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Effect of Urban Pollutants on Physiological and Biochemical Parameters of Leaves of Hardwood Trees and Shrubs in Urban Green Spaces

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Abstract: Trees and shrubs of green spaces can tolerate some physiological parameters. They can adapt to climatic conditions, hence, reduce air pollution and can be used as biological indicators in various researches. The present study aimed to explore the impact of urban air pollution on some parameters such as fresh, dry and turgor weight, amounts of chlorophyll and carotenoid pigments, and electrolyte leakage of 18 ornamental tree and shrub species in marginal and downtown parks of Rasht, Iran. The results revealed significant differences in turgor weight, photosynthetic pigments, and chlorophyll *a/b* ratio, but insignificant differences in electrolyte leakage between the marginal and downtown sites. The results indicated that all quantitative variables of the leaves differed significantly ($p \leq 0.01$) among the species, except for fresh weight.

Key words: Landscape, air pollution, carotenoid, ornamental trees.

Introduction

Clearing of wastes and the absorption of air pollutants are among the advantages of trees and shrubs of urban green space (Beckett et al., 1998). Pollution refers to the presence of one or more solid, liquid, gas and radioactive pollutants in free air, which are harmful to humans, animals and plants depending on concentration, duration, and properties and disturb the life of all living organisms (Olumi et al., 2016). The main atmospheric pollutants are SO_2, NO_2, Pb, O_3, fluorine compounds, soot, and <10 suspended particles (PM10) (Nezami et al., 2008). The most common impact of air pollution on plants is the gradual disappearance of chlorophyll and the chlorosis of leaves, which may be related to the decline of photosynthetic capacity (Joshi and Swami, 2009). Higher air pollution disrupts the performance of different parts of plants including chlorophyll by influencing the structure of the plants and affecting the soil of root zone by creating acid rains, thereby reducing leakage pH and the outflow of nutrients from the soil and replaces hydrogen ions for the nutrients that are absorbable to plants (Vallero, 2014). After entering the leaves through stomata, pollutants such as ozone are dissolved in the aquatic layer generating the radicals of hydroxyl (OH), superoxide (O_2^-), and H_2O_2. They can pass across membranes and impair macromolecules (Loreto and Velikova, 2001). It has been documented that air pollution significantly increased membrane peroxidation in plant species on the sidewalks of the contaminated and non-contaminated areas of Tehran, Iran (Ghorbanli et al., 2009). Ion leakage was reported

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to be higher in the leaves of plants that were closer to contaminated industrial areas than in control plants (Areington et al., 2015). The present study investigates the effect of urban pollutants on physiological and biochemical parameters of trees and shrubs in the urban green space of Rasht, Iran.

Materials and Methods

The study was carried out on four sites in Rasht, Guilan province, Iran on July 17, 2018. The sites were two marginal parks including Mafakher and Daneshjoo Parks and two downtown parks including Mohtasham and Mellat Parks. The marginal two parks with light traffic included Mafakher Park (or Chamarsara Park), in the west of Rasht (Lat. 37º27’ N, Long. 49º56’ E.), and Daneshjoo Park located at the entrance of the city in west (Lat. 37º16’ N, Long. 49º35’ E.). The downtown site included Mohtasham Park (the oldest park of Rasht) located in the southern part (Lat. 37º26’ N, Long. 49º58’ E.) and Mellat Park (the second biggest park of Rasht), located in the northern part (Lat. 37º29’ N, Long. 49º59’ E.). The leaves of 18 plant species that were common among the four sites were sampled. Following the Ritchie et al. (1990) method, 0.5 g of fresh leaves were weighed to obtain their fresh weight (FW). Then, they were floated in distilled water at 4°C in darkness for 24 hours. that the leaves were filtrated through a filter paper, dried and weighed to obtain their turgor weight (TW). Finally, they were oven-dried at 75°C for 24 hours to obtain their dry weight (DW). According to Lichtenthaler’s (1987) procedure, 0.4 g of fresh leaves were ground in liquid nitrogen, extracted with 10 mL of 80% acetone, adjusted to a final volume of 20 mL and centrifuged at 4500 rpm (F= 34.9 g) for 15 minutes. Its absorbance range was read with a spectrophotometer (Z-2000, Hitachi Ltd., Japan) at 470, 645 and 663 nm to determine chlorophyll *a* (Chl.a), chlorophyll *b* (Chl.b), and carotenoid (C_{x+C}) by the following equations:

$$\text{Ch1.a (mg/ml)} = (12.25A_{663.2} - 2.79A_{646.8})$$

$$\text{Ch1.b (mg/ml)} = (21.50A_{646.8} - 5.10A_{663.2})$$

$$C_{x+C} = \frac{1000\ A_{470} - 1.8\ \text{Chl.a} - 85.02\ \text{Chl.b}}{198}$$

Using Kumar and Dey’s (2011) method, 0.1 g of fresh leaves were washed and floated in 15 mL of distilled water for 24 hours. Its initial electrical conductivity (EC_1) was measured. The secondary electrical conductivity (EC_2) of the samples was measured at room temperature after keeping it for 24 hours in a freezer (–20°C), finally, the electrolyte leakage was calculated by the following equation:

$$EC = EC_1/EC_2 \times 100$$

Data were analysed by MSTATC and graphs were drawn by MS-Excel 2010 and the means were compared by Least Significant Difference (LSD) test (p< 0.05).

Results and Discussion

The average daily concentration of air pollutants in Rasht city was recorded in Table 1 on June 22, 2019 (Anonymous, 2019). These pollutants were found to be in the concentration order of $PM_{2.5} > PM_{10} > CO > O_3 > SO_2 > NO_2$.

Fresh Weight, Dry Weight, Turgor Weight

The simple and reciprocal effects of site and ornamental species were not significant on fresh weight. The simple effect of plant species was significant ($p \leq 0.01$) on dry weight, however, the simple effect of site and the reciprocal effect of site and plant species was not significant on this trait (Table 2). The highest dry weight was 0.28 g for the leaves of *Zelkova carpinifolia*. The lowest leaf dry weight was observed in *Yucca filamentosa* (0.12 g) (Table 3). The results showed that the dry weight of *Z. carpinifolia* leaf was twice as great as that of *Y. filamentosa* (Table 3). Since the dry weight is an important trait of a plant species that is controlled by genetics; the results seem to be close to reality. The increased leaf dry weight of *Z. carpinifolia* in the present study supports this report. We obtained similar findings for *Y. filamentosa*. A study on the effect of pollution shows the significant decline of dry weight in *Robinia pseudoacacia* and *Nerium oleander* (Ghorbanli et al., 2008) in polluted areas. We also observed a significant decline in dry weight in *Yucca*, which can imply the plant response to pollution and its strategy to cope with pollution.

The simple and interaction of site and species were significant ($p \leq 0.01$) on turgor weight (Table 2). The highest turgor weight was observed in the marginal sites (Mafakher and Daneshjoo parks) with an average of 0.75 g and the lowest was 0.7 g observed in the downtown sites (Mellat and Mohtasham parks) (Table 3). Among the species, *Z. carpinifolia* had the highest turgor weight (0.87 g), which was significantly ($p \leq 0.01$) higher than *Ligustrum texanum*, *C. siliquastrum*, and *Q. castaneifoli*a with turgor weights of 0.80, 0.80, and 0.84 g, respectively. In contrast, the lowest turgor weight

Table 1: The concentration range of air pollutants in Rasht city (June 22, 2019)

Pollutants	*$PM_{2.5}$ (mg/m^3)*	*PM_{10} (mg/m^3)*	*SO_2 (mg/m^3)*	*NO_2(mg/m^3)*	*O_3 (mg/m^3)*	*CO (mg/m^3)*
Daily average	49	32	11	10	16	20

Table 2: Analysis of variance for the effect of treatments on the traits of the studied ornamental trees and shrubs

Source of variance	*df*	*Means of squares*						
		Fresh weight	*Dry weight*	*Turgor weight*	*Chl.a*	*Chl.b*	*Carotenoid*	*Electrolyte leakage*
Site	1	0.001^{ns}	0.002^{ns}	0.059^{**}	1.212^{ns}	0.249^{ns}	0.042^{ns}	195.854^{ns}
Species	17	0.001^{ns}	0.011^{**}	0.033^{**}	4.243^{**}	2.577^{**}	0.606^{**}	940.926^{**}
Site × species	17	0.001^{ns}	0.001^{ns}	0.015^{**}	3.416^{**}	0.962^{*}	0.342^{**}	326.89^{ns}
Error	70	0.001	0.001	0.006	0.72	0.523	0.153	351.261
C.V. (%)		3.85	14.66	10.63	29.07	37.03	27.73	32.01

**, *, and ns show significance at the $p \leq 0.01$ and $p \leq 0.05$ levels and non-significance, respectively.

Table 3: Means comparison for the effect of ornamental species on the measured traits

Treatment	*Dry weight*	*Turgor weight*	*Chl.a*	*Chl.b*	*Carotenoid*	*Electrolyte leakage*
Lagerstroemia indica	0.203 ef	0.68 dg	2.91 df	3.11 ab	0.64 f	68.2 ac
Chaenomeles japonica	0.23 ce	0.79 ac	2.07 fh	1.43 df	1.39 bd	62.77 ad
Quercus castaneifolia	0.27 ab	0.84 a	3.1 cd	3.68 a	0.95 ac	61.41 ad
Acer negundo	0.16 fg	0.69 dg	2.77 dg	2.24 cd	1.61 a	74.9 ab
Laurus nobilis	0.25 ad	0.62 fg	3.16 bd	2.102 ce	1.92 ac	50.45 cf
Ligustrum texanum	0.22 ce	0.803 ab	2.62 dh	1.74 cf	1.47 ab	66.64 ac
Acer pseudoplatanus	0.22 de	0.74 bd	4.51 a	2.51 bc	1.75 ac	77.16 a
Cercis siliquastrum	0.26 ac	0.804 ab	2.21 dh	1.38 ef	1.56 ab	38.38 ef
Zelkova carpinifolia	0.28 a	0.87 a	3.94 ac	1.68 df	1.706 bc	51.17 cf
Berberis thunbergii	0.15 gh	0.74 be	1.77 h	1.15 f	1.45 bc	53.67 be
Fraxinus excelsior	0.2 ef	0.701 dg	2.79 dg	1.6 df	1.34 be	44.05 df
Euonymus japonicus	0.24 bd	0.708 cf	1.91 gh	1.307 ef	1.21 ce	57.68 ae
Pyracantha coccinaea	0.25 ad	0.71 be	4.13 ab	2.26 cd	1.57 ac	74.57 ab
Ligustrum vulgare	0.23 ce	0.71 be	3.09 cd	2.05 ce	1.22 ce	60.67 ae
Hibiscus syriacus	0.17 fg	0.65 eg	3.06 ce	1.74 cf	1.55 ac	61.99 ad
Prunus ceracifera	0.18 fg	0.72 be	2.11 eh	1.36 ef	1.46 bc	66.04 af
Cedrus deodara	0.23 ce	0.619 fg	4.24 a	2.12 ce	1.57 ac	30.07 f
Yucca filamentosa	0.12 h	0.615 g	2.07 fh	1.62 df	0.92 ef	53.56 be

Means with similar letter(s) did not differ significantly at the $p \leq 0.01$ or $p \leq 0.05$ levels according to LSD test.

(0.62 g) was related to *Y. filamentosa*, which was lower than that of *A. negundo*, *Lagerstroemia indica*, and *H. syriacus* (0.69, 0.68, and 0.65 g, respectively; Table 3). Among the interactions, the highest turgor weight (0.94 g) was related to *Q. castaneifoli*a in the downtown sites, which was significantly (≤ 0.01) higher than *Z. carpinifolia* in the downtown sites and *L. texanum*, *C. siliquastrum*, *Chaenomeles japonica*, and *Z. carpinifolia* in the marginal sites with turgor weights of 0.81, 0.85, 0.87, 0.91 and 0.93 g, respectively. *Y. filamentosa* in the marginal sites exhibited the lowest turgor weight (0.6 g) and the second-lowest turgor weight was 0.61 g observed in *H. syriacus* in the downtown sites (Table 3). Plants that can survive in polluted air exhibit less intensive disruptions in their physiological traits including relative permeability of the plasma membrane of leaves (Bai et al., 2006). This is supported in our study by the decreased turgor weight of *Yucca* and the increased turgor weight of *Z. carpinifolia*, which may be associated with disruptions in physiological traits under polluted conditions.

Pigments: Chlorophyll *a*, Chlorophyll *b*, and Carotenoid

The results revealed the significant effect of species and 'site × species' on chlorophyll *a* content, but the simple effect of site was not significant on this trait (Table 2). The highest chlorophyll *a* content was observed in *Acer pseudoplatanus*, which was significantly higher than that of *P. coccinaea* and *C. deodara*. The lowest chlorophyll *a* content was related to *B. thunbergii*, found to be significantly ($p \leq 0.01$) lower than that of *Euonymus japonicus* (Table 3). Among the studied species, *A. pseudoplatanus* in the marginal sites showed the highest chlorophyll *a* content, which was significantly ($p \leq 0.01$) higher than that of *C. deodara* in the downtown sites, *Z. carpinifolia*, and *P. coccinaea* in the marginal sites. *E. japonicus* in the marginal sites and *B. thunbergii* in the downtown sites had the lowest chlorophyll *a* content (Table 4; Figure 1).

Study on the leaves of concocarpus in polluted areas by Lordifard et al. (2012) revealed a decline in chlorophyll *a*. In addition to this, Joshi and Swami (2009) reported a significant decline in chlorophyll *a* content of coffee, mango, sagon, and *Malotus philippinensis* in the polluted area consistent with our finding on a significant decline in chlorophyll *a* content in *B. thunbergii*. A significant reduction in chlorophyll *a* content was also seen in *E. japonicus* and *C. japonica* in the marginal sites and *B. thunbergii* in the downtown sites, the significant increase in chlorophyll *a* content in *A. pseudoplatanus* had significantly increased showing that photosynthesis of this species is not impaired by the pollution, which can be regarded as a biological response to environmental stress.

The simple effect of species and the interaction of 'site × species' were significant on chlorophyll *b* content, however, this trait was not significantly different among the studied sites (Table 2). *Q. castaneifoli*a exhibited the highest chlorophyll *b* content among the studied species, which was significantly higher than that of *A. pseudoplatanus* and *L. indica*. The lowest chlorophyll *b* content was 1.15 mg g^{-1} FW related to *B. thunbergii* (Table 3). Among the interactions, '*Q. castaneifoli*a × marginal sites' contained the highest amount of chlorophyll *b*, this was higher than *A. negundo* and *Q. castaneifoli*a in the downtown sites, and *P. coccinaea*, and *L. indica* in the marginal sites. The lowest chlorophyll *b* content was 0.79 mg g^{-1} FW related to *E. japonicus* in the marginal sites and this was lower than that of *B. thunbergii* in the downtown sites (Table 4). A study on the effect of air pollutants on the physiological activities of *Pongamia pinnata* in urban, forest, and industrial areas revealed that the higher air pollution was related to lower amounts of major and minor pigments except for chlorophyll *b* (Bamniya et al., 2012). Amini et al. (2016) reported that chlorophyll *b* content in *F. excelsio*r was significantly increased in the vicinity of aluminium factory in Arak. A similar study demonstrated a significant increase in the amount of chlorophyll *b* in *Tevatia neralfoia*, *Zygophyllom album*, and *Moltkiopsis ciliate* in response

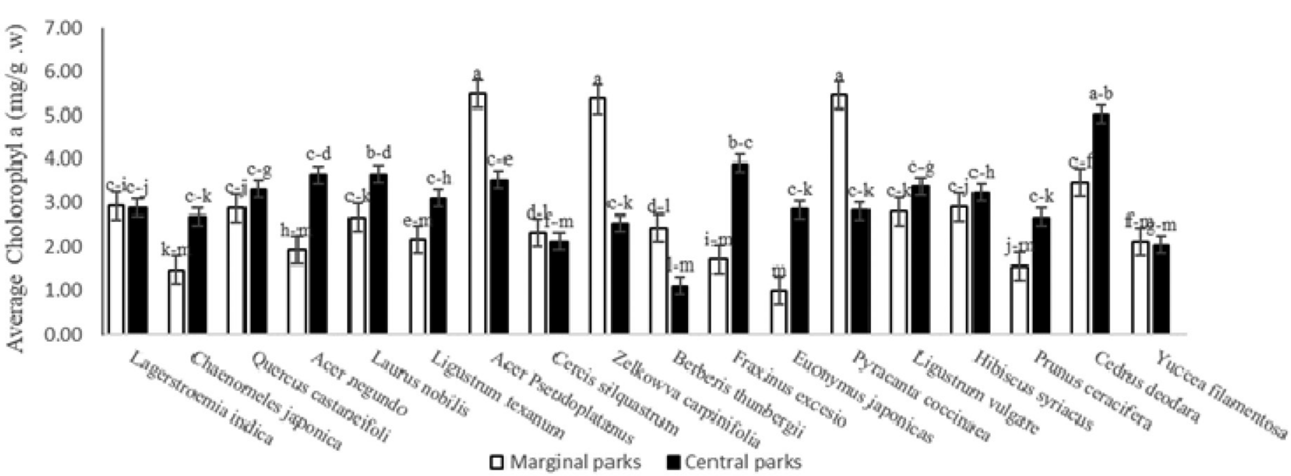

Figure 1: The effect of species on leaf chlorophyll *a* content of the studied ornamental trees and shrubs.

Table 4: Means comparison for the interactive effect of site and species on the measured traits

Treatments	*Turgor weight*		*Chlorophyll* a		*Chlorophyll* b		*Carotenoid*	
	Marginal sites	*Downtown sites*	*Marginal sites*	*Downtown sites*	*Marginal sites*	*Downtown sites*	*Marginal sites*	*Downtown sites*
Lagerstroemia indica	0.75 di	0.62 jl	2.93 ci	2.88 cj	3.58 ab	2.65 bf	0.306 k	0.98 gj
Chaenomeles japonica	0.91 ab	0.66 fl	1.45 km	2.68 ck	1.41 gj	1.45 gj	1.43 bh	1.35 bi
Quercus castaneifolia	0.74 dj	0.94 a	2.88 cj	3.31 cg	3.88 a	3.49 ab	1.53 jn	0.37 bi
Acer negundo	0.67 fl	0.72 el	1.91 hm	3.64 cd	1.44 gi	3.04 ad	1.83 ad	1.39 bi
Laurus nobilis	0.62 hl	0.62 il	2.67 ck	3.65 bd	2.506 bg	1.69 fj	2.14 a	1.704 af
Ligustrum texanum	0.85 ad	0.75 cg	2.14 em	3.09 ch	1.47 gj	2.01 ci	1.36 bi	1.58 ah
Acer pseudoplatanus	0.76 cf	0.73 dk	5.49 a	3.52 ce	2.94 ae	2.09 ci	1.91 ac	1.59 ah
Cercis siliquastrum	0.87 ac	0.73 dk	2.308 dl	2.11 fm	1.35 gj	1.42 gj	1.78 ae	1.39 ci
Zelkova carpinifolia	0.93 ab	0.81 be	5.36 a	2.53 ck	2.07 ci	1.29 hj	2.08 a	1.32 ci
Berberis thunbergii	0.76 cf	0.72 el	2.43 dl	1.1 lm	1.37 gj	0.93 ij	1.72 ae	1.17 ei
Fraxinus excelsior	0.703 el	0.7 el	1.701 im	3.89 bc	1.24 hj	1.97 ci	1.07 fj	1.62 ag
Euonymus japonicus	0.68 fl	0.73 dk	0.99 m	2.84 ck	0.79 j	1.82 ej	1.16 ej	1.26 di
Pyracantha coccinaea	0.77 cf	0.65 fl	5.45 a	2.81 ck	3.14 ac	1.38 gj	1.54 ah	1.6 ah
Ligustrum vulgare	0.75 ch	0.68 el	2.81 ck	3.37 cg	1.87 dj	2.23 ch	1.15 ej	1.29 ci
Hibiscus syriacus	0.69 el	0.61 kl	2.91 cj	3.22 ch	1.85 ej	1.63 fj	1.36 bi	1.73 ae
Prunus ceracifera	0.76 cf	0.68 fl	1.55 jm	2.67 ck	1.17 hj	1.54 fj	1.51 ai	1.4 bi
Cedrus deodara	0.61 gl	0.62 jl	3.46 cf	5.02 ab	2.18 ch	2.07 ci	1.17 ei	1.98 ab
Yucca filamentosa	0.6 l	0.63 gl	2.11 fm	2.03 gm	1.72 fj	1.53 fj	0.88 ik	0.97 hj

Means with similar letter(s) did not differ significantly at the $p \leq 0.01$ or $p \leq 0.05$ levels according to LSD test.

to air pollution (Ali and El-Yemeni, 2010). Consistently, we found a significantly higher amount of chlorophyll *b* in *Q. castaneifoli*a in the marginal sites.

Carotenoid was influenced by the simple effect of ornamental species and the interaction of species and site at the $p \leq 0.01$, but site solely could not change significantly this trait (Table 2). The highest carotenoid content was related to the leaves of *L. nobilis*. *L. indica* exhibited the lowest carotenoid content. Data analysis showed that the leaf carotenoid content of *L. nobilis* was over three times as great as that of *L. indica* (Table 3). '*L. nobilis* × marginal sites' and '*Z. carpinifolia* × marginal sites' showed the highest leaf carotenoid content. These were higher than '*A. pseudoplatanus* × marginal sites' and '*C. deodara* × downtown sites' with a leaf carotenoid content of 1.91 and 1.98 mg g^{-1} FW. Leaf carotenoid content was the lowest in *L. indica* in the marginal sites and this was lower than that of '*L. indica* × downtown sites', '*Y. filamentosa* × downtown sites', '*Y. filamentosa* × marginal sites', and '*Q. castaneifoli*a × marginal sites' (Figure 2). We observed similar results for the loss of carotenoid in *L. indica* in the marginal sites. In a study on the pigments of plants in polluted areas, Joshi and Swami (2007) found that carotenoids declined the most in the leaves of *Eucalyptus* and the least in the leaves of coffee trees. In our study, *L. nobilis* showed the highest carotenoid loss while *L. indica* showed the lowest.

Electrolyte Leakage

Although the effect of species was significant ($p \leq 0.01$) on this trait, the simple effect of site and the interaction between site and species were not significant (Table 2). The highest electrolyte leakage was observed in *A. pseudoplatanus*. In contrast, the lowest electrolyte leakage was related to *C. deodara* (Figure 3). Air pollution, chilling and drought stresses have adverse effects on cell membranes. It has been reported that the measurement of electrolyte leakage can reflect a proper pattern of stress tolerance even in the early stages

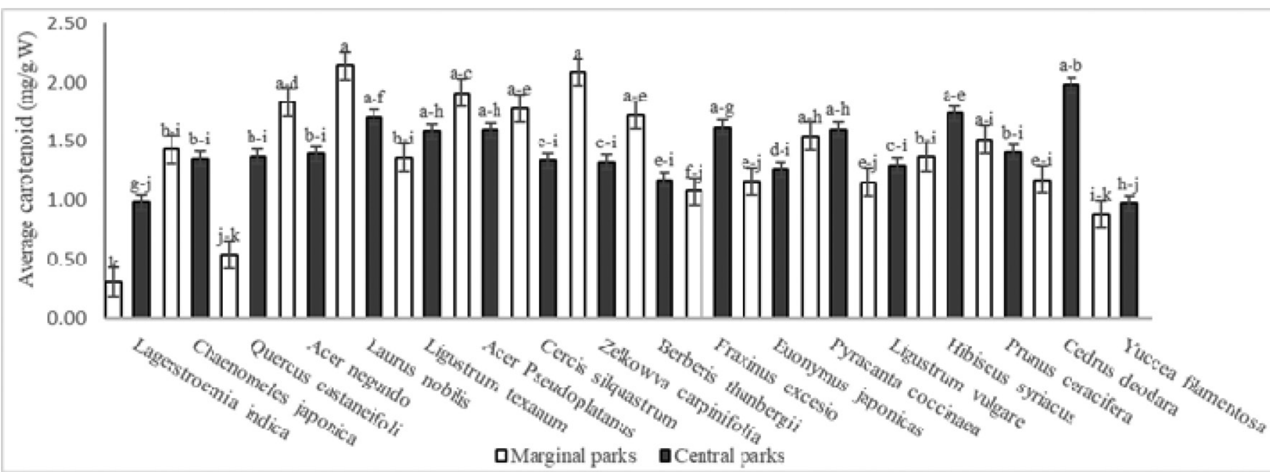

Figure 2: The interactive effect of site and species on leaf carotenoid.

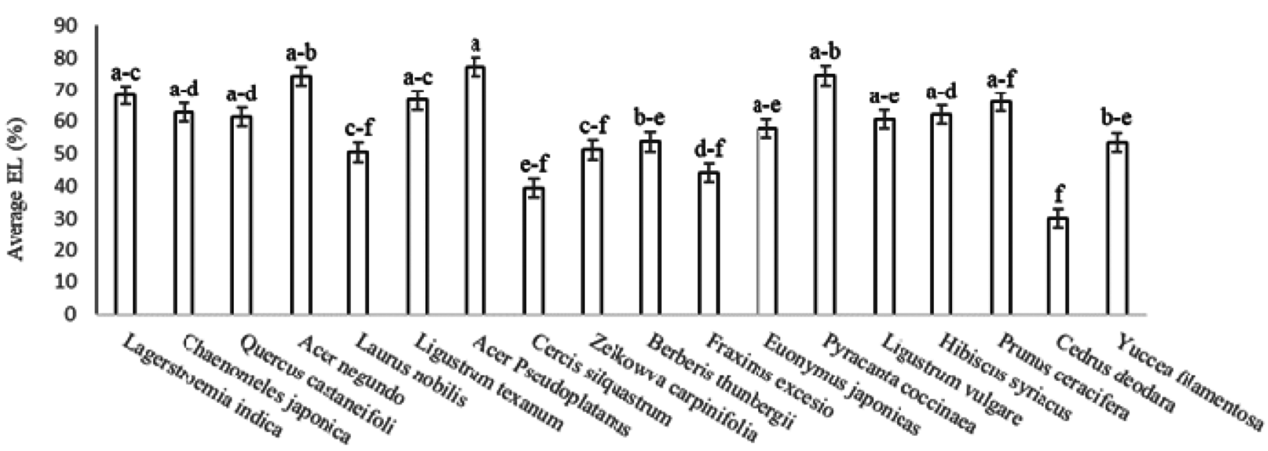

Figure 3: The effect of species on leaf electrolyte leakage of the studied ornamental trees and shrubs.

of stress (Candan and Tarhan, 2003). Air pollution significantly increased membrane peroxidation in *Lavandula officinalis* Chaix and *Ligustrum vulgare* L. grown in the sidewalks in polluted and non-polluted areas of Tehran, Iran (Ghorbanli et al., 2009). Leaves of the species *Brachylaena discolor* grown in the vicinity of polluted industrial areas showed higher electrolyte leakage than control (Areington et al., 2015). This is similar to our finding for *A. pseudoplatanus*, which had the highest electrolyte leakage.

Conclusions

Air pollutants have become a key problem in most cities of Iran. Its severity has risen with the development of urbanisation and industrialisation and the growing expansion of innovation. As a result, examining the impacts of pollutants on plants can enhance our knowledge of species sensitivity to pollution. Vegetation cover, resistant trees, and the management of green space in urban parks can help to curb the effects of these pollutants. It is concluded that the species *Z. carpinifolia* can act as a suitable indicator for the green spaces of Rasht because its dry weight, turgor weight, chlorophyll *a* content, chlorophyll *a/b* ratio, and carotenoid content were found to increase in polluted areas. This paper can provide officials and researchers an insightful study that are already conducted on the effect of air pollution on plant species in urban parks of Rasht. This will help them carry out better research in the future for developing an effective and novel strategy for plant species selection for green spaces. The current study concludes that every plant species responds to air pollutants differently. Some plant responses can be regarded as attempts to adapt to pollution stress while others resist adverse environmental conditions. It is recommended to focus on supplementary experiments in future by studying on physiological responses under urban condition in seasonal ornamental plants including landscape seasonal plants, pot flowers, foliage plants and grasses.

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