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Original Article

Investigating the Effect of Dust on the Rate of Photosynthesis and Air Pollution Tolerance Index of the Leaves of *Ziziphus spina-christi* (L.) Willd. in the City of Ahvaz

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Abstract

Background & Aims: Dust is one of the phenomena affecting the environment's quality. The change in the vital indices of plant species is one of its important effects. The present study was conducted to investigate the effect of dust on the rate of photosynthesis and air pollution tolerance index (APTI) of the leaves of *Ziziphus spina-christi* (L.) Willd. in the city of Ahvaz in 2020.

Materials and Methods: A number of 10 *Z. spina-christi* (L.) Willd. trees were selected in each municipal district of Ahvaz metropolis. From each tree, one leaf sample was selected in the 4 main geographical directions, i.e., 4 samples from each tree species; overall, 40 leaf samples were collected in the whole region and sent to the laboratory to investigate the level of dust particles deposited in the four seasons of the year. Photosynthesis was measured based on the amount of chlorophyll by a spectrophotometer, and the APTI was calculated using Agbaire et al.'s method.

Results: The results of measuring the weight of dust deposited on the leaves of *Z. spina-christi* (L.) Willd. trees in the city of Ahvaz showed that the greatest amount of dust was deposited on the leaves in the geographical north direction. The highest rate of photosynthesis was in the fall (53.76), and the lowest was in the spring (35.97). The mean APTI score of *Z. spina-christi* (L.) Willd. species in Ahvaz was 4.77.

Conclusion: These results indicate that this species has a favorable APTI against environmental pollutants, including dust. In general, the results of the present study confirm the significant association of the dust deposited on the leaves of *Z. spina-christi* (L.) Willd. with the rate of photosynthesis and the APTI.

Keywords: Air pollution, Indoor, Chlorophyll, Environment, Dust, Ahvaz, Iran

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1. Introduction

Today, dust is considered one of the emerging phenomena of the most critical environmental problems, which has disrupted the daily life of the people of the affected territories [1]. The occurrence of dust storms usually accompanies the carrying of enormous masses of solid and suspended materials [2]. The extreme increase in the concentration of suspended particles due to this phenomenon has considerable effects on humans, plants, and animals [3,4]. Given that plants are the only living beings that always have to endure pollution due to being fixed in their habitats, the persistence of the effects of this pollution on them is more than on humans and animals [5]. The results of various studies have indicated that plants constantly exposed to environmental pollutants accumulate these particles in their systems and show observable changes depending on the level of sensitivity [6-9]. There is much evidence that when air pollutants enter plant tissues, such as leaves, they initially function through the production of reactive oxygen species (known as oxidative free radicals) [10,11]. Air pollutants impact in different ways, such as stomatal damage, premature aging, reduced photosynthetic activities, disrupted membrane permeability, and reduced growth and productivity in sensitive plant species [12,13]. Dust as a pollutant can have similar impacts on growth and its physiological and biochemical factors. Numerous factors affect the amount of dust absorption and dust deposited on the leaves of plants, including the geometric form, leaf's cross-sectional surface, phyllotaxis, and external characteristics such as fuzzes, cuticle, petiole length, height and canopy of the plant species, as well as wind condition, direction, and speed [14]. Other factors, such as twisted surface or coarseness, cell order, and abundant cilia, also influence the ability of dust movement by various plant species and culminate in the occurrence of noticeable biological consequences [15]. Some of these consequences include the decreased rate of photosynthesis and air pollution tolerance of plant species [16]. The impact of dust deposited on tree leaves on the rate of

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photosynthesis and air pollution tolerance has been evaluated in various studies. Abuduwaili et al estimated salt deposited from dust on the leaves of silk-cotton trees and its effect on reducing the rate of photosynthesis of this species in northwest China to be 45% to 65% [17]. In the investigation of dust deposited on the leaves of roadside plants in the northeastern regions of India, Rai and Panda also observed a significant correlation between the level of dust deposited and the reduced rate of photosynthesis [18]. Shah et al also suggested that the dust particles deposited on tree leaves led to creating stress and reducing different photosynthetic pigments and their derivatives in plants [19]. Molnár et al pointed out that considering the direct relationship of the air pollution tolerance index (APTI) of plants to the level of dust deposited on plant organs, plants can be used as an index for the level of air pollution in the medium term time intervals [20]. Javanmard et al also mentioned that the increased dust levels result in decreased APTI of plants, the phenomenon of leaf shattering, and premature death of the plant [21]. Due to being located in the world's arid and semi-arid belt, Iran is constantly exposed to numerous local and synoptic dust systems [22]. Khuzestan province, one of the southwestern provinces of Iran, has been influenced by the dust phenomenon of domestic and foreign origin in the past years [23]. From 2010 to 2020, 45 days of dust (the concentration of suspended particles above 300 micrograms per cubic meter) has been averagely recorded for the cities of Khuzestan province, which is the highest amount of pollution in Iran, both regarding the frequency of occurrence of the phenomenon and the mean concentration of suspended particles [24]. Such pollution can effectively contribute to the components of the rate of photosynthesis and APTI of plant species. One of the native species of Khuzestan province, which is favorably adapted to the climate of this region, is the *Ziziphus spina-christi* (L.) Desf. species from the *Rhamnaceae* family [25]. The *Z. spina-christi* (L.) Willd. is naturally a shrub or a thorny tree, and its height varies from 2.5 to 15 meters. Among the features of this family are simple leaves, often alternating, ordered flowers, complete male or hermaphrodite, and dioecious and monoecious polygamous [26]. This species is of great biological value in the area of Khuzestan province. The present study is conducted to investigate the effect of dust deposited on the leaves of *Z. spina-christi* (L.) Willd. on the APTI and photosynthesis indices.

2. Materials and Methods

This study was conducted in 2020 to assess the effect of dust on the rate of photosynthesis and APTI of leaves of *Z. spina-christi* (L.) Willd. trees in the city of Ahvaz. Based on the objectives and to achieve the expected statistical accuracy, 10 *Z. spina-christi* (L.) Willd. trees were selected in different regions of Ahvaz metropolis by random sampling. One leaf sample was selected from the central part of each tree in the 4 main geographical directions (north, south, east, west), i.e., 4 samples from each tree species; overall, 40 leaf samples were collected in the whole region and sent to the laboratory to investigate the level of dust particles deposited. Sampling was performed in four seasons. The preparation of leaf samples and the research process to investigate the level of dust particles deposited were performed in the research laboratory of Khuzestan Agricultural and Natural Resources Research and Education Center. For this purpose, after transferring the leaf samples to the laboratory, each sample was distilled twice with 50 cc of distilled water, washed using a soft brush, and the extracted solution was accumulated in 100 cc falcons. The acidity of the dust solution was measured using a pH meter device. Then, the falcons were placed in a centrifuge device at 8000 rpm for 10 minutes so that the dust particles of the solution were deposited entirely. Afterward, the samples were placed in the oven for 48 hours at 70°C until the water of each falcon evaporated entirely [27]. Finally, the deposited dust was scraped and collected using a proper tool to be weighed using a digital scale with an accuracy of 0.0001. In order to investigate the rate of pure photosynthesis, young mature leaves were sampled. In order to calculate the total amount of chlorophyll, 0.5 g of fresh leaves were crushed and extracted with 10 mL of distilled water. The chlorophyll level was then measured using the Minolta Chlorophyll meter model SPAD-502. Two physiological parameters, such as relative water content and pH, and two biochemical parameters of ascorbic acid and total chlorophyll were measured in the studied samples to measure the APTI. The relative water content was first determined to assess the plants' physiological parameters. For this purpose, the fresh and dry weights of the plants' leaf samples were determined, and the values of these parameters were placed in equation 1.

$$RWC\% = (W_t\text{-}Wd/W_t) \times 100 \qquad \text{Eq. 1}$$

In this equation, W_t is the wet weight of the plant's fresh sample, and W_d is the dry weight of the plant's leaf sample placed in the oven for 24 hours at 70°C. After determining the relative water content to measure the pH of the leaf extracts, first, 2 g of fresh leaves were taken and ground in 20 mL of double distilled water in a Chinese mortar. Then they were transferred to 50 mL falcons and centrifuged for 5 minutes at 4000 rpm.

The parameters were placed in the following equation. Two mL of the solution was mixed with 10 mL of acetone, and its amount was read in the spectrophotometer. The obtained extract was passed through a filter paper and transferred into special containers. Finally, it was read using a calibrated digital pH meter. In order to measure ascorbic acid in plant samples, 1 g of fresh plant leaves was first ground in 20 mL of 5% metaphosphoric acid. The obtained mixture was then centrifuged for 20 minutes at 8000 rpm at 4°C. In the following, 0.5 mL of 2,6-dichlorophenol and 3 mM phenol solution was added to 1 mL of the filtered solution to oxidize ascorbic acid to dehydroascorbic acid. Afterward, 1 mL of 1% thiourea was added to the samples, and the samples were left for 20 minutes. Then 1 mL of 2,10,4-dinitrophenylhydrazine solution was added so that 2,4-dinitrophenylhydrazine derivative was formed from dehydroascorbic acid. After fulfilling the mentioned steps, the samples were placed in a water bath for 1 hour at a temperature of 50°C and then in an ice bath for 20 minutes. After that, 50 mL of 85% sulfuric acid was slowly added to each sample. Also, 1 mL of 20% sulfuric acid was added to the samples. After preparing the proper solution, a UV-VIS spectrophotometer was used to read the ascorbic acid at a wavelength of 520 mm. For this purpose, 1 mL of 5% metaphosphoric acid was added to 0.5 mL of DCIP 3 mM ; then 1 mL of 20% sulfuric acid was added to the control solution and just similar to the previous steps, it was placed in a water bath at 50°C for 1 hour and then in an ice bath for 20 minutes. After completing all the steps, it was tried to read the ascorbic acid in 1 hour because the complexes formed during the experiment would be stable in this duration of time. In order to measure total chlorophyll as the last parameter of APTI, 0.5 g of the plant's fresh leaves were first ground with 10 mL of 80% acetone in a Chinese mortar. After the samples were ground entirely, the obtained extracts were poured into the falcon and centrifuged at 4000 rpm for 10 minutes. The filtered extract content was poured in sufficient amounts into the spectrophotometer's specific cuvette and read at 645 and 663 nm wavelengths. The absorption rate at two measured wavelengths was placed into equations 2, 3, and 4, and the values of chlorophyll a, chlorophyll b, and total chlorophyll were calculated according to Equations 2, 3, and 4, respectively [28].

$$\text{Chlorophyll a} = (19.3 \times A663 - 0.86 \times A645)V/100W \quad \text{Eq. 2}$$

$$\text{Chlorophyll b} = (19.3 \times A645 - 3.6 \times A663)V/100W \quad \text{Eq. 3}$$

$$\text{Total chlorophyll} = \text{chlorophyll a} + \text{chlorophyll b} \quad \text{Eq. 4}$$

After measuring the above four parameters, the APTI was calculated based on the following equation:

$$APTI = [AA(Tcl + pH) + RWS] \div 10 \quad \text{Eq. 5}$$

In this equation, *AA* shows the amount of ascorbic acid in mg/g, *pH* shows the amount of the leaf's active acidity, *RWS* denotes the percentage of relative humidity, and *Tcl* denotes the total chlorophyll content in mg/g.

3. Results

The results of measuring the weight of dust deposited on the leaves of the *Z. spina-christi* (L.) Willd. trees in Ahvaz indicated that the highest amount of dust was deposited on the leaves in the geographical north direction (0.040081 g), and the lowest amount was on the leaves in the geographical west direction (0.02514 g). According to Figure 1, the results of Duncan's multi-response mean comparison concerning the weight of dust deposited in the crown of *Z. spina-christi* (L.) Willd. showed that in municipal districts 1, 2, 3, and 4, which are in the geographical western and northern directions, a greater weight of dust was deposited on leaf surfaces; in this sense, it has deposited in regions 5, 6, 7, and 8 which are in the eastern and southern directions (Figure 1).

3.1. Photosynthesis calculation

One of the objectives of the current study is to evaluate the effect of dust deposited on tree leaves on the rate of photosynthesis. For this purpose, the leaf photosynthesis rate was measured twice before and after washing with distilled water (Figure 2). This process was administered in 4 seasons (spring, summer, fall, and winter; 2019) and in the hours of 10 am to 3 pm (during these hours, the photosynthesis of plants is at the maximum level). Its purpose is also to assess the effect of temperature conditions on the rate of photosynthesis and the dust

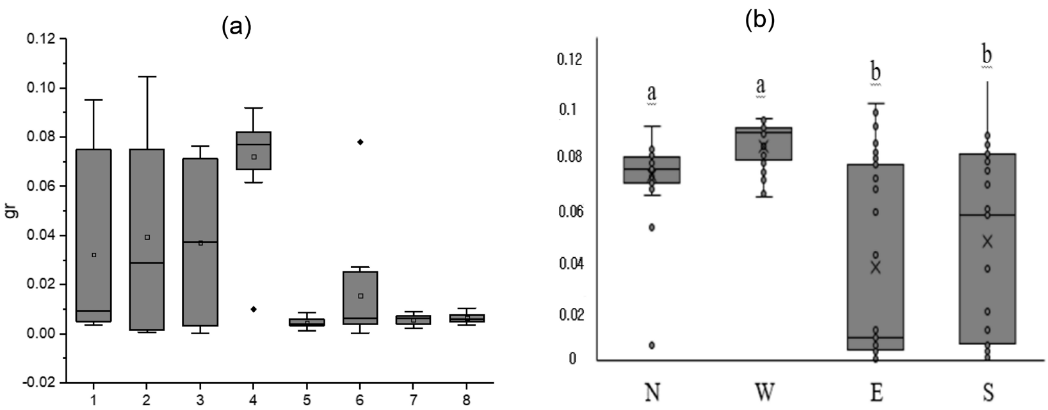

Figure 1. Comparing the mean values of the weight of dust deposited in 8 municipal districts (a) and the geographical directions of the crown of *Ziziphus spina-christi (L.) Willd.* (b)

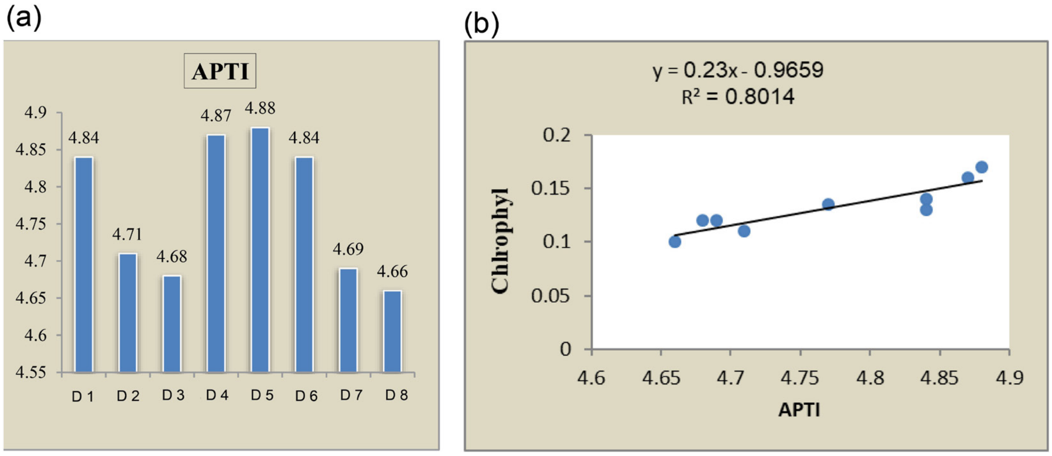

Figure 2. Changes in the mean APTI score of the leaves of *Ziziphus spina-christi (L.) Willd.* trees in the 8 municipal regions of Ahvaz (a) and the regression relationship between the APTI values and the amount of chlorophyll in tree leaves (b). D, District

deposited. The results indicate that in all samples, after the leaf washing process, the rate of photosynthesis increases. The highest rate of photosynthesis was in the fall (53.76), and the lowest was in the spring (35.97). The highest percentage of changing photosynthesis after leaf washing was related to winter (8.13%).

One of the results of the current study is that the decreased rate of photosynthesis in the tree leaves is affected by the dust deposited on them. The biggest difference in the rate of photosynthesis affected by dust occurred in the fall. The results of one-way analysis of variance (ANOVA) of the rate of photosynthesis between the leaves of *Z. spina-christi* (L.) Willd. trees indicated no significant difference between the rates of photosynthesis in winter and summer; however, there were significant differences between other seasons ($P<0.05$).

3.2. APTI calculation

An essential index in investigations regarding the role of plant species in controlling pollution is APTI calculation. In this study, APTI was used, the value of which is shown in Table 1. The mean values of this index in *Ziziphus spina-christi* (L.) Willd. species were calculated in 8 municipal districts (Table 1). The mean APTI score of *Ziziphus spina-christi (L.) Willd.* is 4.77. These results indicate that the *Z. iziphus spina-christi* (L.) Willd. species has an acceptable and favorable air pollution tolerance against environmental pollutants.

According to Figure 2, the results of the linear regression test indicated a significant relationship between APTI values and the amount of chlorophyll (photosynthesis) ($P\leq0.05$). The R^2 index was estimated to be equal to 0.801, suggesting a strong relationship between these two variables. According to Table 2, these results reveal that changes in the amount of dust at the level of Ahvaz affect the APTI and photosynthesis of the leaves of *Ziziphus spina-christi* (L.) Willd. trees.

Table 1. The components of the air pollution tolerance index of the *Ziziphus spina-christi* (L.) Willd. species

Sample	pH	Relative Water %	Ascorbic acid Mg.g^{-1}	Total chlorophyll Mg.g^{-1}	APTI
District 1	5.2	41.1	1.38	0.13	4.84
District 2	6.1	37.94	1.48	0.11	4.71
District 3	6	37.91	1.46	0.12	4.68
District 4	5.7	40.53	1.41	0.16	4.87
District 5	5.6	40.72	1.4	0.17	4.88
District 6	5.1	41.3	1.37	0.14	4.84
District 7	6	37.9	1.47	0.12	4.69
District 8	5.9	37.92	1.45	0.10	4.66
Mean	5.7	39.4	1.42	0.135	4.77

APTI, Air Pollution Tolerance Index.

Table 2. The test statistic of the association between APTI and photosynthesis in the leaves of *Ziziphus spina-christi (L.) Willd.* trees in the city of Ahvaz

Statistic	Test result
Pearson's correlation	0.895
R^2	0.801
Intercept	0.9659
Line gradient	0.23
Significance	0.001

4. Discussion

As the main species, trees are applied for biological monitoring. Thus, the development of plant species compatible with the region and the dust absorbent are among the most influential available and environment-friendly solutions; in addition, APTI is one of the suitable criteria for selecting plants for being applied in polluted regions [29]. The ability to absorb dust is not equal in different species [30-32]. The results of various studies, including Abuduwaili et al and Rai and Panda, also confirm the reduced rate of photosynthesis affected by the dust phenomenon [17,18]. In the current study, the rate of photosynthesis in the leaves of *Z. spina-christi* (L.) Willd. trees enhanced between 4.51 to 8.13% after washing the dust on the leaf surfaces. The greatest change in the photosynthesis percentage after leaf washing was related to winter (8.13%). Given that most dust events in Khuzestan province are related to winter, these results were predictable. In Chaturvedi and colleagues' study, roadside plants' photosynthesis rate has reduced by 25% [33]. In Arvin and colleagues' study, the chlorophyll level of sugarcane species has decreased by 14% affected by dust [34]. Tomašević and colleagues' study revealed that the short leaf washing with distilled water significantly increased the amount of chlorophyll [35]. Geographical directions are also among other components influencing the level of dust deposited on the leaves of *Z. spina-christi* (L.) Willd. trees. This index is related to the wind direction component. Kang and colleagues' study also confirmed the role of wind direction in the dust deposited on tree leaves [36]. Therefore, conducting a study to assess the association of wind speed and direction with the level of dust deposited in Ahvaz is recommended. The presence of a significant relationship between chlorophyll level and APTI was another result of the current research. Hence, it can be concluded that with the increase in the level of dust deposited on the leaves of the *Z. spina-christi* (L.) Willd. trees, the amount of chlorophyll and, ultimately, pollution tolerance will reduce significantly.

5. Conclusion

In general, the results of the current study confirm the presence of a significant relationship between the dust deposited on the leaves of the *Z. spina-christi* (L.) Willd. trees and the rate of photosynthesis and APTI. The results of the present study can underlie the determination of the cultivable species suitable for the climate of Ahvaz and the dust storm phenomenon. One of the proposed research fields of the present study is to carry out a similar study on other plant species in the city of Ahvaz and other cities facing the dust phenomenon.

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Competing Interests

The authors declared no conflict of interest.

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