IMPACT OF REGIONAL DISTRIBUTION AND AIR POLLUTION ON INTERNAL STRUCTURE OF *MELIA AZEDARACH* L. LEAVES

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ABSTRACT

This study was performed to investigate the impact of air pollution on leaf area and anatomical features of *Melia azedarach* L. trees, in urban areas with three demographical classes :location (I) industrial area, location (II) roadside area and free parts (control area) as a location (III) of Duhok city/Kurdistan Region-Iraq, during July 2021. The results demonstrated that the leaf area of selected plants' leaves in location I had reduced with no noticeable change in the average stomata density in the three locations I, II and Ⅲ. Meanwhile, the results of the most anatomical features of the blade (blade, lower cuticle, epidermis (both upper and lower) thickness, palisade layer height and spongy parenchyma width) in addition to midrib parameters (epidermis thickness (upper and lower), collenchyma and parenchyma layer width, phloem and xylem width and pith diameter) were decreased in both locations I, II, and with well-developed anatomical features in location III.

Keywords: *Melia azedarach* L., air pollution, leaf blade, midrib, stomata density, leaf area.

تأثير التوزيع المناطقي و تلوث الهواء في التركيب الداخلي لأوراق السبحبح .*Melia azedarach* L

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المستخلص

اجريت هذه الدراسة بهدف دراسة تأثير تلوث الهواء على مساحة الأوراق والسمات التشريحية لأشجار *Melia azedarach L.*، في المناطق الحضرية بثلاث مواقع ديموغرافية: الموقع (I) المنطقة الصناعية والموقع (II) جوانب الطرق فضلا عن المناطق المفتوحة والمتمثلة بالموقع (III) لمدينة دهوك / إقليم كردستان العراق، خلال شهر تموز 2021. أظهرت النتائج على أن مساحة الأوراق في الموقع الاول قد تقلصت مع عدم وجود تغيير ملحوظ في متوسط كثافة الثغور في المناطق الثلاثة (المواقع I و II و III). في الوقت ذاته بينت نتائج معظم الصفات التشريحية للنصل (نصل الورقة، البشرة السفلية، سمك البشرة (العلوية والسفلية)، ارتفاع طبقة العمادية وعرض الطبقة الإسفنجية) فضلا عن الصفات التشريحية للعرق الوسطي (سمك البشرة العلوية والسفلية، عرض النسيج الكولنكيمي والبارنكيمي، عرض اللحاء والخشب وقطر اللب) في كلا الموقعين و II قد تقلصت مع وجود تطور جيد في الخصائص التشريحية في الموقع III.

الكلمات المفتاحية: .*Melia azedarach* L، تلوث الهواء ، نصل الورقة ، العرق الوسطي ، كثافة الثغور ، مساحة الورقة.

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INTRODUCTION

Chinaberry tree (*Melia azedarach* L.) belongs to Meliaceae family which includes 50 genera with 1400 species, it considered as deciduous trees with high reach up to 12m and pinnate leaves, opposite arrangement leaflets and drupe fruit (24). Mature leaves gain a stink smells and poisonous effects. The fruit color progress from green at juvenal age to light yellow and brown at repining stage (8). The native habitat of this tree is tropical region originated in Asia, spread and transplants in a various tropics and subtropical countries, such as United States of America, Argentine, Brazil, Philippines and Africa, it has been broadly cultivated in various area of Kurdistan region (3). This species adapts well to poor soils, warm temperatures, and seasonally dry conditions. It is widely dispersed, dries quickly, and is easy to process without cracking or warping (2). Although the fruits might be poisonous for the humans and definite mammals, it may use in many countries as a traditional medicinal product for anti-parasitic and anti-fungal properties. Nevertheless, overdose could lead to death (20). Trees are mainly used by roadsides and in urban green areas due to their different functions the yellow color of leaves in autumn, aromatic flowers and in winter its aesthetical fruits (19). The plant also has the ability to improve the environmental quality by accumulating several pollutants in their leaves, roots, trunks, barks or fruits, these accumulations are used as appropriate in order to monitor the traffic-related air pollution (17), to reduce the effect of the presences of high concentration of heavy metals through agrochemical fertilizer, insecticides and waste products and gases of factories that considered as the main source of pollution of soil and atmosphere (25). Pollutants from a various source, including industrial processes, paved and unpaved roads, building and demolition sites, cause substantial internal and exterior harm to plants and it`s cells, these contaminants mostly affect plants, particularly trees growing along roadsides, through their leaves “rather than the stem or roots” is the most sensitive component of the body to air pollution (14). Mitu *et al* (16) showed that the epidermis, palisade, and spongy parenchyma had reduced cell size of plants in polluted areas, with a black dot-like substance deposited in those parts, on the other hand, the leaves of control site plants, had normal anatomy. Moreover, Kushwaha *et al* (15) found that the anatomical properties of *Nerium oleander* leaves had changed significantly in contaminated region samples on both the upper and lower surface of the leaf, by increasing in the number of stomata and epidermal cells, along with a decrease in the length and width of guard and epidermal cells. This study was investigated to examine the leaf area and anatomical features of *Melia azedarach* L. trees as a result to air pollution in addition to their demographical distribution in different areas of Duhok province.

MATERIAIS AND METHODS

Samples of *Melia azedarach* L. leaves have been taken from trees growing at urban areas of Duhok city Kurdistan Region-Iraq. The urban area was divided into three demographical classes: location (I) industrial area, location (II) roadside area and free parts of the Duhok with thin population and with no industrial activities (control area) as a location (III) at the same age nearly 8 years. At each area, 25 samples of leaves were collected from each site during July 2021. The samples were kept in plastic bags then, transported to the laboratory for morphological and anatomical investigations. The samples were prepared in order to determine the leaf area (cm^2) according to Abdel (1). Stomata density which include the number of stomata in one mm^2 has been calculated. For anatomical study and slide preparation, the samples were fixed in Formalin, Glacial Acetic Acid, Alcohol (70%) FAA (5:5:90) ml, respectively (4). The cross sections of samples were stained with safranin and fast green (9), then they were mounted in Canada balsam for 24 hours. The observed cross sections parameters include stomata density, thickness of blade, cuticle (upper and lower), epidermis (upper and lower) and in mesophyll layers (palisade layer height and spongy parenchyma width). For leaf midrib, the thickness of upper and lower cuticle, the upper and lower epidermis, collenchyma and parenchyma layer width, phloem and xylem width in addition to pith diameter were examined under light microscope (Motic) for description of anatomical features and estimating the measurements of its components. For suitable images were photographed by using Dino-eye digital camera.

RESULTS AND DISCUSSION

Leaf area and stomata density: The average leaf area and the range in Table (1) shows a reduction in the leaf area of *Melia azedarach* L. which was collected from locations I and II (5 and 4.40 cm^2) (2.14-9.36 and 2.43-6.98 cm^2) respectively, while the highest average of leaf area was (10.14 cm^2) recorded in location III. These differences in leaf size and color can easily be observed in Figure (1). The biggest leaf area with dark green appeared very clear in the control leaf samples collected from location Ⅲ. On the other hand, there was no difference in average of stomata density among the three locations and it was quite clear that the highest average stomata density was (113.13 mm^2) and the range reached (90-140 mm^2) found in location I, as shown in Table (1).

Table 1. The measurements of leaf area and stomata density of *Melia azedarach* L. from three different locations*

Characters	Statistics	*Melia azedarach* L. I	II	III
Leaf area (Cm^2)	Average	5	4.40	10.14
	Range	2.14 – 9.36	2.43 – 6.98	6.98 – 16.5
Stomata density (mm^2)	Average	113.13	108.5	104.6
	Range	90 – 140	80 – 145	82 – 136

***The value in the table represent the average of 25 observations for each quantitative character.**
(Ⅰ = Industrial area, Ⅱ = Roadside area and Ⅲ = control area).

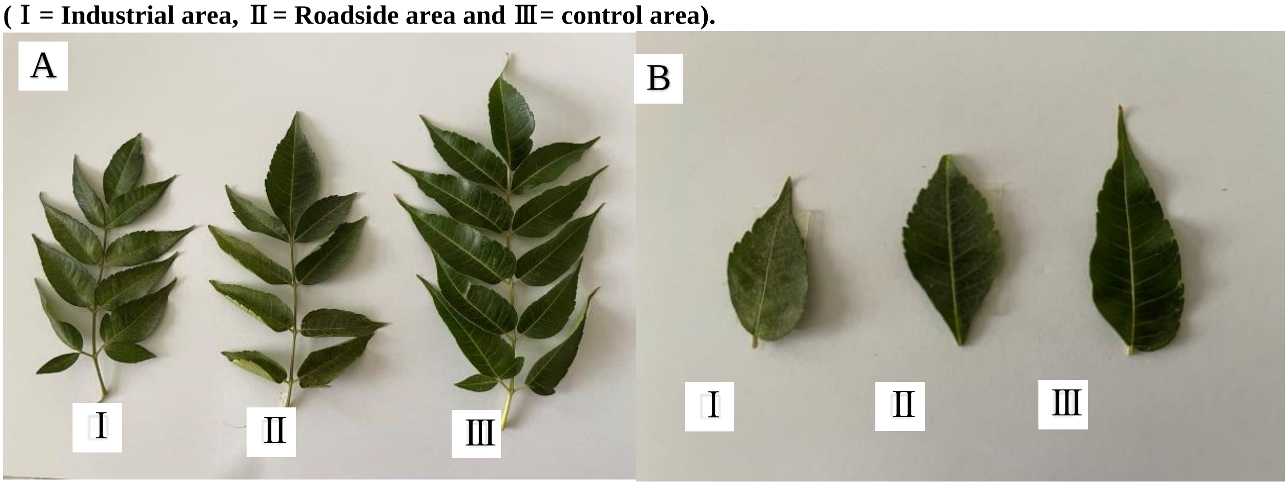


Figure 1. Leaf of *Melia azedarach* L. grown industrial area (Ⅰ), roadside area (Ⅱ) and control area (Ⅲ). A: compound leaf, B: leaflet

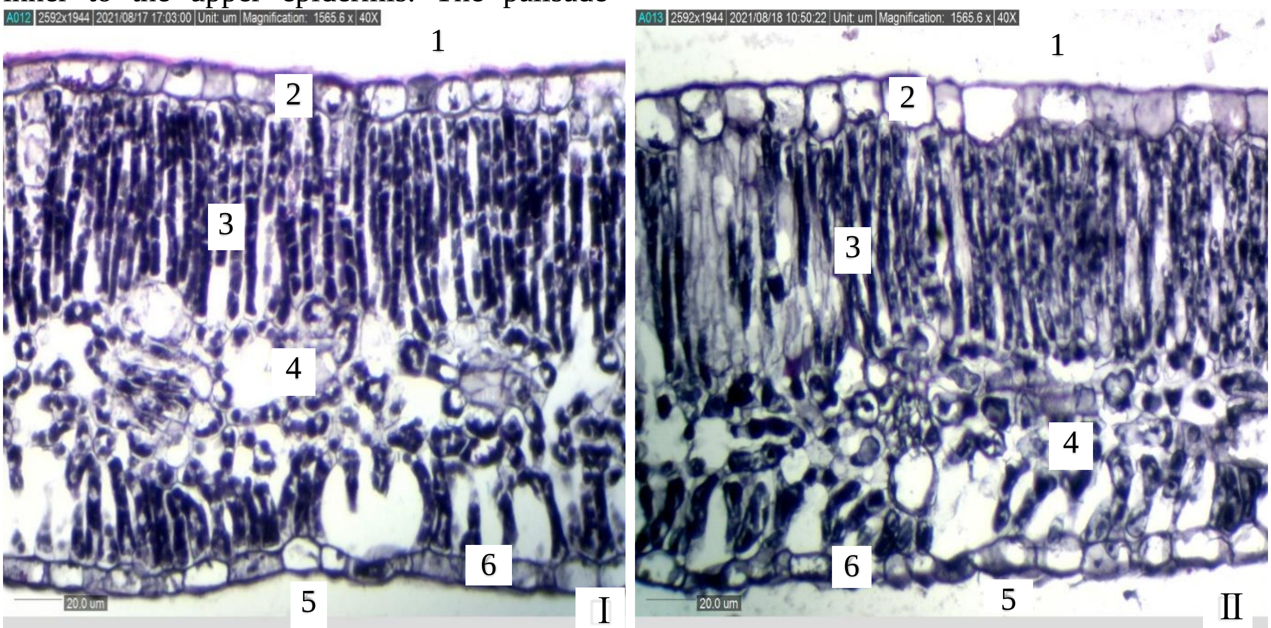
Leaf blade: The average of blade thickness of *Melia azedarach* L. in Table (2) show the thickest blade in location Ⅲ (165.6 µm) and the thinnest in location Ⅰ (140.1 µm) with the range (117.6-251.1 µm). In contrast, the highest average (4.1 µm) of upper cuticle thickness appears in location Ⅰ. However, there was no difference between the locations (I, II and III) in lower cuticle thickness. While the highest average of upper and lower epidermis was recorded (13.4 and 10 µm) respectively in location III, and the lowest one was (9.4 and 5.4 µm) respectively in location I. At the same time, the highest average of palisade layer height and spongy parenchyma width was seen in location Ⅲ and the lowest was in location Ⅰ (74.8, 60.6, 57.9 and 49.4 µm) respectively.

Table 2. The measurements of blade components of *Melia azedarach* L. at three different locations*

Characters	Statistics	*Melia azedarach* L.		
		I	II	III
Blade thickness (μm)	Average	140.1	144.5	165.6
	Range	117.6 – 152.7	129.5 – 167.2	118.3 – 251.1
Upper cuticle thickness (μm)	Average	4.1	3	2.9
	Range	2.2 – 4.7	1.6 – 4.7	1.2 – 4.6
Lower cuticle thickness (μm)	Average	2.5	2.7	2.8
	Range	1 – 3.5	1.6 – 4.3	1.9 – 3.5
Upper epidermis thickness (μm)	Average	9.4	11.5	13.4
	Range	6.5 – 13.2	8.9 – 15.1	9.8 – 17.8
Lower epidermis thickness (μm)	Average	5.4	6.5	10
	Range	3.6 – 7.2	5.4 – 9.7	7.4 – 13.8
Palisade layer height (μm)	Average	57.9	63.6	74.8
	Range	50.5 – 69.9	47.8 – 95.2	60.1 – 85.6
Spongy parenchyma width (μm)	Average	49.4	52.9	60.6
	Range	32.4 – 65.6	42.4 – 66.2	40.9 – 78.3

***The value in the table represent the average of 25 observations for each quantitative character. (Ⅰ = Industrial area, Ⅱ = Roadside area and Ⅲ = control area).**

The blade transverse section of *Melia azedarach* L. in figure (2) shows that the adaxial (upper) surface is glossy covered with a thick waxy layer of cuticle in location Ⅰ as compared with locations Ⅱ and Ⅲ. Beneath the upper cuticle, a single compact raw of almost rectangular shape of upper epidermis layer. The mesophyll layer is differentiated into palisade and spongy parenchyma layers, inner to the upper epidermis. The palisade layers consist of one longitudinal cell layer free of intercellular space and rich of chloroplasts, while loose spongy parenchyma is formed by several irregular shape cell layers. Lower epidermis consists of a single rectangular layer of compact cell smaller than the upper epidermis. Abaxial (lower) surface covered with almost thin waxy cuticle layer, Stomata are Anomocytic type and hypostomatic condition



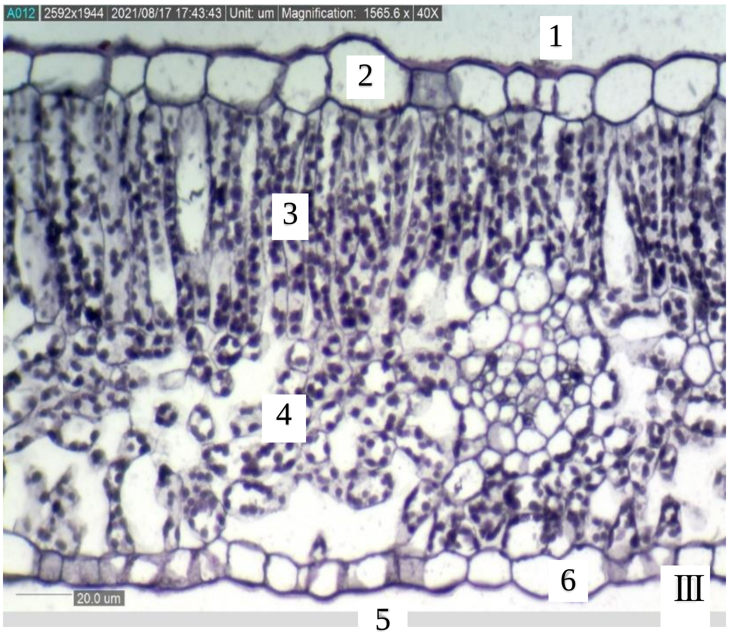


Figure 2. Blade transverse section of *Melia azedarach* L. grown industrial area (Ⅰ), roadside area (Ⅱ) and control area (Ⅲ). 1. Adaxial surface (upper), 2. Upper epidermis, 3. Palisade layer, 4. Spongy layer, 5. Abaxial surface (lower), 6. Lower epidermis

Midrib: The anatomical features of the midrib in table (3) illustrate that the average of upper cuticle thickness was high in location Ⅰ. (4.5µm). However, the lowest average of upper cuticle thickness was seen in location Ⅱ (3.3µm) while the average of lower cuticle thickness was nearly the same in three locations. On the other hand, the average of each of the following features: upper and lower epidermis thickness, collenchyma and parenchyma layer width, phloem and xylem width and pith diameter in location Ⅲ was the highest (11.4, 7.5, 25.9, 96.9, 60.3, 88 and 65.5 µm) respectively, but the lowest average of them was fluctuating between location Ⅰ and location Ⅱ.

Table 3. The measurements of midrib components of *Melia azedarach* L. at three different locations*

Characters	Statistics	*Melia azedarach* L.		
		I	II	III
Upper cuticle thickness (µm)	Average	4.5	3.3	3.9
	Range	2.8 – 5.9	2.3 – 4.7	3 – 4.9
Lower cuticle thickness (µm)	Average	3	2.8	3.1
	Range	2.1 – 4.2	1.6 – 4.6	2.1 – 4.9
Upper epidermis thickness (µm)	Average	9.9	9.3	11.4
	Range	7.2 – 14.6	7.2 – 11.5	8.7 – 15.3
Lower epidermis thickness (µm)	Average	6.4	6.6	7.5
	Range	3.7 – 8.8	4.7 – 9.5	4.3 – 9.7
Collenchyma layer width (µm)	Average	20.5	21.3	25.9
	Range	12 – 28.9	12.8 – 42.8	15.9 – 37.1
Parenchyma layer width (µm)	Average	71.8	64.8	96.9
	Range	56.2 – 138.4	43 – 117.7	69.7 – 154.4
Phloem width (µm)	Average	45.6	45.7	60.3
	Range	34.8 – 63.6	27.1 – 74.4	37.1 – 80.6
Xylem width (µm)	Average	65.9	56.1	88
	Range	44.8 – 80.9	36.4 – 75.2	58.9 – 112
Pith diameter (µm)	Average	42.8	46.5	65.5
	Range	32.9 – 59.1	36.1 - 63	53.6 – 74.6

***The value in the table represent the average of 25 observations for each quantitative character.**
(Ⅰ = Industrial area, Ⅱ = Roadside area and Ⅲ = control area).

The cross sections of *Melia azedarach* L. (Figure 3) reveals that the outline of midrib is nearly elliptic in shape. The adaxial (upper) surface is approximately in the form of a hump, while the abaxial (lower) is a semicircle. Both the cuticle and the epidermal layers of the adaxial surfaces are thicker than the abaxial surfaces. The adaxial surface of the midrib is made up of a single compact epidermis layer that is virtually rectangular in shape, while the abaxial surface is made up of a single epidermis row that is smaller than the upper epidermis. Underneath both epidermis layers are collenchyma layers. The collenchyma and the vascular bundle are separated by parenchyma layers. The central large vascular bundle consists of xylem elements toward the adaxial and phloem elements toward the abaxial. The stomata are present only on the lower surface.

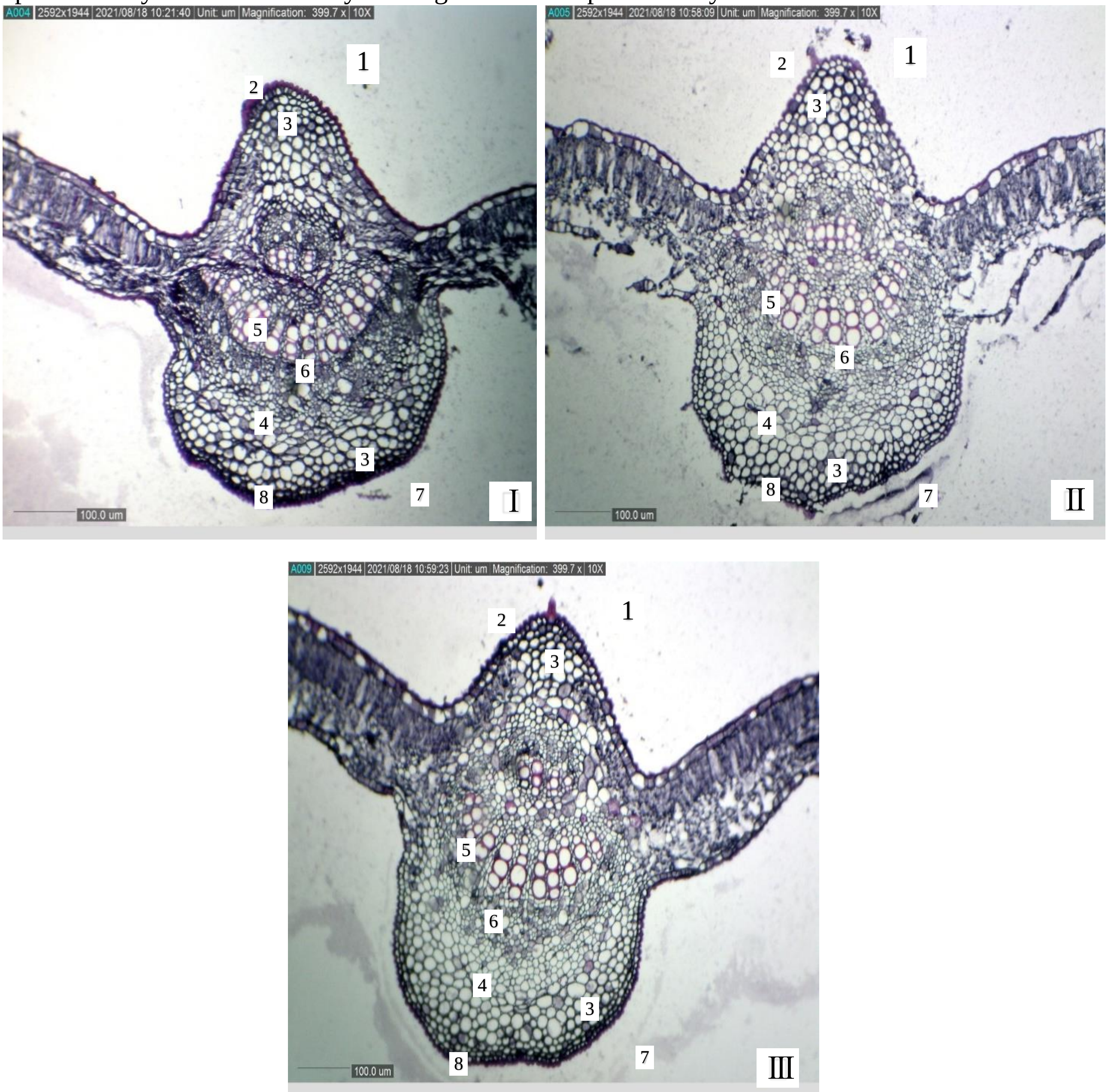


Figure 3. Midrib transverse section of *Melia azedarach* L. grown in industrial area (Ⅰ), roadside area (Ⅱ) and control area (Ⅲ). 1. Adaxial surface (upper), 2. Upper epidermis, 3. Collenchyma layer, 4. Parenchyma layer, 5. Xylem, 6. Phloem, 7. Abaxial surface (lower), 8. Lower epidermis

rban and industries area constantly charge the environment with toxic compounds, thousands of organic trace contaminants have been generated and partially discharged into the environment over the previous few decades (18). Numerous industrial activities damage various environmental components such as water, air, soil, and plant growth (5). Plants growing in polluted environmental conditions, such as industrial area or by the highway, have morphologically and anatomically different leaves, stems, and roots as a result of air and soil pollution than plants growing in more favorable conditions (12). Results of leaf area and anatomical features of *Melia azedarach* L. leaves showed that the leaf area and the most of anatomical features of leaf blade and midrib growing at different polluted locations (I = industrial area, II = roadside area) were reduced when compared to the location (III) control area. Plants respond to industrial and vehicular air pollution stress through a number of active morphological and anatomical responses, including changes in stomata and epidermal cell size and stomata frequency, as well as the stomatal index (28). This reduction in stomata density and size could be viewed as a plant's adaptive response to avoid toxic ingredients from industrial and vehicle exhaust from entering the plant, which could otherwise have negative consequences (23). Moreover, El-Khatib *et al* (11) found that the stomata were denser and smaller in size than those in the control group also, the cuticle, epidermis, palisade tissue, mesophyll tissue, and parts of the vascular cylinder (xylem and phloem) of olive leaves reflected the deterioration impacts of cement dust air pollution. A similar reduction in leaf area and epidermal cells is found at polluted urban sites compared to the reference site (26 and15). Rai (21) showed that air pollution induced significant alterations in foliar morphology as well as biochemical parameter abnormalities. In addition, similar results have been recorded by each of (27, 7, 13 and 10). Due to the stress of automotive exhaust emissions with high traffic density in metropolitan areas, the morphological traits of plants and the leaf surface properties, including stomata and epidermal cells, of plant species growing along road sides are significantly affected (6 and 22).

CONCLUSION

Plants generally respond to air pollution in the similar way they respond to drought and other environmental stresses. It is concluded that the low-polluted areas reduce the changes in the phenotypic and anatomical characteristics of the plant, unlike the more polluted areas, which affect the anatomical structure and thus the plant physiology.

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