phosphate (SSP), with varied phosphorus pentoxide (P2O5) content of the air particles, based on the type of acid use to pulverize phosphate rock [3]. Any plans to update or to reduce pollution or technology for this industry had not developed yet, despite the application of such techniques since long time [4]. Assiut superphosphate fertilizer plant is a branch of the Egyptian Financial and Industrial Company (EFIC). Due to the absence of ecological regulation, its presence in close proximity to the agricultural and residential areas lead to a significant loss in farms, atmospheric radiation hazard and the spread of diseases among the population [5, 6, 7, 8, 9].

The continuous monitoring of the environmental quality in this area should be taken into account. One of the proposals under evaluation is the introducing of biomonitoring through using the leaves of the currently growing urban trees, which their survival may correlate with structural and metabolic adaptations to the stressful environmental conditions. Accordingly, the main objective of this study was to assess the potential use of some anatomical and physiological leaf characteristics as effective parameters in this concern. In addition, due to the lack of addressing this subject in this area, the obtained results could be used as preliminary baseline data for future assessment and studies.

2 Materials and methods

Study area description: Superphosphate Fertilizer Plant erected on an area of 0.26 km^2 (27.205175N 31.1208344E) at Manquabad Village, which is a suburban area far from Assiut City by 7 km and includes densely population and agricultural areas (Area= 11.35 km^2, No. inhabitants = 52286) (Mape1). According to the Egyptian Environmental Affairs Agency [10], the climatic characteristic of the study area reveals that air temperature is ranging from 15.3°C to 30.3°C; the annual mean of wind velocity is 7.5 knots/hour, with maximum peak during spring (8.3 knots/hour) in the N-W direction, and. the annual mean of relative humidity is 38% and the annual mean of rain fall is 0.7 mm.

*Corresponding author e-mail: aaelkhatib@yahoo.com

Map (1): showing the location of Manquabad village, where the Superphosphate Fertilizer Factory.

Sampling of dust and tree leaves: This study conducted during 2009–2010 in four seasons. Two common tree species namely: *Eucalyptus globulus* (Myrtaceae) and *Ficus nitida* (Moraceae) (Buolus, 2000) were sampled from the close surrounding area of the plant [11]. Control plants collected from area located away from the impact of the pollution emission. Three individual trees from each species were taken into account. A total of 384 young leaves from each tree species were selected from polluted and control area for the study of different parameters (forty eight leaves / season). The selected leaves were cleaned and marked. At the end of each season, the surface dust load of marked sixteen leaves per tree individual were collected on pre-weighed tracing paper with utmost care and the on pre-weighed tracing paper with utmost care. Then these leaves were detached from the petiole, After this, leaves were cut from the petiole. To study the leaf anatomical structure of the studied trees, three leaf samples were fixed in FAA (formaldehyde: acetic acid: alcohol, 5: 5: 90, respectively) during the field, other detached leaves were kept in ice-box, and brought to the laboratory for further analysis and investigation.

Physiological investigation: Dry weight of leaf samples was determined by using an electronic balance with an accuracy of 0.001 g (Electronic balance ER-120A, A&B company limited, Tokyo Japan) according to the method of Agbaire and Esiefarienrhe [12]. The leaf area (m2) estimation was proceed according to Vora and Bhatnagar method [13]. The method adopted by Metzner *et al.* [14] was used to determine the photosynthetic pigments with the help of a Unico®1200 Spectrophometer.

Microtomy and maceration: Depending on the hardness of the material, the field fixed samples were preserved in either mixture of alcogycerol (1:1 mixture, 70% ethyl alcohol and glycerol) or in 70% ethyl alcohol. Leaf samples were treated by nitric acid to facilitate the study of cell size variation in the vascular tissues [15]. To investigate the complete leaf anatomy using binuclear vertical microscope (Olympus BS-2), leaf transverse section of 10 mm thickness were immersed in ethanol series, stained with Heidenhain's haematoxylin and safranin/Bismarck brown, then mounted by Canada balsam on glass slides to be ready for investigation. The sections photographed by digital camera (HDCE-50B) and the macerated vascular element were measured using Image-Pro Plus 6.1 [16].

Statistical analysis: Data were analyzed using SPSS15.0 for Windows (SPSS Inc., Chicago, USA). Significance of differences between mean (n=3) for each set of parameters obtained using MANOVA Model and Pearson's linear correlation coefficient (r). A probability level of 0.05 was considered statistically significant.

3 Results

Field observations declared the presence of leaf visible injury in the studied tree species. These symptoms (chlorosis, yellowish, and necrosis) were more obvious in the samples of *F. nitida* tree. The present results (Fig. 1) reflect the drastic effects posed by airborne particle mass on the leaf area of the studied species, where there is a negative correlation in between (r = -0.442).

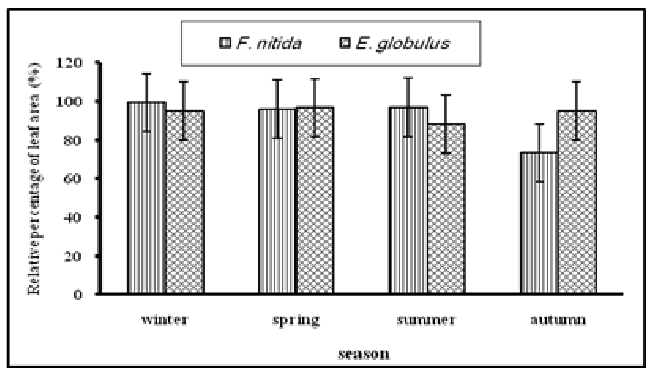

Figure (1) seasonal variation in the relative percentage of leaf area in the studied tree species.

Maximum relative percentage value of the leaf area was recorded during the spring season, being 96.4% (*E. globulus*), while the minimum value being 73.2% (*F. nitida*) during the autumn season. In general, the interaction between season and plant species exhibited a significant effect on the leaf area variations. Dry weight of leaf samples exhibited significant variations (P<0.01) between seasons, and tree species as well (Fig. 2). Its relative percentage reached a minimum value of 48% in *F. nitida*, during spring season and maximum value o f 89.5% in *E. globulus* during summer season.

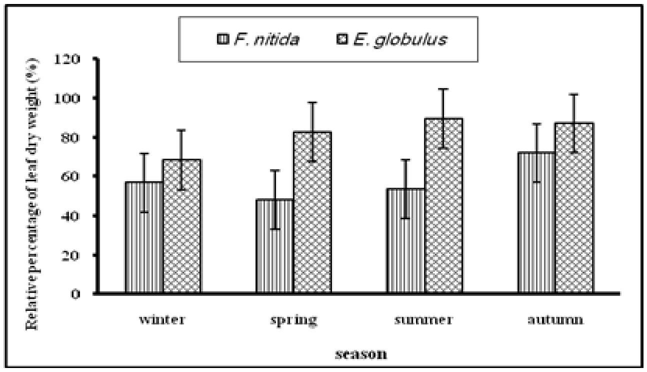

Figure (2) Seasonal variation in the relative percentage of leaf dry weight in the studied tree species.

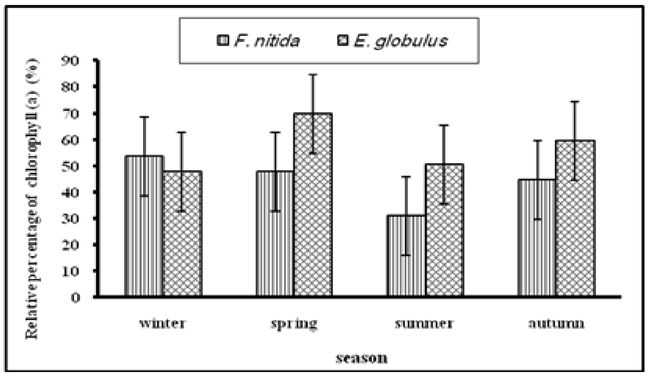

Figure (3) Seasonal variation in the relative percentage of chlorophyll (a) in the studied tree species.

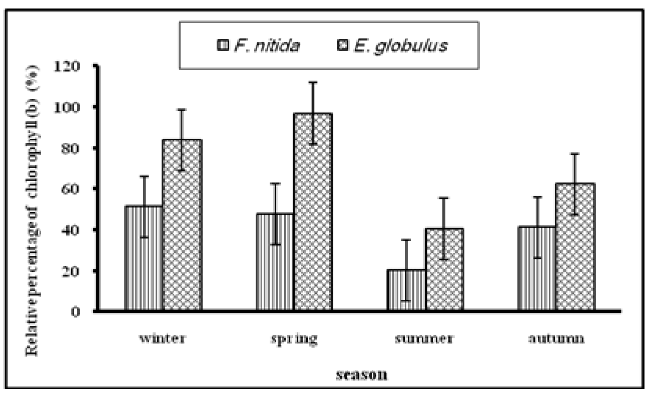

Figure (4) Seasonal variation in the relative percentage of chlorophyll (b) in the studied tree species.

The leaf pigments content mirror the prevailing conditions of air pollution at the study area. In response to the load of air pollution chlorophylls (a) and (b) exhibited varied contents in the different species (Figs. 3, 4). Both chlorophylls inversely correlated with deposit particle mass, expressing significant correlation coefficient of -0.866 and -0.912, respectively. The maximum value (69.7%) of chlorophyll (a) recorded in samples of *E. globules* during the spring season, while the minimum value (30.8%) was in *F. nitida*, during summer season. By the same way as in chlorophyll (a), chlorophyll (b) at the polluted sites exhibited marked variations than those at the control site, and decline trend was recorded (Fig. 4). Higher relative percentage (96.7%) recorded in samples of *E. globulus* during spring season. Contrary to what is explained with chlorophyll, carotenoids showed an increasing trend in the polluted leaf samples, in comparison with those of control (Fig. 5).

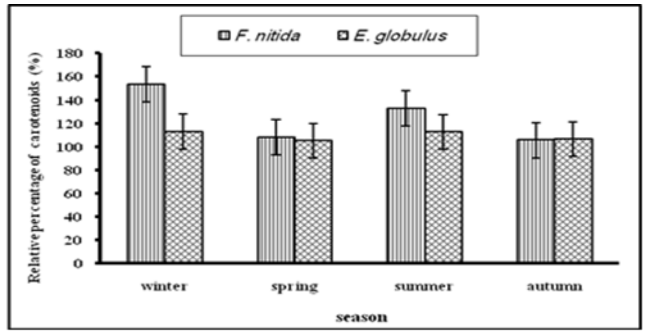

Figure (5) Seasonal variation in the relative percentage of carotenoids in the studied tree species.

Samples of *F. nitida* dominated other leaf samples in their content of carotenoids. Season exhibited order as winter >summer >spring >autumn, with values of 133.1%, 115.3%, 107.7% and 105.4%, respectively. In general, the interaction between season and plant species has a highly significant effect ($P< 0.01$) on the carotenoids content.

As shown in Table (1) and plate (1 A), anatomical structure of control leaves of *F. nitida* (scale bar=10 µm and X= 100; 400) showing the epidermis covered with thin cuticle (10.1 µm), epidermal cell of healthy shape with diameter of 50.01 µm. The cystolith was small in size (200.5 µm), the plaside and spongy tissue rich with chloroplast, the vascular bundle with normal shape and xylem vessel with thin wall (2.68 µm). Xylem vessel showed a wide diameter (27.6 µm) and phloem tissue of thickness of 55.9 µm (plate 1B). Samples of *F. nitida* collected from the polluted sites (Plate 1 C) showed thick cuticle (13.03 µm). Abnormal shape of epidermal cells with diameter of 40 µm was recorded. The cystolith exhibited large size (1003.3 µm), xylem vessels have thick wall of 10.3 µm. Diameter of vessels was 20.9 µm. The phloem tissue of abnormal appearance and condensation of its cell constituents was observed (plate 1D). Phloem tissue was small in thickness (40 -43.21 µm), Scale bar=10 µm and X= 400.

Plate (2 A) illustrates some anatomical characteristics of the *E. globulus* leaves collected from control site. The epidermis cell has a thin cuticle (16.7 µm). The phloem tissue had large thickness (70 µm). The xylem vessel appeared with thin wall (1.1 µm) and large diameter (18.3 µm) (plate 2B). In the leaves from polluted site (Plate 2 C), the epidermis cell is covered by thick cuticle (22.0 µm). The xylem vessel has diameter of

Table (1): Leaf anatomical measurements of the studied tree species.

Tree species	Cuticle thickness	Epidermis thickness	Xylem vessel diameter	Vessel wall diameter	Phloem layer thickness	Cystolith
Polluted areas						
F. nitida	13.03±0.015	40±5	20.9±0.854	10.3±1.3	41±6.68	1103.3±3.7
E. globulus	22±1.127	16.29±0.753	18.03±1.002	4±0.2	40±4.35	
Control areas						
F. nitida	10.1±0.15	50.01±1	27.6±2.17	2.68±0.58	55.9±5.43	200.5±2.18
E. globulous	16.7±2	16± 2.0	13±2	1.1±0.1	70±2.64	

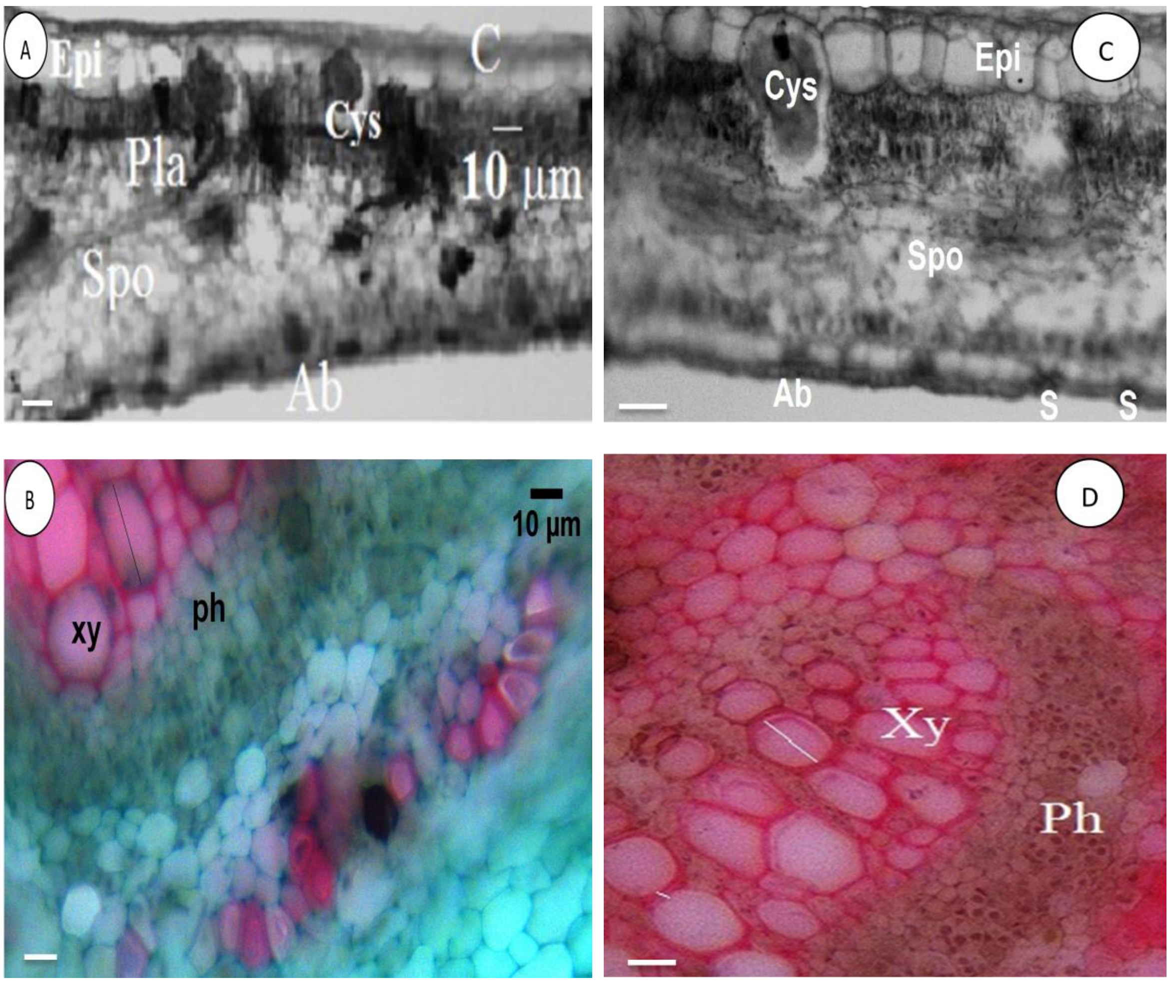

Plate (1): Cross section of control (A and B) and polluted (C and D) leaf sample of *F. nitida* tree showing: C: cuticle, Epi: epidermis, Cys: cystolith, Pla: plasid tissue, Spo: spongy tissue, X: xylem vessel, (□□□): diameter of xylem vessel, ph: phloem (scale bare= 10µm, A, B magnification= 100x; C, D magnification = 400x).

13.0 µm with thick secondary wall (4 µm). The phloem cells showed different degeneration symptoms including accumulation of secondary compounds and some cells were collapsed, the thickness of phloem tissue appeared small (40 µm) (plate 2D).

Analysis of variance results showed significant variation in anatomical structures between plants from polluted site and control (P<0.05 for cuticle, P<0.05 for xylem vessel diameter, P<0.01 for vessel wall, P<0.005 for phloem). There is no significant difference in the thickness of epidermis between samples of free and polluted sites.

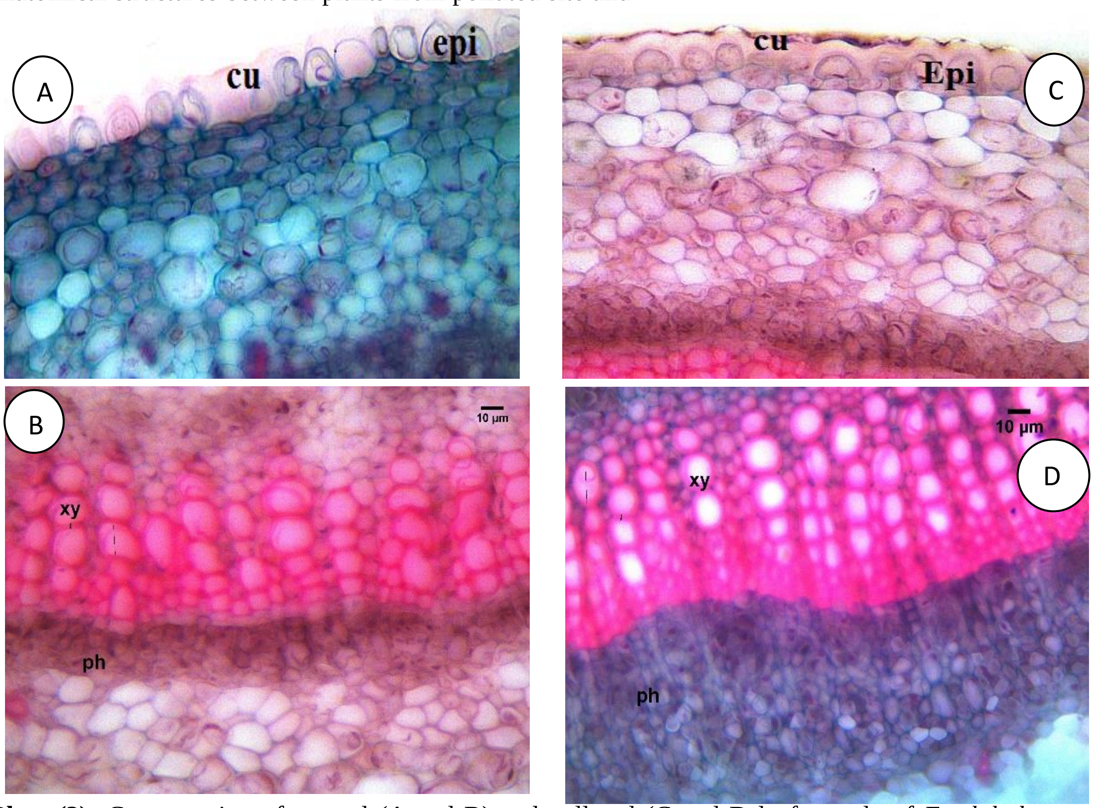

Plate (2): Cross section of control (A and B) and polluted (C and D leaf sample of *E. globulus* tree showing: C: cuticle, Epi: epidermis, Xy: xylem vessel, (□□□): diameter of xylem vessel, ph: phloem (scale bare= 10µm, magnification= 100xx).

4 Discussion

Data published by El-Khatib *et al.* [8] on the bio-monitoring of airborne particles pollutants by urban tree in the surrounding area of Assiut Superphosphate Fertilizer Factory confirm the presence of a high percentage of PM2.5 and PM10 particle species in comparison to PM50, in the leaf deposit samples. This was considered by Vallius [17] as indicators for the anthropogenic source of air pollution, and might reflect the main contribution of Superphosphate Fertilizer Factory emission in the prevailing air pollution status at the study area. From bio-monitoring point of view, the perennial habit of the studied trees species make them greatly stressed by pollutants, where they showed visible injuries and physio-anatomical alterations in their leaves. The fate of these injuries and alterations appeared to be affect with the area pollution load, the climate conditions and tree species.

Dry weight of leaf samples from the different tree species exhibited significant (P<0.01) variations between seasons and tree species. Pearson correlation matrix estimated a negative relationship between dry weight and deposit particle mass (r = -0.616), reflecting the role played by deposition in reducing the metabolic activities of the studied species. Deposit dust particles appeared to responsible for decreasing the total chlorophyll content through their alkaline effects which posed its degradation; or altering of its synthesis through shading effects [18] and/or their interference with essential enzymes for chlorophyll biosynthesis.

The reduction of chlorophyll synthesis and enhance its degradation under the effect of pollution was reported by many authors [19, 20, 21, 22], In agreement with what has been published by many authors [23, 24], the present results recommend the using of chlorophyll content as biomarker for the airborne particulate pollution. It is well known that under pollution stress, the plant species seeks to develop mechanisms to cope the stressful conditions, one of these is the production of carotenoid, which considered by many authors as anti-oxidative stress. This is clear, when the data of the correlation matrix was considered, where positive correlation coefficient (0.404) was recorded between carotenoids and deposit airborne particle mass.

Leaf visible injuries like necrosis, chlorosis and drying were considered by many authors [25, 26, 27, 28] as signs on the air pollution induced by airborne particles. Air pollutants are known to causes plant growth reduction; consequently reduction in leaf area [29]. El-khatib *et al.* [30, 31] reported that pollution stress altered the leaf structure of their studied species. In agreement with the finding of Reig-Arminana *et al.* [32], the recorded anatomical alterations include increasing of cuticle thickness in the two studied species.

The reduction in the epidermis tissue in the polluted leaves was obvious when it compared with those of the control leaves. The increase of cystolith size in the leaf samples of *F. nitida* was recorded under pollution conditions. In agreement with the results of Rajput *et al.* [33] and Gostin [34, 35], the polluted leaf samples exhibited reduction in vessel width when compared to those of control leaf samples. Youssef [36] attributed the reduction in the vessel width to the increase of xylem vessel lignifications. In the studied species, the phloem tissue showed abnormal appearance. This may be attributed to the drastic effects imposed by the prevailing air pollution in the study area.

5 Conclusion

From the results it can be concluded that alterations in growth, physiological investigation and anatomical features of urban vegetation can be used as biomarkers of air pollution stress posed by the Superphosphate Fertilizer if the studied species (*Ficus nitida* and *Eucalyptus golobulus*) have nominated to use for this purpose.

References

[1]UNEP (1998). Mineral fertilizer production and the environment. Technical report No 26, Part 2. Environmental management system, PP 35-57.

[2]Daly A., Zannetti P. (2007) An Introduction to Air Pollution Definitions, Classifications, and History: Ambient Air Pollution, Published by: The Arab School for Science and Technology (ASST) and the Environ Comp Institute.

[3]U.S. Environmental Protection Agency (EPA) (1979) Source Assessment: Phosphate Fertilizer Industry, May 1979, EPA-600/2-79-019C. Office of Research and Development, Research Triangle Park, NC.

[4]Egyptian Environmental Affairs Agency (EEAA) (2002) The Inspection Handbook for fertilizers industry, EPAP, 2002, Egypt.

[5]Saad N. M., Ibrahim Th. A, Shehata A, Seddek A. Sh (1990) Estimation of some industrial pollutants in milk and milk products. Assiut Veterinary Medicin 23, 46-51.

[6]Khallil A M, Abdel-Satar M (1993) Fungi from water, soil and air polluted by the industrial effluents of Manquabad Superphosphate Factory (Assiut, Egypt). Journal of Basic Microbiology 33, 83-100.

[7]Gadallah M A A (1993) Phytotoxic effects of industrial and sewage wastewaters on growth, chlorophyll content, transpiration rate and relative water content of potted sunflower plants. Water, Air and Soil Pollution 89, 33-47.

[8]El-Khatib A. A, El-Shanawany A. A, El-Amery E. M (2010) Significance of leaf surface of urban trees to monitor airborne particle pollutants nearby Superphosphate Fertilizer Plant. Journal of Environmental Studies [JES] 5:57-69.

[9]El-Taher A, Makhluf S (2010) Natural radioactivity levels in phosphate fertilizer and its environmental implications in Assiut Governorate, Upper Egypt. Indian Journal of Pure and Applied Physics 48, 697-702.

[10]Egyptian Environmental Affairs Agency (EEAA) (2006) Ecological description of Assuit. page1-2

[11]Boulus, L. (2000) Flora of Egypt, Volume II, Al Hadara Publising, Cairo, Egypt.

[12]Agbaire P.O., Esiefarienrhe E. (2009) Air pollution tolerance indices (APTI) of some plants around Otorogun Gas Plant in Delta State, Nigeria. Journal of Applied Science and Environment 13, 11 – 14.

[13]Vora A. B., Bhatnagar A. R. (1986) Comparative study of dust fall on the leaves in high pollution areas of Ahmedabad. IV. Altered proline contents of leaves. Pollution Research 5, 153-157.

[14]Metzner H, Rau H, Segner H (1965) Untersuchungen zur synchronisierbarkeit einzelnerpigment-mangel mutanten von chlorella. Planta 65:186-194.

[15]Ghouse A.K.M., Yunus, M. (1972) Preparation of epidermal peels from leaves of gymnosperms by treatment with hot 60% HNO3. Stain Technology 47, 322–324.

[16].Ruzin S.E (2000). Plant Microtechnique and Microscopy. Oxford University Press, New York, pp 334.

[17]Vallius, M. (2005). Characteristics and source of fine particulate matter. Publication of National Public Health Institute, Kuopio, Finland, pp 13-67.

[18]Singh B., Singh Y. J.K, Ladha K.F, Bronson V., Balasubramanian J, Singh C,. Khind C.S (2002). Chlorophyll meter and leaf color chart-based nitrogen management for rice and wheat in Northwestern India. Agronomical Journal 94, 821-829.

[19]Migahid, S., El-Darier S (1995) Effect of cement dust on three halophytic species of the Mediterranean salt marshes in Egypt. Journal of Arid Environments 30: 361-366

[20]Sandelius A.S., Naslund K, Carlson A. S, Pleijel H, Sellden G (1995) Exposure of spring wheat (*Triticum aestivum*) to ozone top chambers. Effects on acyl lipid composition and chlorophyll content of flag leaves. *New Phytolgist* 131, 231-239.

[21]Prajapati S.K., Tripathi B. D (2008) Seasonal Variation of leaf Dust accumulation and pigment content in plant species exposed to urban particulates pollution. Journal of Environmental Quality 37, 865–870.

[22]Chauhan A., Joshi P.C. (2010). Effect of ambient air pollutants on wheat and mustard crops growing in the vicinity of urban and industrial areas. New York Science Journal 3, 52-60.

[23]Pawar K.,. Dubey P.S (1985) Effects of air pollution on the photosynthetic pigments of *Ipomea fistulosa* and *Phoenix sylvestris*' All India seminar on Air Pollution Control Indore, Abs. 19-21.

[24]Gilbert O.L. (1968) Biological indicators of air pollution. Ph.D. Thesis, University of Newcastle upon Tyne, Great Britain.

[25].Mishra, G.P. (1990) Effect of industrial discharge on agricultural crops along the penalization gradient of ACC Kymore. Environmental Ecology 8, 38-42.

[26]Prasad M. S. V., Inander J. A (1990) Effect of cement klin dust pollution on black gram (*Vigna mungo* L.). Proceeding of Indian Academy of Science. Plant Science 100, 435-443.

[27]Pandey D.D., Prasad S.N, Sinha C.S, Nath R (1991) Response of species composition, life forms, biological spectrum and importance value index of grassland ecosystem to cement dust pollution. Environmental Ecology 9, 321-324.

[27]Darley E. F. (1966) Studies on the effect of cement-kiln dust on vegetation. Journal of Air Pollution Contr. Assoiation 16, 145-50.

[28]El-Khatib A.A. (2003) The response of some common Egyptian plants to ozone and their use as biomonitors. Environmental Pollution 124, 419-426.

[29]Winner W.E. (1989) Photosynthesis and transpiration measurements as biomarkers of air pollution effects on forests. Pages 303-316 in Biologic Markers of Air-Pollution Stress and Damage in Forests. National Academy Press, Washington, DC.

[30]El-Khatib A. A., Abdelkalek K. N, Naglaa U. (2008) Dust particulate Air Pollutants and the Structural Features of Urban Vegetation. 4th International conference for Development &Environment 2008, King Saud University, KSA.

[31]El-Khatib A. A.,. abdel-Khalek K. N, Naglaa U. (2011) Bio-monitoring of Airborne Dust Particles Pollutants by Morpho-anatomical Reactions of Urban Tree Leaves Under Dry Climate. Egytian Journal of botany vol 51 No.1, 1-12.

[32]Reig-Arminana J., Calatyud V, Cervero J., Garcia-Breijo F.J, Ibars A, Sanz M.J (2004) Effect of ozone on the foliar histology of the mastic plant (*Pistacia lentiscus* L.). Environmental Pollution132, 21-331.

[33]Rajput K.S., Rao K.S, Kim Y.S (2008) Cambial activity and wood anatomy in Prosopis spicigera (Mimosaceae) affected by combined air pollutants. IAWA Journal 29, 209–219.

[34]Gostin I. N. (2009a) Air Pollution Effects on the Leaf Structure of some *Fabaceae* Species. Notulae Botanica Hort Agrobotanici Cluj-Napoca 37, 57-63.

[35]Gostin I. N. (2009b) Structural modification induced by air pollutants in *Plantago lanceolata* leaves. Tom. XVI / 1, pp. 61-65

[36]Youssef N. (2009) The effect of particulate air pollutants on anatomical structure and antioxidant content of urban tree leaves; the possibility for their using in the biomonitoring processes and air quality improvement. M.Sc Thesis. Botany Department of Botany, Faculty of Science, Sohag University, Egypt.