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Original Paper

Morpho-Anatomical Characteristics of Olive (*Olea europaea* L.) Trees Leaf as Bio-indicator of Cement Dust Air Pollution in Libya

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Abstract
Comparisons were made between the anatomical and morphological changes in olive tree leaves from a site with relatively clean air (Al-Khadra area), and two sites (al-Khums and Zelatin) near to cement factories in the area east to Tripoli, Libya. Olive tree leaves exhibited marked variations in their morphological and anatomical characteristics, in relations to variations in the site cement dust air pollution load. Under high pollution load, leaf visible injuries were recorded. In addition, stomata appeared in higher density and smaller size than those of control. The anatomical characteristics of olive leaf including cuticle, epidermis, palisade tissue, mesophyll tissue, and elements of vascular cylinder (xylem and phloem) reflected the deteriorate effects of cement dust air pollutants, the subject which recommend their using as bio indicators.

Keywords: *Olea europaea***,** epidermis, stomata, xylem, morphology, cement dust.

Introduction

Cement dust results from the grinding of a clinker, which is produced by burning a mixture of limestone, clay, and gypsum at high temperatures (1450–16001C) in specially designed kilns (Suess *et al.,* 1985). A cement industry offers an excellent opportunity for studying the impact of dust, during the process of cement manufacture considerable amounts of dust are emitted from handling, spillage and leakages. Dust is produced from quarrying of the major raw material limestone and ending with the packing and dispatch of cement from the industry (Abdul-Wahab, 2006). Cement dust is a gray powder with an aerodynamic diameter ranging from 0.05 to 5.0 mm (Kalacic, 1973).

Cement dust can cause illness by skin or eye contact as well as inhalation. Risk of injury depends on duration and level of exposure and individual sensitivity. Moreover, different cements have different ingredients. Many of them contain substances that can be hazardous, like crystalline silica (quartz), lime, gypsum, nickel, cobalt, and chromium compounds (Green N8 Residents Group, 2004). Inhalation of silica dust can cause silicosis or other potentially fatal lung diseases. In addition, inhalation of chromium compounds found in some cement dusts can cause cancer. Hence, cement dust can be an important pathway for potential human exposure. High concentrations of particles emitted from cement plant may affect the health and property of homeowners living adjacent to the plant. There are numerous complaints about cement plant from nearby residents. They include specific problems about odors, blasting, noise, respiratory problems and corrosive dust on cars.

Plant physiological parameters have been used as bio-indicators of urban habitat quality. For example, highly alkaline dust-like cement visibly injures plant leaves; even chemically inert dust physically affects photosynthesis and transpiration when it accumulates on leaf surfaces. Covering and plugging of stomata (Ricks & Williams, 1974). shading (Peirce, 1910; Thompson *et al.,* 1984). increasing leaf temperature (Eller, 1977; Borka, 1984)**.** and removal of cuticular wax (Eveling & Bataille, 1984; Eveling, 1984). had been used to characterize local air pollution (Moraes *et al.,* 2002). Less attention has been given to morphological and anatomical parameters of plants as indicators of long-term responses to changing (urban) habitat quality, although parameters as specific leaf area, stomatal density and pore surface were recognized to

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vary depending on microclimatic conditions (Barber *et al.,* 2004). Moreover, sampling and analysis of these parameters are relatively easy and inexpensive.

Trees act as a sink for air pollutants and thus reduce their concentration in the air especially in urban environments (Woo and Je, 2006; Tewari, 1994; Rawat and Banerjee, 1996). Dust interception capacity of plants depends on their surface geometry, phyllotaxy, and leaf external characteristics such as hairs, cuticle, leaf shape and size, texture, length of petioles, and canopy of trees etc., weather conditions and direction and speed of wind and anthropogenic activities (El-Khatib, 2007; 2011; Santosh and Tripathi, 2008).

The olive tree (*Olea europaea* L.) is one of the major crops in the Mediterranean region. Whilst its cultivation has spread to other regions around the world, olive production is of vital importance to the economy of Mediterranean countries, including Libya. The marked reduction in the growth and yield of olive trees in the polluted area may be explained in terms of the shading effect of the foliar cement crust as well as through the changes in soil characteristics that had been brought about by the cement factory effluents. Thus the uncontrolled emissions of a cement kiln can affect the growth of the adjacent vegetation through both the air and the soil (Khalid *et al.,* 2009). This paper was to investigate the feasibility of using the changes in anatomical of olive tree leaves in the studied areas as bio-indicators for cement dust air pollution.

Materials & Methods

The study area:

Three sites located in Libya were chosen for the purpose of this study. They were coastal cities located east of Tripoli, their names are Alkhums (Site I) (latitude 32º 38" N and longitude 14º 13" E), Zliten (site II) (latitude 32º 25" N and longitude 14º 29" E) and Al-khadra (Site III) (latitude 32º 26" N and longitude 13º 42" E). The two first sites are located at distance of 0.5 km from the cement factories, while the third one is located far from any pollution sources (distance of 40 km) and considered to be as control. These sites covered by olive trees as main crop, besides fragment vegetation of vegetables and wild species. As reported by Libyan National Meteorological Center Climatologically Department, (2009), the temperature of this area is ranging between 14.66°C and 25.36°C. The annual mean of wind speed is 6.88 knots/hour, the annual mean of relative humidity is 73.17 %, and the annual mean of rain fall is 24.81 mm.

Sampling

At each site, leaf samples were collected from olive trees growing around the cement factories at site I and Site II, in four directions to cover the different directions of the plant load emissions as: location (1): west of the factory; location (2) north-west of the factory, location (3) south of the factory, and location (4) south-east. Sampling collection was during summer of 2010 and winter of 2011. At each location, three samples of olive tree leaves were collected, resulting in 12 leaf samples for each study site. Sampling conducted according to Lau & Luk, (2001) method. At each site, by wearing polyethylene gloves, 36 leaves were detached from each tree at 1.5-2 m above the ground by pruning shears from the outer part and inner part of the canopies and from the four directions for the tree (E, W, N, S; nine leaves per each space direction) kept in plastic bags, placed in icebox, and transported to the laboratory for the next preparation.

Anatomical investigation

To study the leaf anatomical structure of the studied trees, leaf samples were fixed in FAA (formaldehyde: acetic acid: alcohol, 5: 5: 90, respectively) then preserved in 70% ethyl alcohol. Transversal sections (7 µm) were obtained using microtome. The sections were stained with safranin. (0.5gm/500 ml ethyl alcohol) for 30 minutes and washed by different concentrations of ethyl alcohol (50%, 70% and 95%) then the sections stained with light green (0.5 gm/1000 ml ethyl alcohol) for 30 seconds followed by washing with 95% ethyl alcohol .The sections were mounted in canada balsam, dried at 55-60°C for 3 days and examined under light microscope (Olympus-BX51) for description of anatomical structures .The sections photographed by digital camera (Olympus –DP12) and measured by Image-Pro Plus 6.1 (Ruzin, 2000).

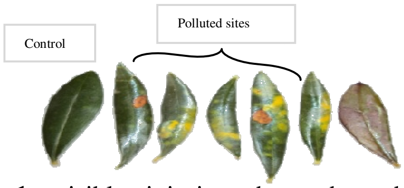

Plate.1. visible injuries show that chlorosis, yellowish, necrosis and drying on the upper surface of leaves, collected from polluted sites (I and II) and control site (III) during summer and winter seasons.

Statistical Analysis:

Data were subjected to statistical analysis using Minitab®14. Comparisons of means were carried out using the analysis of variance (MANOVA, Two-way). Differences were considered to be significant at level *P<0.05, and P<0.01*.

3. Results and discussion:

Trees act as a sink for air pollutants and thus reduce their concentration in the air, especially in urban environments (Woo and Je 2006; Tewari, 1994; Rawat and Banerjee, 1996). Dust interception capacity of plants depends on their surface geometry, phyllotaxy, and leaf external characteristics such as hairs, cuticle, leaf shape and size, texture, length of petioles, and canopy of trees etc., weather conditions, direction and speed of wind and anthropogenic activities (El-Khatib, 2007; El-Khatib *et al.*, 2011; 2012; Santosh and Tripathi 2008). The results of the present investigation are well in agreement with the previous findings, where the recorded morphological and anatomical variations in the leaf of cultivated olive trees at the study sites markedly reflected the significant variations in the leaf dust load which stated to have relations with the cement dust air pollution burden at this area. Based on the recrded data for the leaf deposits of olive trees collected from this area during the same period of investigation (Table, 1), the study sites arranged in the order as: Al-khums (Site I) >Zelitin (Site II) > Al-Khadra (Site III).

Site	Season	
	Summer	Winter
I	18.53±6.36	17.04±6.62
II	13.65±3.07	15.38±5.07
III	0.79±0.12	0.58±0.14

Table.1. Deposit particulate mass (gm/m^2) captured by olive leaf at different sites during the period of study; each value is Mean ±SD; n=3.

Compared with leaf samples those were collected from the control site (Al-Khadra), leaf visible injuries such as necrosis, chlorosis/yellowish and drying appeared very clear in the polluted leaf samples collected from Alkhums (Site I) and Zelitin (SitesII) (Plate 1). Visible leaf injuries were stated by many authors (Mishra, 1990; Prasad and Inander, 1990; Pandey *et al.,* 1991, Darley, 1996) as bio-indicators for alkaline and acidic dust air pollutants. Differences in stomatal density were highly significant (P<0.001) between sites. It was obvious that under cement dust air pollutants olive leaf exhibited higher stomatal density in comparison with those of control.(Table 2). The average stomatal density exhibited its maximum value (458. 28 stomata/mm^{-2}) in leaves collected from site (II) during winter season, and the minimum value was recorded (290.61 stomata/mm^{-2}) in those collected from control site (III). Under similar conditions of cement dust air pollution, Ayanbamiji (1996). reported the increment of stomatal frequency as an adaptive mechanism by leaf to compensate the blockage ones and protect its gaseous exchange and transpiration rate. The present results are in agreement with those of many authors (Antonio. M. *et al.,* 1999; Ade-Ademilua and Obalola 2008; Rezeq, *et al.,* 2004; Artemios, and George, 2002). who reported that stomata tend to be more numerous and smaller in size under pollution conditions when compared to those of control.

The anatomical characteristics of the leaves collected from the different study sites are presented in Plate (2 and 3). Wax layer of cuticle and a few trichomes appeared to cover the upper epidermis. Significant variations (P<0.05) in cuticle thickness were recorded at the different sites (Table 2). Its maximum value (33.11 µm) was at site (II) and the minimum one (6.17 µm) was at site (III). Generally, it exhibited the following patterns: site (II) >site (I)> site (III) during the whole period of study. In this concern, Reig-Arminana *et al.,* (2004) recorded an increase in cuticle thickness in plants of polluted sites, in comparison with those of control site. The width of upper epidermis of olive trees leaf under cement pollution circumstances exhibited significant variations (P<0.001) compared to those of control (Table 2, Plate 2). Concerning the upper epidermis**,** the maximum value (22.86 µm) was recorded at site (III) and the minimum value (13.75 µm) was recorded at site (II). There weren't significant differences among seasons. The lower epidermis showed significant seasonal and site variations ($P<0.001$). The maximum value (17.89 µm) was recorded at site (III) and the minimum one (12.05 µm) was recorded at site (I) (Table 2).

Sites	**Seasons**	**Upper cuticle µm**	**Upper Epidermis µm**	**Upper Palisade µm**	**Spongy Tissue µm**	**Lower Palisade µm**	**Lower Epidermis µm**	**Vessel wall diameter µm**	**Xylem vessel diameter µm**	**Phloem tissue µm**	**Stomatal Density (stomata/mm2)**
I	**Summer**	20.87±1.89	18.2±1.6	170.25±5.82	161.32±8.52	32.18±6.71	14.69±1.33	6.19±1.03	13.1±2.01	67.01±12.81	395.31±15.43
	Winter	22.22±2.71	17.89±2.1	168.87±6.19	164.13±6.72	33.81±3.89	14.2±1.51	6.51±0.97	12.74±2.61	70.08±13.68	419.51±22.08
II	**Summer**	21.16±2.13	16.86±1.43	167.63±5.69	163.28±8.46	30.8±7.19	15.36±1.07	5.73±0.69	10.04±1.72	70.98±9.2	388.24±15.79
	Winter	23.27±2.59	16.57±1.39	169.34±4.16	165.33±6.28	34.41±5.52	14.87±1.76	6.28±0.88	11.19±1.68	68.23±7.29	412.84±16.67
III	**Summer**	9.25±1.39	20.08±2.18	173±8.12	172.64±7.62	34.34±5.12	16.12±1.48	3.46±0.81	15.63±1.64	90.47±11.18	367.82±13.54
	Winter	9.98±1.61	19.32±1.85	172.1±4.53	173.24±7.56	37.76±4.43	15.6±1.29	3.21±0.67	16.05±1.45	87.12±10.87	354.41±32.69

Table.2. Mean values ±S.D of measured leaf tissues parameters of *Olea europaea* L. at polluted sites (I and II) and control site (III) during the study period.

Palisade tissue of polluted olive leaf exhibited significant reduction in its width compared to those of control. The maximum value (190.02 µm) of upper palisade was recorded at site (III), while the minimum (150.58 µm) was at site (I). Non -significant variation in upper palisade tissue was recorded among seasons (Table2, plate 2). Concerning the lower palisade tissue, significant site variations ($P<0.05$) were recorded. The maximum lower palisade value was recorded (44.24 µm) at site (III) and the minimum value (20.18 µm) was at site (I). Under cement dust air pollution, olive leaf exhibited reduction in the width of its mesophyll spongy tissues (Table 2, Plate 2). This reduction exhibited significant variations ($P<0.001$) between sites, reaching its minimum value (143.34 µm) at site (I) and maximum value (192.5 µm) at site (III). Spongy tissue followed this pattern: Site (III) > Site (II) > Site (I). In their tested species, several authors were described damage symptoms similar to those observed here in the mesophyll tissue as a result of air pollution exposure (e.g. Sutinen and kaivisto, 1995, Mikkelsen and Heide- Jorgenson, 1996, Gunthardt- Goerg and McQuattie, 1998 and Reig-Arminana *et al.,* 2004).

Olive leaf showed marked variations in structure of xylem vessel under cement dust air pollution (Table 2, Plate 3). The thickness of vessel wall varied significantly ($P<0.001$) between sites, reaching its maximum value (7.85 µm) at site II. Tanja, *et al.,* (2010) reported an increase in the thickness of the xylem vessel wall due the deposition of lignin and suberin when the tested species exposed to air pollution conditions. Generally, the sites were arranged in the following order: site II > site I > site III. Xylem vessel diameter showed a decrease in its value under pollution conditions, where the rate of deposition of lignin and suberin increased the vessel wall diameter, resulting in vessel cavity reduction (plate 3). In comparison, sites II appeared more affected than site I and site III, respectively. Non-significant variation was recorded among seasons. The effects of air pollution on wood formation and on the dimensions of wood cells have been evaluated in few tree species (Pozgaj *et al.,* 1996; Jabeen and Abraham, 1998; Iqbal *et al.,* 2000). Gupta and Iqbal (2005) recorded that the size of vessel elements decreased under the influence of coal-smoke pollution as the pollution stress altered the process of cambial activity leading to a lesser wood production in some trees.

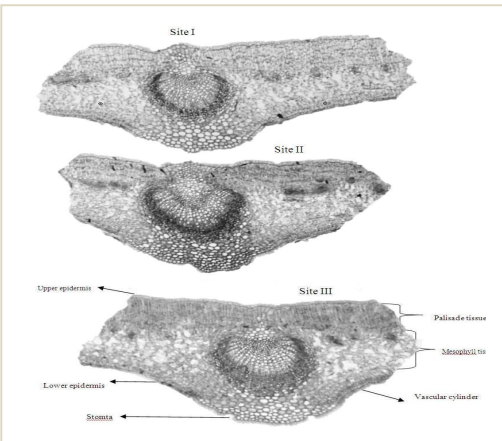

Plate (2): Transverse sections of the leaf of *Olea europaea L* under pollution load, Site I (Al-Khums), Site II (Zelitin) and Site III (Al-Khadra).

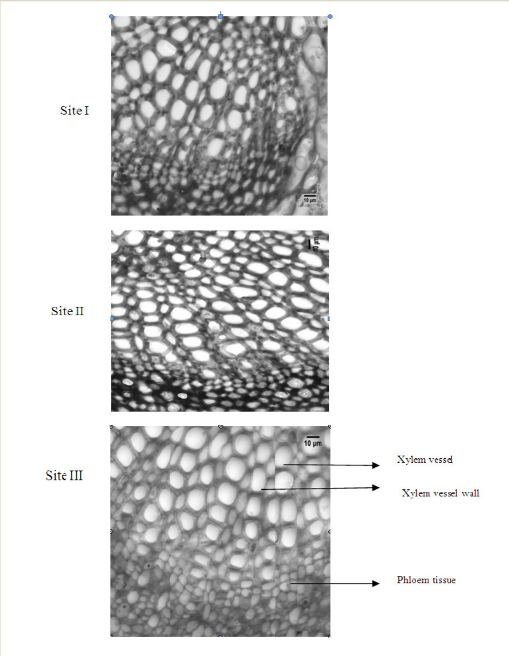

Plate (3): Vessel and phloem elements changes under different cement dust pollution load at Site I, Site II and Site III.

Phloem tissue exhibited decreased in its size under pollution conditions. In comparison, phloem tissue size of polluted leaf was lower than those of control (Table2, plate 3). Minimum value (48.78 µm) of phloem size was recorded in leaves collected from site II during winter, while maximum one (130.49 µm) was recorded in samples collected from control site (III) during summer. Phloem cell wall appeared to be slightly thicker, thus reducing the lumen, in polluted samples than those of control. A remarkable increase in the wall thickness of the phloem fibers was also evident in samples of polluted sites. Generally, changes in the phloem tissue varied significantly (P<0.001) between sites. Accordingly, the sites were arranged as: site II > site I > site III.

In conclusion, air pollution was described as an additional stress on plants since they often respond to atmospheric contamination in the same way as they respond to drought and other environment stress. The role of air pollutants causing injury to plants either by direct toxic effect or modifying the host physiology rendering it more susceptible to infection. In severe case of pollution, the injury symptoms were expressed as foliar necrosis or completely disappearance of the plant. In that way the present research work carried out cement dust pollution on anatomical structure of olive tree leaves, which appeared markedly affected. The fate of anatomical changes appeared to strongly increase with the intensity of cement dust air pollution, reflecting significant variations among sites. Generally, the appearance of such anatomical changes and increasing their intensity under these conditions recommend their using as bio-indicators for cement dust air pollutions.

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الملخص العربى

الخصائص المورفو- تشريحية لورقة أشجار الزيتون كمؤشرات حيوية على التلوث بغبار الاسمنت فى ليبيا

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الخلاصة
أظهرت الدراسة المقارنة للخصائص المورفو- تشريحية لورقة أشجار الزيتون التي تم جمعها من مواقع ملوثة بغبار الاسمنت وأخرى غير ملوثة في المنطقة شرق مدينة طرابلس، ليبيا اختلافات واضحة فى خصائصها المورفولوجية و التشريحية طبقا للاختلاف الموقعية في معدل التلوث بغبار الاسمنت . لقد تم تسجيل تشوهات مرئية في الورقة و زيادة في عدد الثغور مع صغر حجمها تحت تأثير المعدلات العالية من التلوث بغبار الاسمنت و ذلك مقارنة بعينات الضابطة. عكست الخصائص التشريحية لورقة أشجار الزيتون المتضمنة الكيتوتيكل، البشره، النسيج العمادى، النسيج الوسطى، و عناصر الاسطوانة الوعائية التأثيرات الضارة لملوثات غبار الاسمنت بطريقة تزكى من استخدام هذه التأثيرات كمؤشرات حيوية.