Albanian j. agric. sci. 2020;19 (1): 21-28   *Agricultural University of Tirana*

RESEARCH ARTICLE   **(Open Access)**

Effects of Air Pollution on Stomatal Responses, Including Paleoatmospheric CO_2 Concentration, in Leaves of *Hedera helix*

ILVA ALUSHI[1*], XHESIKA VEIZI[1]

[1]Biology Department, Faculty of Technical Sciences, University of "Ismail Qemali" Vlore, Albania

Abstract

Stomata, small holes in the epidermal surface of the leaves, are important organs in plant phylogenetic relationship studies, responsible for exchanging CO_2 and transpiration. They are easily affected by external environmental conditions. Leaf stomata in different plants, from generation to generation, possess significantly different characteristics such as density, size, and shape. Consider that, bivariate correlation and descriptive statistics was performed in this study, aming to detect significant differences for stomatal density (stomata per mm^2) and stomatal morphologic parameters obtained from plants growing in ambient and elevated CO_2 treatments. We have chosen *Hedera helix* plant to examine stomatal density (stomata per mm^2), long axis length (length), and short axis length (width), evaluating at once even the status of environment in different area in Vlora city, Albania. The plant material (leaves) was collected in urban and rural populations growing in a free pollution environment near the village of Drashovic, Vlore and in urban agglomeration in Vlora city. In terms of the rensponses of stomata width and density to CO_2 enrichment, we noticed asignificant positive correlation and decrease of those parameters. Whereas CO_2 elevation does not affect the length of stomata in our research. Given that the increase of CO_2 concentration in atmosphere is associated with a decrease in Stomata Density, we estimated that rural environment is less polluted than urban one.These results indicate that simulated climate change influences leaves structure and function somehow.

Keywords: *Hedera helix*; CO_2; stomata density, stomata morphological parameters.

1. Introduction

Morphological characters of plants are good indicators of habitat quality, since they poses vary depending on microclimatic conditions [4]. Both stomata cells (the tiny plant structures on the epidermis which form the main system that controls photosynthesis and respiration) and stomatal index and morphological parameters (stomatal density, stomatal apparatus and guard cell architecture) respond to environmental and physiological cues [18,11].

Leaves need a way to easily exchange gasses with the plants environment. Stomata are also responsible for transpiration causing pressure that allows the roots to absorb water in the process of water and nutrients uptake.

Stomata pores open and close depending on different environmental conditions, in association with light availability and osmotic changes, allowing CO2/ O2 exchange during photosynthesis to enter the plant for photosynthesis.

Most stomata on a leaf are concentrated on the dorsal part, although some can be found on the ventral surface of the leaf of some species. Some plants' stomatal density changes from generation to generation depending on the concentration of carbon dioxide in the environment [21] - showing phenotypic plasticity, other species only change over many generations due to long term changes in carbon dioxide concentration, and other species stomatal densities do not show a change at all. These changes in stomatal density have proved to be very important to scientists interested in climate change as changes in transpiration due to less overall numbers of stomata have to be included into any model with the purpose of predicting future climate condition.

Some ecological factors such as light, water, humidity, CO_2 concentration can effect directly or indirectly, in or outside the stomata depending on the environment of the plant occurrence [2, 3].

Stomatal density responses to concentrations of atmospheric CO_2, have been documented for 109 vascular plant species, with 60 % of these species showing a reduction in density with increasing CO_2concetration [5]. Based on above results, we have done estimation about the less or the more polluted

*Corresponding author: Ilva Alushi; E-mail: ilva.alushi@yahoo.com
(Accepted for publication April 27, 2020)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

area, among our study areas in Vlora city using leaves of *Hedera helix*, thus estimating variations of Stomata Density and stomata morphological parameters. The plant material (leaves) was collected in urban and rural populations growing in a free pollution environment near the village of Drashovic, Vlore and in an urban agglomeration in Vlora city, as a way to compare them in terms of air pollution.

2. Material and Methods

This study was carried out on August 2018, in Vlora city, Albania. The plant that we have chosen was *Hedera helix.* Several factors are known to control stomatal conductance, such as light, soil water potential, internal CO_2 concentration as well as sink strength in trees [7].

This study aimed to determine the variations of Stomatal Density of *Hedera helix* in different areas in Vlora city based on ecological factors such as carbon dioxide (CO_2), temperature, humidity and light level (illumination), which directly or indirectly affect the number and size of the stomata. Based on above factors, we have done estimation about the less or the more polluted area, among our study areas [13, 6].

The leaves of plants were collected collected from four different populations of *Hedera helix* in both urban and rural populations growing in a free pollution environment near the village of Drashovic, Vlore and in an urban agglomeration in Vlora city (Figure 1) and we labeled them clearly.

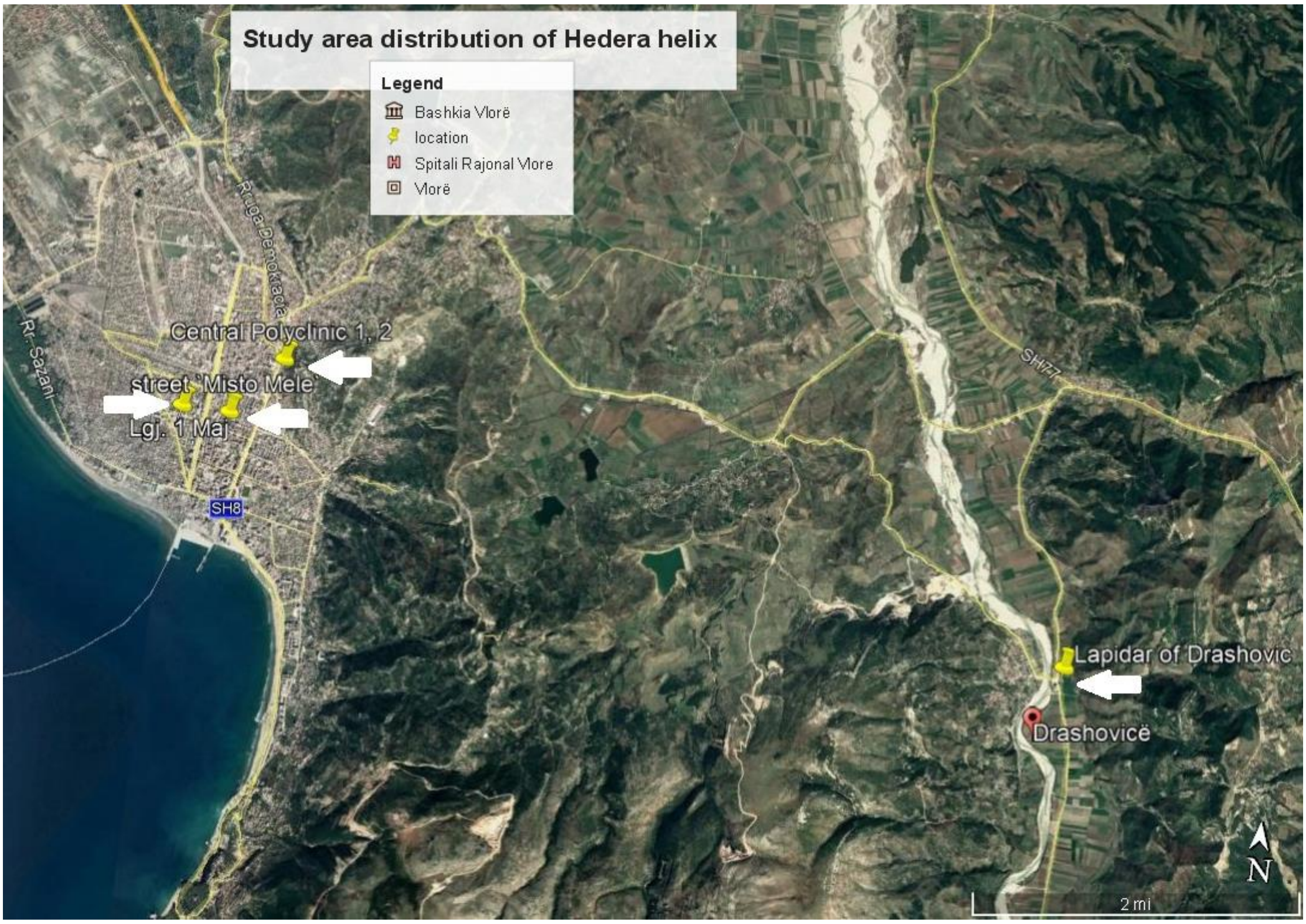


Figure 1. The map of our study areas distribution

During the sampling, we have used WIKILOC (Outdoor Navigation GPS) software to determine the exact locations and data such as: location, date, time, geographical coordinates (latitude and longitude) and altitude. We attempted to keep differences in factors during the environment work that might influence stomatal density to a minimum, such as general temperature, humidity, daily atmospheric pressure and the rate of daily UV radiation, by using the program AccuWeather (local & global weather maps).

The chosen trees were located 5-10 meters from a roadway. For each plant of the site under consideration, four leaves were taken from the bottom layer of each tree crown (1/3 of crown length), two of which were in the shadow exposition (north) and the other two facing sunny exposition (south).

H. helix is hypostomatous plant, therefore for stomata counting we painted the epidermis layer of the abaxial side of leaf with clear fingernail polish, and a piece of transparency sheet (2 cm^2) was pressed on it by fingers. Later, the imprint of stomata was obtained by striping the transparency sheet from the leaves [22]. Three imprints were taken from dorsal side of the main vein of a leaf. In each strips, five microscopic fields were analyzed and a maximum of 10 stomata were analyzed for each field, measuring stomata's lengths and widths by using a light microscope [13]. We have also calculated stomatal density (Stomatal density = number of stomata in entire FOV / area (mm^2)).

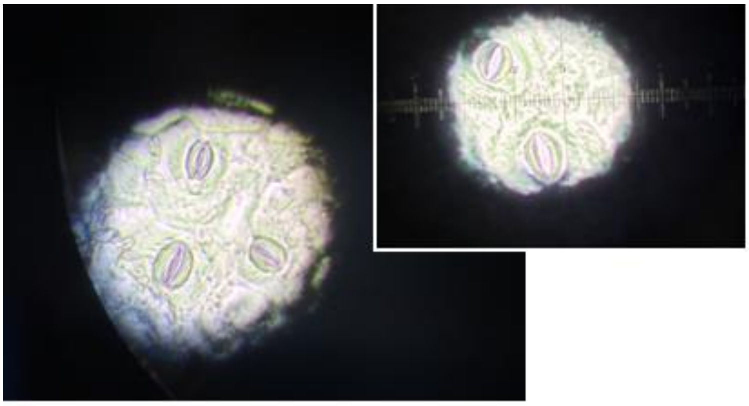


Figure 2. Leaf epidermis stomatal morphology of Hedera helix

In total we have analyzed: 4 (locations) x 4 (leaves analyzed per each location) x 3 (strips at the dorsal part of a leaf) x 5 (Fields observed for each sheet) x 10 (stomata for each field) = 2400 stomata analyzed for width and length variables.

Microsoft Office Excel, IBM SPSS statistics was perfomed to estimate the impact of carbon dioxide`s concentration in stomatal density and morphologic parameters using bivariate correlation and by comparing means using descriptive statistics.

3. Results and Discussion

All data analyzed in this study were sufficient and fully measurable, because the minimum number of leaves to be analyzed is ten, whereas we have analyzed more than ten. We have estimated two stomata factors that indicate environmental pollution. Stomata morphological parameters (stomata width and length) and Stomata Density. The results of our study are presented below:

The stomata width increases at high light intensities compared to those positioned in the shade, resulting in an increase of the CO_2 concentration in leaves (table 1). However, the stomata parameters are affected beside of CO_2, even by other abiotic factors such as temperature, humidity and wind (wind speed) [2]. During the sampling, it was also obtained wind speed parameter, where the maximum speed was 4.1 m/ s, which is almost negligible. At a low or negligible wind speed the stomata always stay open, thus having the highest width. On the other hand, the increase in wind velocity increases the rate of transpiration but very high wind velocity decreases the rate of transpiration because it leads to stomata closure due to mechanical effect, drying and cooling of the transpiring organs.

http://www.biologydiscussion.com/transpiration/factors-affecting-transpiration-10-factors/70625

Based on the positive correlation, observed between the stomata width and Stomatal Density with respect to the light-shade exposition, we conclude that the increase of Stomatal Density is associated with an increase of stomata width and vice versa, but exposition does not effect our stomata length.

We have analyzed stomata morphological parameters in our hypostomatous plant by Descriptive statistics (IBM SPSS program) (table 3), showing that the highest average of stomata width was found near the central Vlora Polyclinic (15,3 μm) and Lapidari Drashovic (14.9 μm), while the lowest one was along "Misto Mele" Street (13,8 μm).

Furthermore, given that high concentrations of SO_2, CO and CO_2 cause a decrease in Stomata Density and width, [21], and according to Table 1 and table 2, there is a significant positive correlation between stomata width and Density. CO_2 elevation does not affect the length of stomata in our research. We found that the highest number of stomata (213,7 /mm^2) was near Lapidar of Drashovica. While along MistoMele Street were lower stomata frequencies between 179/mm2 (table 3). We can conclude that the most problematic area in terms of pollution is the area near "Misto Mele" Street and Lagjia "1 Maji". The less polluted, is the area of central Polyclinic and near Drashovica`s Lapidar, since the values of Stomatal Density and width appear to be higher.

Table 1. Descriptive Statistical analysis of *Hedera helix* associated with light-shadow position

Position		**Stomata width (µm)**	**Stomata density (no mm^2)**
Shadow	Mean	14,4850	192,90780
	N	150	30
	Minimum	8,25	159,574
	Maximum	19,00	228,723
Light	Mean	14,6950	197,87234
	N	150	30
	Minimum	11,50	154,255
	Maximum	20,25	260,638

Table 2. Correlations between stomata width and density of *Hedera helix*.

Correlation between various characteristic paramters			
		Stomata width (µm)	**Stomata density (no mm^2)**
Stomata width (µm)	Pearson Correlation	1	,277*
	Sig. (2-tailed)		,032
	N	300	60
Stomata density (no mm^2)	Pearson Correlation	,277*	1
	Sig. (2-tailed)	,032	
	N	60	60

*Correlation is significant at the 0.5 level (2-tailed)

CO_2 may be an important factor that has contributed to the reduction of Stomata Densities, including high concentrations of SO_2 and CO, as chemical compounds that are produced by the burning of fossil fuels from motor vehicles [21]. In terms of the rensponses of stomatal density of different areas of leaves, they had a decreased stomata density and stomata width in response to CO_2 enrichment [20].

In 1987, F. I. Woodward [27] published a study in Nature that revealed a decrease of stomatal density in eight species of plants that correlated with the 60 µmol/mol increase of carbon dioxide in the atmosphere over the previous 200 years—most of this increase is accounted for by the industrial revolution. The fact that Drashovica area is less polluted by these pollutants treated above, may be due to the lack of urbanization, thus less emission of fossil fuels.

To find out if exposure to the urban environment compared with rural one, affected functional leaf properties, we determined stomatal density (Table 3). Given that the increase of CO_2 concentration in atmosphere is associated with a decrease in Stomata

Density [27] and since the values of Stomatal Density appeared to be higher for the plant grown in the area near Drashovica`s Lapidar, we can easily estimate that the rural environment is less polluted than urban one may be due to the lack of urbanization, thus less emission of fossil fuels. Similarly, significant growth reductions have also been reported for leaves from other tree species in heavily polluted city areas (*Platanus acerifolia, Ficus bengalensis, Guaiacum officinale* and *Eucalyptus* sp., [19, 14]). Consider our results, we estimate that the high pollution shown in the laboratory tests in these areas is due to the high air pollution from the fossils, harmful gases emitted by vehicles, urbanization and aggravated infrastructure which are occasionally repaired.

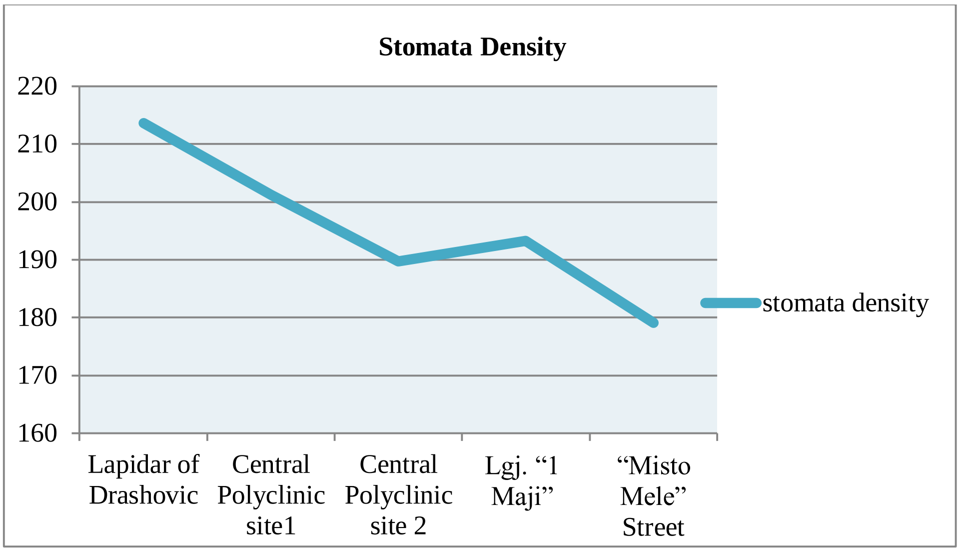
Negative effects of air pollutants on stomata densities and opening have also been found in other species such as *Trifolium repens, Trifolium pratense, Cicer arietenum, Acer saccharum* and *Ipomea pes-tigridis* growing in polluted areas [23,9,10]. A reduction of stomata density is also found in response to elevated CO_2 concentrations, frequently present in city centres [25, 26]. The reduction in stomata densities and their pore size may be important for controlling absorption of pollutants [25] but will limit photosynthesis at the same time.

Consider our results, we estimate that the high pollution shown in the laboratory tests in these areas may be due to the high air pollution from the fossils, harmful gases emitted by vehicles, urbanization and aggravated infrastructure which are occasionally repaired in our study areas. The reduction in stomata densities and their pore size may be important for controlling absorption of pollutants [25], but will limit photosynthesis at the same time.

Table 3. Descriptive Statistical analysis of *Hedera helix* with respect to locations.

Hedera helix		**Stomata width**	**Stomata length**	**Stomata density**
Lapidar of Drashovica	Mean	14,9250	20,8042	213,65248
	N	60	60	12
	Minimum	13,25	18,25	170,213
	Maximum	16,75	24,25	260,638
Central polyclinic site 1	Mean	15,3542	23,3375	189,71631
	N	60	60	12
	Minimum	11,50	18,50	154,255
	Maximum	19,00	26,50	218,085
Central polyclinic site 2	Mean	14,7583	22,1417	201,24113
	N	60	60	12
	Minimum	12,25	17,75	159,574
	Maximum	20,25	25,25	239,362
"Misto Mele" street	Mean	13,8417	22,3667	179,07801
	N	60	60	12
	Minimum	11,25	18,75	159,574
	Maximum	16,25	26,50	202,128
Lgj. '1 Maji"	Mean	14,0708	24,6958	193,26241
	N	60	60	12
	Minimum	8,25	15,75	170,213
	Maximum	18,25	29,50	228,723

Figure 3. Stomata density of *Hedera helix* plants in an urban compared with a rural site



In conclusion, our study shows that urban conditions affected structural leaf properties, which may lead to lower photosynthesis through lower leaf area, lower stomatal densities and pore widths and probably higher sensitivity to drought because of a thin cuticle. However, mainly leaf surface features, but not internal functional anatomy, were affected. These results support that trees can cope with pollution [8] and therefore, are suitable roadside plants for megacities. The observation that leaves in urban environments were strongly covered by dust particles suggests that plane may contribute significantly to improving air quality [24]. To determine the extent of air filtration and tolerance to traffic exhaust, controlled studies have to be performed in future.

4. Conclusions

- The stomata width increases at high light intensities compared to those positioned in the shadow resulting in an increase of the CO_2 concentration in leaves. However, the stomata parameters are affected beside of CO_2, even by other abiotic factors such as temperature, humidity and wind (wind speed) [2].
- Consider that the increase of CO_2 concentration in atmosphere is associated with a decrease in Stomatal Density [21], given that there was a significant positive correlation between stomata width and Density, we found that the lowest number of stomata was along "Misto Mele" Street.We can conclude that the most problematic area in terms of pollution is the area near "Misto Mele" Street and Lgj. "1 Maji".
- CO_2 elevation and other pollutants does not affect the length of stomata in our research.
- From the above results, the less polluted locality is the area near Drashovica`s Lapidar, since the values of Stomatal Density and width appear to be higher. The fact that Drashovica area is less polluted by these pollutants treated above, may be due to the lack of urbanization, thus less emission of fossil fuels.

6. References

1. http://www.biologydiscussion.com/transpiration/factors-affecting-transpiration-10-factors/70625
2. Aphalo Pedro J. And Sanchez Rodolfo A. **Stomatal Responses to Light and Drought Stress in Variegated Leaves of Hedera helix'**. Plant Physiol. 1986. 81, 768-773
3. Apple M.E., Olszyk D.M., Ormrod D.P., Lewis J., Southworth D., Tingey D.T. **Morphology and stomatal function of Douglas ®r needles exposed to climate change: elevated CO2 and temperature**. Int. J. Plant Sci. 2000. 161, 127±132.
4. Barber, J. L., Thomas G. O., Kerstiens G., and K. C. Jones. **Current issues and uncertainties in the measurement and modelling of air-vegetation exchange and within-plant processing of POPs**. Environmental Pollution 2004, 128, 99-138.
5. Beerling D. J., Heath J., Woodward F. I., Mansfield T. A. **Drought-CO2 interactions**

in trees observations and mechanisms. New Phytol. 1996, 134 235–242.

6. Batos B., Vilotić D., Orlović S. and Miljković D. **Inter And Intra-Population Variation Of Leaf Stomatal Traits Of Quercus Robur L. In Northern Serbia.** Arch. Biol. Sci., Belgrade, 2010. 62 (4), 1125-1136, Doi:10.2298/Abs1004125b
7. Chwil S., Kozłowska-Strawska J., Tkaczyk P., Chwil P., Matraszek R. **Assessment of air pollutants in an urban agglomeration in Poland made by the biomonitoring of trees**. J. Elem. 2015, 20(4): 813-826. DOI: 10.5601/ jelem.2015.20.1.742
8. Dickinson NM., Turner AP., Lepp NW. Part III. **Survival of trees in a metal-contaminated environment.** Water Air Soil Pollut. 1991;57–58:627–633. doi: 10.1007/BF00282926.
9. Garg KK., Varshney CK. **Effect of air pollution on the leaf epidermis at the sub microscopic level.**Experientia. 1980;36:1364–1366. doi: 10.1007/BF01960100.
10. Ghouse AKM., Zaidi H., Attique A**. Effect of air pollution on the foliar organs of Callistemon citrinus Stapf**. J Sci Res. 1980;2:207–209.
11. Gitz D. C., Bakern J. T. **Methods for creating stomatal impressions directly onto archivable slides.** Agronomy Journal. 2009. 101, 232-236.
12. Hong Tao, Lin Han, He Dongjin: **Characteristics and correlations of leaf stomata in different Aleurites montana provenances**.https://doi.org/10.1371/journal.pone.0208899. 2018.
13. Ilgin Muruvvet and CaglarSemih. **Comparison of leaf stomatal features in some local and foreign apricot (Prunus armeniaca L.) genotypes**. African Journal of Biotechnology Vol. 8 (6), pp. 1074-1077, ISSN 1684–5315 © 2009 Academic Journals
14. Jahan S., Iqbal MZ. **Morphological and anatomical studies of leaves of different plants affected by motor vehicles exhaust**. J Islamic Acad Sci. 1992;5:21–23.
15. Lammertsma E. I., de Boer H. J., Dekker S. C., Dilcher D. L., Lotter A. F., Wagner-Cremer F. **Global CO2 rise leads to reduced maximum stomatal conductance in Florida vegetation. Proceedings of the National Academy of Sciences,** 2011; DOI: 10.1073/pnas.1100371108
16. Lin Jinxing, Jach M. E. and Ceulemans R. **Stomatal density and needle anatomy of Scots pine (Pinus sylvestris) are affected by elevated CO2**. New Phytologist 2001; 150: 665–674
17. Mott Keith A. & Peak David. **Stomatal responses to humidity and temperature in darkness.** Plant, Cell and Environment 33, 2010; 1084–1090 doi: 10.1111/j.1365-3040.2010.02129.x
18. Nadeau J. A., and Sack F. D. **Control of stomatal distribution on the Arabidopsis leaf surface.** Science 2002. 296, 1697-1700.
19. Ninova DJ., Dushkova PI., Kovacheva CV. **Anatomical, morphological studies of Platanus acerifolia at various degrees of air pollution.** Ekologiya (Sofia) 1983;6:35–47.
20. Pourkhabbaz Alireza, Rastin Nayerah, Olbrich Andrea, Langenfeld-Heyser Rosemarie, and Polle Andrea. **Influence of Environmental Pollution on Leaf Properties of Urban Plane Trees, Platanus orientalis L. Bull Environ Contam Toxicol.** 2010 Sep; 85(3): 251–255.
21. Royer D.L. **Stomatal density and stomatal index as indicators of paleoatmospheric CO2 concentration**. Review of Palaeobotany and Palynology, 2001, 114 1±289
22. Schechter I., Proctor JTA., Elfving DC. **Morphological difference among apple leaf types.** Hortscience1992, 27: 101-103.
23. Sharma GK, Butler J. **Leaf cuticular variations in Trifolium repens L. as indicators of environmental pollution**. Environ Pollut. 1973;5:287–293.
24. Smith WH, Staskawicz BJ. **Removal of atmospheric particles by leaves and twigs of urban trees: some preliminary observations and assessment of research needs**. Environ Manag. 1977;1:317–330. doi: 10.1007/BF01865859
25. Verma RB, Mahmooduzzafar, Siddiqui TO, Iqbal M. **Foliar response of Ipomea pes-tigridis L. to coal-smoke pollution.** Turkish J Bot. 2006;30:413–417
26. Williams WE, Garbutt K, Bazzaz FA, Vitousek PM. **The response of plants to elevated CO2. IV. Two deciduous- forest**

tree communities. Oecologia.1986;69:454–459.

27. Woodward F.I. **Stomatal numbers are Sensitive to increases in CO2 from Pre-Industrial levels**. Nature, 1987; 327, 617-618. http://dx.doi.org/10.1038/327617a0.