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Car Traffic Pollution and its Impact on the Anatomical Characteristics of *Carissa Macrocarpa* (Eckl.) A. DC. Leaves in the City of Hit

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Abstract

An experiment was conducted to study the effect of pollution caused by vehicle emissions due to increased traffic on the leaves of *C. macrocarpa* on the main street in Hit. on the main street of Hit, located approximately 70 km. west of Ramadi, the center of Al Anbar Governorate, Where the traffic is highly concentrated due to the presence of markets and medical clinics. Three locations were selected based on varying levels of traffic congestion along the main street (Alchery): Site 1 had low congestion, Site 2 had moderate congestion, and Site 3 had high congestion. A control sample was stored away from pollution sources. This pollution measure in the plant indicates the effects of pollutants on living organisms. Various anatomical changes in the leaves were studied. The study lasted 40 hours over 10 days, with 4 hours daily observation. The results showed that plants in the most congested areas were the most affected by a significant increase in the reduction of all studied anatomical traits, such as stomatal diameter, guard cell size, epidermal cells, and stomatal number. The greatest decrease was observed at Site 3, with the decrease lessening as traffic congestion reduced in Sites 2 and 1, respectively, compared to the control sample. These results were consistent for both the upper and lower surfaces of the leaves.

Introduction:

Air, in its natural composition, is vital for all living organisms. If its composition changes for any reason or if other gases mix with it, that air becomes polluted and harmful to human health and the lives of living organisms [1-3]. Air pollution is the imbalance in the proportions of atmospheric air components or the result of releasing large amounts of gaseous and solid elements into the air, leading to a significant change in the characteristics and volume of air elements [2]. There are many different types of air pollutants, such as gases (including ammonia, carbon monoxide, sulfur dioxide, nitrous oxides, methane, carbon dioxide, and chlorofluorocarbons), suspended particles (organic and inorganic), and biological molecules [4]. The development in industry in all fields in the present era has led to an increase in air pollution with harmful particles and substances, especially in developing countries [5, 6].

The car has become the main means of transportation in cities, where the operation of a car engine leads to exhaust emissions resulting from fuel combustion [7][8]. Vehicle emissions are one of the main causes of air pollution, as about 60% of urban air pollution is caused by vehicles alone [9, 10]. Global statistics indicate that the number of cars in the world did not exceed 20 million cars in 1918 but reached 750 million cars in 2005. Currently, more than 50 million cars are being introduced annually.

Plants are among the living organisms most affected by this pollution [11]. Car exhaust is a mixture of small solid bodies and inhalable liquid droplets that reach deep into the lungs. This mixture results from the gasoline or diesel combustion process that causes the car to move [12]. The exhaust emitted from the internal combustion of the car engine into the atmosphere contains large amounts of pollutants, including carbon monoxide (CO), carbon dioxide (CO_2), sulfur oxide (SO_2), and lead compounds. The European Commission has reported that nearly 12% of total CO_2 emissions are produced by passenger cars, the dominant means of internal transportation, representing 83.2% of the total number of passengers in Europe in 2013 [13-16]. The proportion of these pollutants varies according to the speed of the car and the quality of the engine [17]. Geographical factors also play an important role in the concentration level of these pollutants, as topography significantly impacts the increase in pollutant quantities. A car driving in flat areas pollutes the environment less than one climbing to higher areas because the car needs more thrust and thus more fuel in the latter case [18, 19].

Car exhaust pollution on plant leaves along roadsides can be studied anatomically by analyzing changes in the number of guard cells, stomata, and other anatomical aspects [20]. Pollution leads to changes in leaf structure and function [21][22], affecting plant health and its ability to grow and reproduce [23].

Car exhaust pollution can also affect the number of stomata by increasing the concentration of carbon dioxide and suspended particles in response to enhancing the photosynthesis process. However, suspended particles may clog the stomata and reduce gas exchange efficiency [24, 25]. Stomata are openings on the leaf surface that allow gas exchange between the plant and the surrounding environment. A stressed plant cannot bloom as it will use all its resources to fight the threat and survive, so most plants exposed to car exhaust are usually delayed in blooming [26].

Materials and methods:

C. macrocarpa is a shrub native to tropical and subtropical regions. The plant belongs to the Apocynaceae family, genus Carissa, and the species macrocarpa. It is part of the vascular plants division, true leaves section, seed plants division, and dicotyledonous class. It is a perennial tree characterized by green, glossy, round leaves and white flowers, commonly known as Natal plum [27]. ***C. macrocarpa*** is a plant that tolerates environmental pollution, especially air pollution, due to its strong physiological and morphological characteristics, particularly its often thick and waxy leaves [28]. Therefore, it has been selected for this study.

Study Location

The city of Hit is located approximately 70 km west of Ramadi, the center of Al Anbar Governorate. Three locations were selected based on varying levels of traffic congestion along the main street in Hit. Site 1 had low congestion, Site 2 had moderate congestion, and Site 3 had high congestion. This selection was made to ensure the impact of traffic on pollution levels could be accurately measured. Three groups, each consisting of three plant samples, were labeled according to site and sample number. A control sample, not exposed to pollutants, was also maintained.

Samples were spaced 300 meters apart. The study spanned 40 hours over 10 days, with 4 hours of observation daily during peak traffic times (2:00 – 6:00 PM). Plants were moved from storage to the street for these periods and then returned to storage, away from pollution sources. The control sample remained in storage throughout the study. Plants were watered as needed to maintain optimal moisture for stomatal function.

Laboratory work was conducted at the University of Anbar, College of Applied Sciences, Hit, in the biology laboratory, using advanced equipment (microscope – OPTO-EDU). Leaves were collected from various parts of the plant (top, bottom, middle), focusing on young leaves, and plant dissection tools were used.

Preparation of Epidermis

The epidermis was prepared from fresh leaf samples of *C. macrocarpa* following the method of [29], with slight modifications. A section of the leaf was taken from a fixed position (middle of the leaf near the central vein, including part of the lamina and edge). Both peeling and stripping methods were used to obtain upper and lower epidermises using a scalpel and fine-tipped forceps. The epidermis was placed on a slide with a drop of glycerin, covered with a coverslip, and examined. Five samples per type were studied, and measurements of stomatal dimensions and epidermal cell shapes were taken using a compound microscope (OPTO-EDU) and an ocular micrometer under 40x magnification.

Stomatal Size and Cell Dimensions

Stomatal openings are crucial for the leaf as they control water vapor loss relative to water absorption from the soil, directly impacting plant growth. The stomatal size was measured on the leaf surface under a 40x objective lens. Length and width of stomata were measured using a micrometer unit.

Stomatal number

Stomata per centimeter were counted by using a compound microscope (OPTO-EDU) and an ocular micrometer under 40x magnification.

Results and discussion

Stomatal Diameter

The results in Table 1 showed that planting in areas far from the source of fumes helped reduce the decrease in the size of stomatal pores compared to plants grown near the source of fumes, for both the upper and lower leaf surfaces. For the upper surface plants grown in site 1 had a stomatal pore size close to that of the control plants, with an average pore size of 194.51 μm, while the control plants had an average pore size of 262.21 μm. The reduction in pore size between plants in site 1 and the control plants was less significant compared to plants in other areas, with a reduction of 67.7 μm. This reduction in pore size increased as the proximity to the source of fumes increased, with reductions of 80.48 μm and 102.84 μm in sites 2 and 3, respectively. The release of gases from car exhausts also causes the leaf surface to become smaller to minimize the number of stomata, which are the main entry points for these gases, leading to changes in the shape and size of the stomata. This is consistent with the findings of [30][31]. The results also showed that the plants grown far from polluted areas had larger stomatal sizes on the lower surface of the leaves compared to plants closer to polluted areas, which experienced increased traffic congestion. The stomatal diameter decreased significantly in site 3, with an average stomatal size of 202.56 μm, compared to the control treatment, which had the highest average stomatal size of 356.69 μm. The stomatal diameter for sites 1 and 2 were 241.17 μm and 236.45 μm, respectively.

Table 1: Explains the effect of car traffic on the stomatal diameter on the leaf surfaces

Sample Number	Upper Leaf Surface	Lower Leaf Surface
Control	262.21 μm ± 1.28	356.69 μm ± 1.25
1	194.51 μm ± 1.43	241.17 μm ± 1.41
2	181.33 μm ± 1.52	236.45 μm ± 1.20
3	159.37 μm ± 1.13	202.56 μm ± 0.87

Stomatal Cell Size

Table 2 shows the results obtained for measuring the size of stomatal cells in plants grown in more congested sites compared to the control sample. A decrease in the size of stomatal cells was observed in site 3, and the decrease continued as we moved towards sites with less traffic. The epidermal cells of the leaves of the studied plant decreased in size as they got closer to the source of fumes in site 3, caused by car exhaust, compared to the epidermal cells taken from the control sample. The results shown in Table 2 indicate that the average length of stomatal cells in site 3 was 188.89 μm, decreasing by approximately 40 μm compared to the stomatal cells of the control sample, which had an average length of 228.81 μm. In contrast, the average length of stomatal cells in sites 1 and 2, which are further from the source of fumes, was 206 μm and 196 μm, respectively, with a reduction of 22 μm and 32 μm compared to the control sample. This reduction in cell length was also observed in the width of the stomatal cells, with the smallest width recorded in site 3 at 166.65 μm, a reduction of 35.33 μm compared to the control sample. The smallest reduction in cell width was observed in site 1, with a decrease of 18.19 μm, while site 2 showed a reduction of 29.39 μm compared to the control sample. This decrease in stomatal cell size as proximity to the fumes increases is consistent with findings by [32][33], which indicate that air pollution from vehicle and industrial emissions leads to a reduction in stomatal cell size, thus affecting leaf size in general. This might be due to the coagulation of colloidal substances in the cytoplasm and disruption of nucleic acid synthesis in growth centers, as well as interference with photosynthesis, as noted by [34][35]. The accumulation of heavy metals absorbed by the plant may also contribute to overall reduced plant growth. The same applies to the stomata on the lower surface of the leaf, as shown in table 2.

Table 2: Explains the effect of car traffic on the stomatal cells (guard cells)

Sample Number	Upper Leaf Surface		Lower Leaf Surface	
	Length	Width	Length	Width
Control	228.81 µm ± 1.11	201.98 µm ± 1.16	273.24 µm ± 1.26	241.39 µm ± 1.18
1	206.49 µm ± 1.42	183.79 µm ± 1.47	220.74 µm ± 1.31	192.63 µm ± 1.24
2	196.36 µm ± 1.15	172.59 µm ± 1.51	205.95 µm ± 1.15	186.34 µm ± 0.95
3	188.89 µm ± 1.11	166.65 µm ± 1.25	197.25 µm ± 1.18	183.29 µm ± 1.02

Epidermal Cells

Table 3 shows that the epidermal cells in the leaves of the studied plant decreased in size, with the average length of epidermal cells in site 3 being 196.58 µm, decreasing by approximately 120.18 µm compared to the epidermal cells of the control sample, which had an average length of 316.76 µm. The average length of epidermal cells in sites 1 and 2 was 256.29 µm and 203.21 µm, respectively, with reductions of 60.47 µm and 113.55 µm compared to the control sample. This reduction in epidermal cell size was also observed in cell width, with the smallest width recorded in site 3 at 174.62 µm, a reduction of 126.87 µm compared to the control sample. The smallest reduction in epidermal cell width was observed in site 1, with a decrease of 61.11 µm, while site 2 showed a reduction of 163.03 µm compared to the control sample. This decrease in epidermal cell size as proximity to the fumes increases is consistent with findings by [36][37][38], indicating that the reduction in epidermal cell size is a general response to air pollution. This decrease in the size of epidermal cells, both in cell length and width, on the upper surface of the leaves of plants grown in the most traffic-congested locations was also observed in the cells on the lower surface of the leaves of the same plants. The size of the epidermal cells decreased in site 3 compared to the control treatment, and the plants grew in sites 1 and 2, according to traffic density, as shown in Table 3.

Table 3: Explains the effect of car traffic on the size of the epidermal cells

Sample Number	Upper Leaf Surface		Lower Leaf Surface	
	Length	Width	Length	Width
Control	316.76 µm ± 1.13	301.49 µm ± 1.36	330.45 µm ± 1.56	308.72 µm ± 1.01
1	256.29 µm ± 1.27	240.38 µm ± 1.29	294.15 µm ± 1.75	262.88 µm ± 1.36
2	203.21 µm ± 1.43	183.46 µm ± 1.31	254.61 µm ± 1.41	231.93 µm ± 1.32

3	196.58 µm ± 1.15	174.62 µm ± 1.36	217.73 µm ± 1.38	196.94 µm ± 1.08

Number of Stomata

By comparing the obtained results shown in Table 4 with the control sample, we observe a decrease in the number of stomata in the most congested site for traffic as well as in sites with less traffic, both on the upper and lower surfaces of the leaves of the planted plants. The number of stomata on the upper surface of the leaf in the most congested site 3 was 13.33, while this number increased as traffic decreased to Site 1, which had 16.66 stomata on the leaves of the plants, and the control treatment had 22.37 stomata. Similarly, on the lower surface, the number of stomata decreased as we moved from the least congested site 1 to the most congested site 3, where the number of stomata ranged from 6.34 to 4.33, respectively, compared to the control treatment, which had 8.56 stomata on the lower surface of the leaves. This decrease is likely due to increased pollution in the most congested area, caused by vehicle exhaust emissions and the slow movement of cars, leading to greater emission and accumulation of fumes in the exact location [39][40].

Table 4: Explains the effect of car traffic on the number of stomata in plant leaves

Sample Number	Upper Leaf Surface	Lower Leaf Surface
Control	22.33 µm ± 0.36	8.56 µm ± 0.32
1	16.66 µm ± 0.43	6.34 µm ± 0.42
2	14.33 µm ± 0.58	5.67 µm ± 0.35
3	13.33 µm ± 0.33	4.33 µm ± 0.37

Conclusions

The study results showed the significant impact of increased car traffic on air pollution, which in turn affected the anatomical characteristics of the plant leaves used in the study along the roadside in Hit City. The increase in car traffic led to a reduction in the size of stomata on both surfaces of the leaves, as well as a decrease in the size of guard cells and epidermal cells, and the number of stomata on both the upper and lower leaf surfaces, compared to the control samples stored away from car traffic. This anatomical reduction in leaf characteristics diminishes progressively from areas with the highest congestion and heavy vehicle traffic to areas with less congestion.

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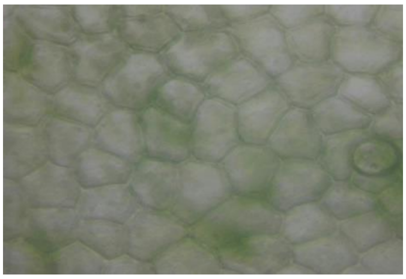
Appendix

C. macrocarpa Site 1

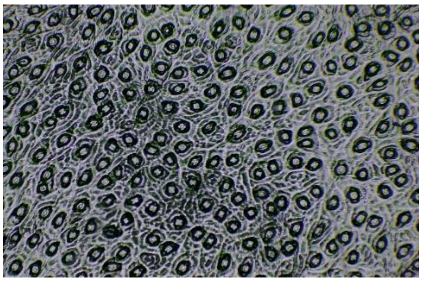
C. macrocarpa Site 2

C. macrocarpa Site 3

C. macrocarpa Control



Epidermal Cells



Stomata Cells

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تلوث حركة السيارات وتأثيره على الخصائص التشريحية لأوراق نبات *Carissa macrocarpa* (Eckl.) A. DC. في مدينة هيت.

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التلوث، حركة السايرات، الخصائص التشريحية، خلايا الاوراق، النبات

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الخلاصة:

أجريت تجربة لدراسة تأثير التلوث الناجم عن انبعاثات المركبات بسبب زيادة حركة المرور على أوراق نبات كاريسا ماكروكاربا في الشارع الرئيسي في مدينة هيت، التي تقع حوالي 70 كيلومترًا غرب الرمادي، مركز محافظة الأنبار. حيث تتركز حركة المرور بشكل كبير بسبب وجود الأسواق والعيادات الطبية. تم اختيار ثلاثة مواقع بناءً على مستويات مختلفة من الازدحام المروري على طول الشارع الرئيسي (الجري): الموقع 1 كان ذو ازدحام منخفض، الموقع 2 ذو ازدحام معتدل، والموقع 3 ذو ازدحام عالٍ. تم الاحتفاظ بعينة مقارنة بعيدًا عن مصادر التلوث. يعتبر هذا المقياس للتلوث في النباتات مؤشرًا على تأثير الملوثات على الكائنات الحية. تم دراسة بعض التغيرات التشريحية في الأوراق. استمرت الدراسة لمدة 40 ساعة على مدار 10 أيام، مع 4 ساعات من المراقبة يوميًا. أظهرت النتائج أن النباتات في المناطق الأكثر ازدحامًا تأثرت بشكل أكبر بزيادة واضحة في انخفاض جميع الخصائص التشريحية المدروسة، مثل قطر الثغور، وحجم الخلايا الحارسة، وخلايا البشرة، بالإضافة إلى عدد الثغور. تم ملاحظة أكبر نسبة انخفاض في الموقع 3، وقل هذا الانخفاض كلما اتجهنا إلى المواقع الأقل ازدحامًا في حركة مرور السيارات (الموقعين 2 و1 على التوالي) مقارنة بعينة المقارنة. كانت هذه النتائج متوافقة على كل من السطح العلوي والسطح السفلي للأوراق.