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Original paper

Stomatal apparatus response of *Tilia cordata* (Mill.) and *Betula pendula* (Roth.) to air quality conditions in the City of Banja Luka (Bosnia and Herzegovina)

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Summary. Comparative analysis regarding micro-anatomic stomatal apparatus parameters of small-leaved linden (*Tilia cordata* Mill.) and birch (*Betula pendula* L.) from the wider area of Banja Luka (entity of Republic of Srpska, Bosnia and Herzegovina) in the course of two seasons was performed in this study. Sampling of selected plant species was made on trees which were located next to the road (city centre) and on trees located in the suburban forest estate of „Velika Gozna", near the city. One of the objectives of this study was to examine the impact of particular air pollutants on stomatal apparatus characteristics (stomata number, size of stomatal apparatus and pore). The obtained results indicate significant differences in the stomatal apparatus characteristics between *Tilia cordata* (Mill.) and *Betula pendula* (Roth.) as well as with respect to the origin of the examined material. Changes in stomata number and stomatal apparatus size in the examined species can serve as an indicator of their resistance to air pollution in urban areas.

Keywords: air pollution, *Betula pendula*, stomata, *Tilia cordata*.

INTRODUCTION

Air pollution is increasingly becoming a global problem due to intensive industrialization and population growth. In the majority of urban areas exhaust gases from motor vehicles constitute 57-75% of total emissions (WHO 2006). In addition, many other particles that can cause disturbances of ecological homeostasis or adverse effects in humans, animals and plants are dispersed into the atmosphere by mechanical disintegration of solid substances (Somers et al. 2004; Ghorbanli et al. 2007; Lindsay et al. 2010). In past few decades many scientific studies have reported the harmful effects of different air pollutants on plants, with a special focus on the effect of sulphur and nitrogen oxides, as well as ozone (Velissariou et al. 1992; Dimitrova and Yurukova 2005; Kosiba 2008). The majority of sulphur and nitrogen oxides reach plant leaves through stomata, thereby causing damage to the photosynthetic apparatus and photosynthesis inhibition (Miszalski and Mydlarz 1990; Jablanović et al. 2003). A particular manifestation of the harmful effect of air pollutants is expressed in their interaction with other environmental factors, such as increased air humidity, intense illumination or increased CO_2 concentration (Mudd and Kozlowski 1975; Pearson and Mansfield 1993; Govindaraju et al. 2010). Increased sulphur oxides concentrations cause stomatal closure through complex signalling pathways, where Ca^{2+} ions have the leading role in guard cells (McAnish et al. 2002). Stomata closing are considered to be an adaptive strategy in plant defence mechanisms, although such conditions decrease the rate of photosynthesis and total yield (Duranni et al. 2004). Therefore, the synergistic effect of SO_2 with increased concentration of CO_2 is considered to be the most harmful, leading to inhibition of stomatal closing (Majernik and Mansfield 1972). Tropospheric ozone is believed to be a very toxic pollutant for plants, also known as "photochemical smog", generated from primary atmospheric pollutants, nitrogen oxides and volatile hydrocarbons, especially under intensive luminosity conditions. It is known that ozone concentrations in urban areas are the highest in the afternoon hours during traffic jams and higher insolation (Kley 1997). Increased ozone concentrations lead to the oxidative damage of biomol-

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ecules that are most prominent in epidermal cells (Musselman and Massman 1999; Jaffe et al. 2008). The majority of plant species increase antioxidant metabolites in response to the harmful effects of ozone, and use stomatal closing as a primary reaction to ozone stress (Moldau et al. 2011).

Regular monitoring of atmospheric pollutants in urban areas is a standard procedure for controlling environment conditions, whereby chemical analysis provides exact data on the concentrations of particular gases and particles. Recently, air quality biological monitoring methods have seen increasing use, possibly because they provide valuable data on the interaction of chemical pollutants with living systems. Originally, air quality biomonitoring primarily involved studying the ecophysiological characteristics of lichens and mosses, which are traditionally considered indicators of clean air (Nash and Gris 2002; Govindapyari et al. 2010). However, in centres of large urban areas, lichens and mosses use is often not possible, since such areas are usually characterized by a complete absence of these organisms. Therefore, studies of deciduous and coniferous woody plant species have become increasingly important in air quality monitoring in urban areas for all biological aspects, including plant biochemistry, physiology and morphology (Hagen-Thorn et al. 2004; Tretyakova and Noskova 2004; Yilmaz and Zengin 2004).

Air quality biomonitoring in the present study includes analysis of two plant species: birch (*Betula pendula* Roth.), referred to as species sensitive to air pollutants according to literature data; and a small-leaved linden (*Tilia cordata* Mill.), as a more resistant species to this type of pollution (Wright 1988; Kosiba 2008). The objective of the present study was to determine the differences in stomata number and stomatal apparatus size between these selected plant species (*Tilia cordata* and *Betula pendula*) sampled in an urban area of the City of Banja Luka and a suburban forest estate over two seasons.

MATERIAL AND METHODS

Leaf sampling was conducted in two seasons (spring and summer), and was performed in the main streets of the city centre in Banja Luka (Mladena Stojanovića, Kralja Petra I Karađorđevića, Vuka Karadžića and Aleja Svetog Save) (Fig. 1). Leaves from individual trees of the urban area were separated into two groups: samples from the crown parts that were not directly exposed to roads were marked as UA 1 (urban area 1) and samples from the other side of the crown which were positioned directly toward the street were marked as UA 2 (urban area 2). All trees were chosen directly next to the street, and were not blocked by other facilities or trees. As control samples, tree leaves were also collected from a suburban area, about 30 km from the city centre in the forest estate "Velika Gozna". For each plant group sampling was performed from three trees: leaves from three different points (base, middle and top part) were taken, and three leaves were taken for each point. All samples were packed in dark plastic bags, marked, transported to the laboratory and forwarded to processing preparation the same day. Determination of stomata number was made by the method of Petrović and Štrbac (1996). Considering that stomata are not equally distributed throughout the leaf plate, and that the examined species have hypostomatic leaves, their prints were taken from the abaxial epidermis, namely from three parts (top, middle and base). Samples were analysed using a Leica DMLB microscope, whereby the following parameters were taken into consideration: stomata number/ mm^2, length and width of stomatal apparatus and stomatal pore, respectively. All parameters were analysed in ten replicates per preparation and results were presented as mean values of three trees. Statistical data processing was performed in the program MAPLE 18 by analysis of variance (one-way ANOVA) with a statistical significance of 5% ($p < 0.05$). Data presented in Table 1 represent average monthly values for meteorological conditions in May and August 2014 (RS Hydrometeorological Institute) and average monthly values for air quality (Department of Public Utilities, Housing and Traffic) of the City of Banja Luka.

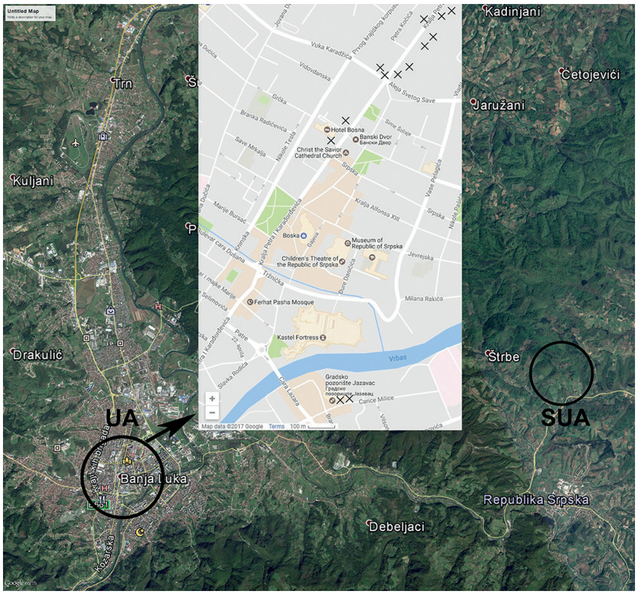


Fig. 1. Map of the City of Banja Luka with marked sampling areas: UA- Urban area in the city centre with magnified sampling points (X) close to the busiest streets; SUA- suburban area located about 30 km from the city centre (source https://www.google.ba/maps).

RESULTS

The obtained results show that *B. pendula* leaves taken from urban areas have statistically significant lower stomata number in comparison to those from suburban areas. The same trend of stomata number decrease with *B. pendula* was noticed during both seasons (Fig. 2). Also, a statistically

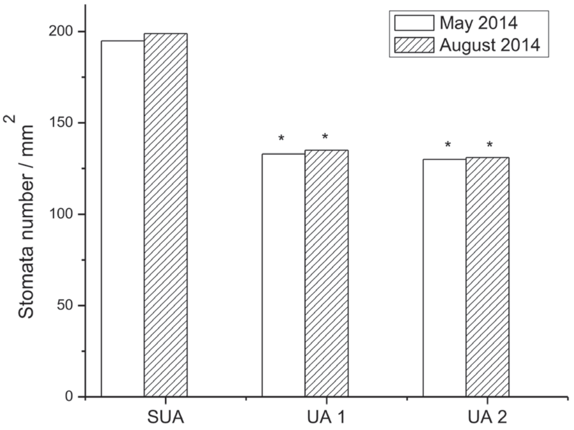


Fig. 2. Stomata number/mm^2 in the leaves of *Betula pendula* sampled in May and August, 2014. SUA- control samples collected in suburban area; UA 1- samples collected in urban area from crown parts not directly exposed to roads; UA 2- samples collected in urban area from crown parts directly exposed to roads; Control samples (SUA) were compared with samples of urban trees (UA 1 and UA 2) by statistical data processing, * indicates statistically significant difference.

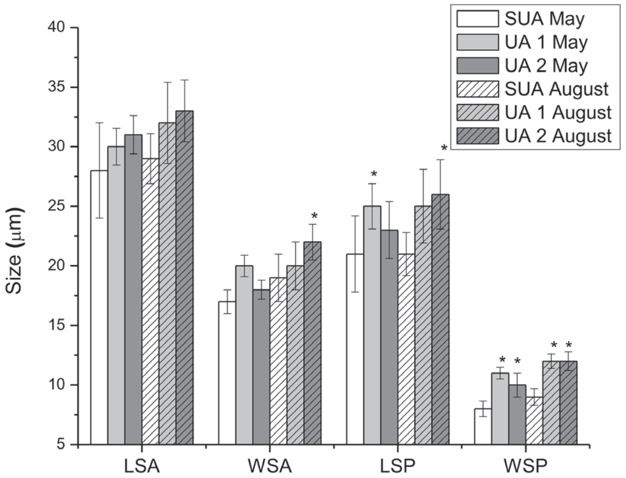


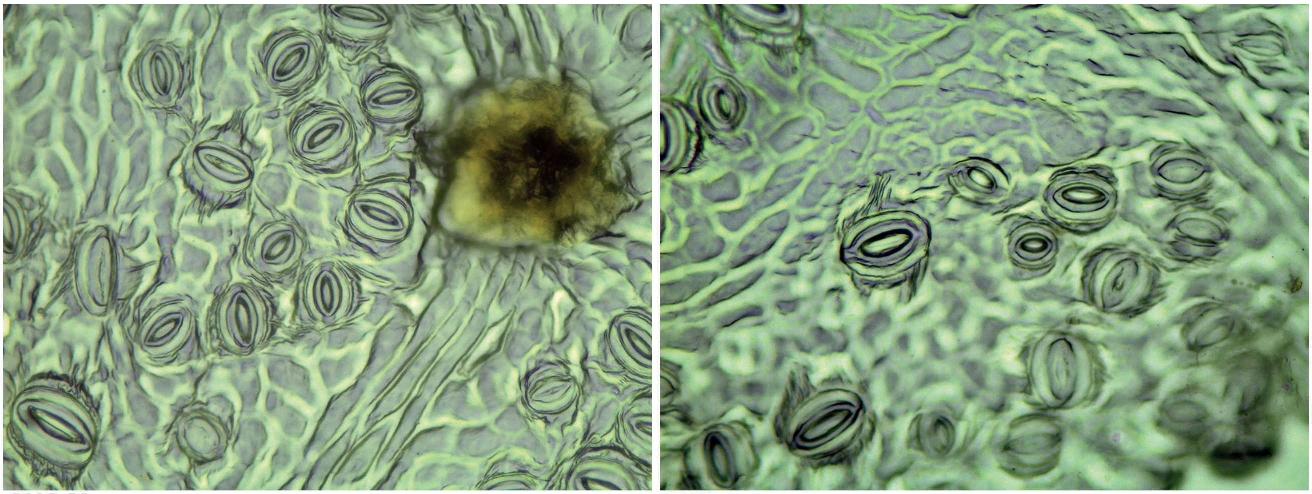
Fig. 3. The size of stomatal apparatus and pore in *Betula pendula* leaves sampled in May and August, 2014. SUA- control samples collected in suburban area; UA 1- samples collected in urban area from crown parts not directly exposed to roads; UA 2- samples collected in urban area from crown parts directly exposed to roads; LSA- stomatal apparatus length; WSA- stomatal apparatus width; LSP- stomatal pore length; WSP- stomatal pore width; Control samples (SUA) were compared with samples of urban trees (UA 1 and UA 2) by statistical data processing, * indicates statistically significant difference.

significant difference was found in stomatal pore length between urban and suburban birch leaves in both seasons (Fig. 3). In addition, stomatal pores in birch leaves sampled in the urban area from both group (UA 1 and UA 2) were wider in comparison with controls during both seasons (Fig. 3). However, statistically significant differences were not found in stomatal apparatus length between urban and suburban birch leaves during both seasons (Fig. 3). On the other hand, during summer, a statistically significant difference was noticed in stomatal apparatus width between control leaves and leaves directly exposed to the road in an urban area (samples UA 2). No significant difference was observed in stomata number between leaves from different crown parts taken in the urban area (samples UA 1 and UA 2). Stomata morphology of *B. pendula* leaves from urban and suburban trees are presented in Figure 4.

The obtained results show smaller changes in the analysed parameters of stomata in *T. cordata* leaves in comparison with *B. pendula*. Namely, for both seasons a smaller stomata number was observed in the leaves of city trees versus control leaves, but this difference was not statistically significant (Fig. 5). Likewise, during the summer none of the analysed parameters for stomatal apparatus size or pore changed significantly in urban area samples in comparison with controls (Fig. 6). However, during May the situation was somewhat different, since longer and wider stomatal pores were noticed in the leaves which were directly exposed to roads in the urban area in comparison with control, and these differences were statistically significant (Fig. 6). Stomata morphology of *T. cordata* leaves from city and suburban trees is presented in Figure 7.

DISCUSSION

Although contradictory results in the literature may be found regarding the effect of air pollutants on stomata number (Gostin and Ivanescu 2007; Kardel et al. 2010), numerous authors believe that morphological and stomatal characteristics of the leaf are reliable indicator of air quality (Kurteva 2008; Balasooriya et al. 2009). Numerous reports confirm that stomata number changes depending on the degree of air pollution by different types of pollutants, primarily sulphur and nitrogen oxides and ozone (Zelitch 1961; Mulgrew and Williams 2000; Shweta 2012). Stomatal response to air pollution is very complex and depends not only on air pollutant concentration and type, but is also very selective depending on plant species (Abeyratne and Ileperuma 2006). In addition, the interaction of plants and chemical pollutants often varies if synergistic effects of several different pollutants is present. With respect to *Tilia cordata* some authors believe that this species is less sensitive to gaseous pollutants and that it is more susceptible to the harmful effects of floating cement particles and heavy metals (Antipov 1979; Kosiba 2007). According to chemical analysis results of gaseous atmospheric pollutants in the City of Banja Luka during May and August 2014, the air was not first class quality in terms of nitrogen oxide and ozone concentrations (Table 1). On the other hand, analysed parameters for *T. cordata* leaf stomata



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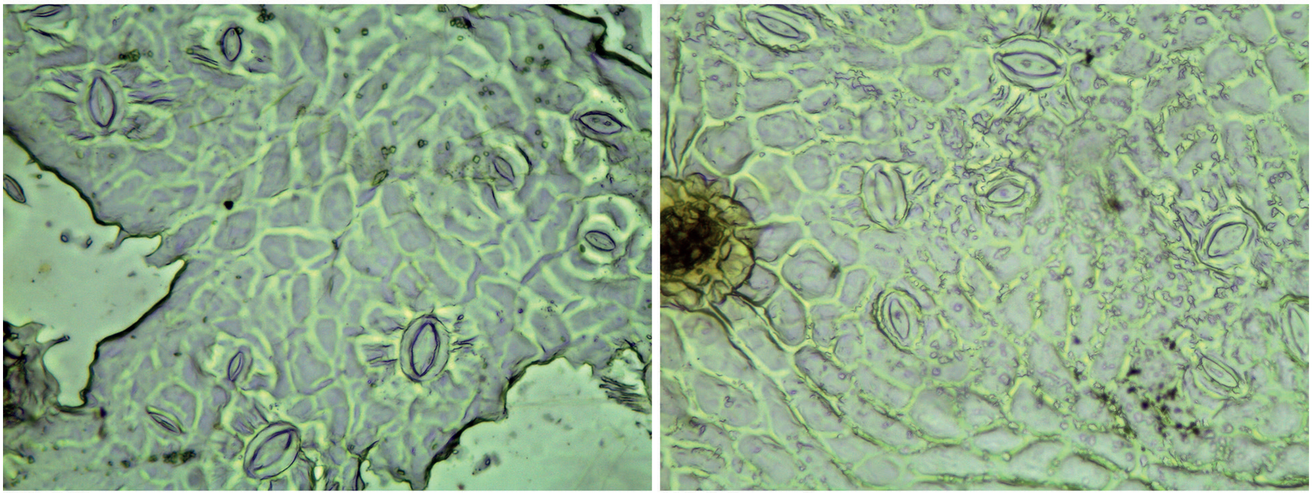

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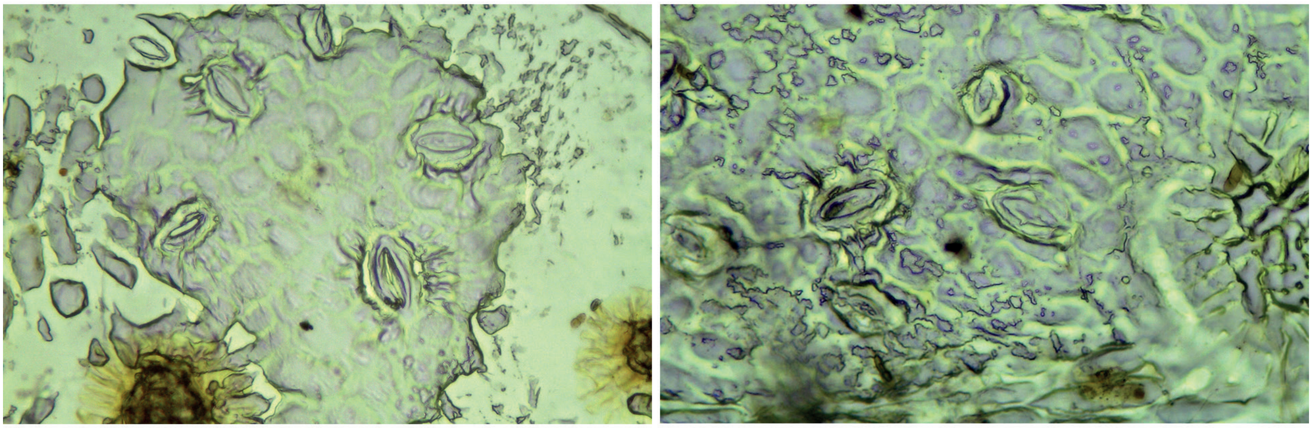

UA 2 May

UA 2 August

Fig. 4. Stomata on abaxial epidermis of *Betula pendula* leaves from city and suburban trees sampled in May and August, 2014. SUA- control samples collected in suburban area; UA 1- samples collected in urban area from crown parts not directly exposed to roads; UA 2- samples collected in urban area from crown parts directly exposed to roads.

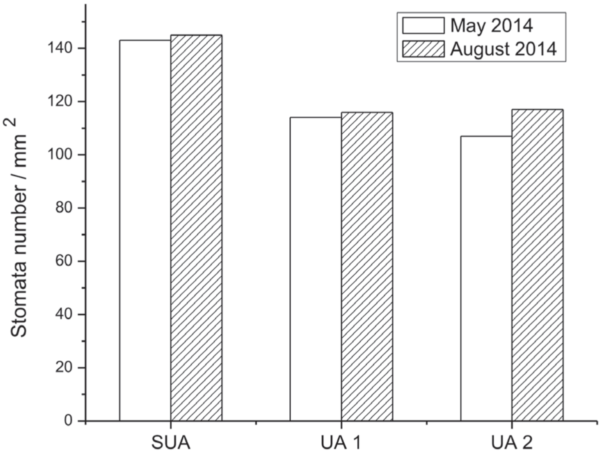

Fig. 5. Stomata number/ mm^2 in the leaves of *Tilia cordata* sampled in May and August, 2014. SUA- control samples collected in suburban area; UA 1- samples collected in urban area from crown parts not directly exposed to roads; UA 2- samples collected in urban area from crown parts directly exposed to roads; Control samples (SUA) were compared with samples of urban trees (UA 1 and UA 2) by statistical data processing, * indicates statistically significant difference.

Table 1. Average monthly values for meteorological parameters and gaseous atmospheric pollutant concentrations measured during May and August, 2014 at the location City of Banja Luka.

Season	May	August
Temperature	25 °C	27 °C
Wind	East, 7 km/h	Northeast, 2.9 km/h
Air humidity	38%	87%
UV index	2	6.4
CO_2 (mg/m^3)	0.23*	0.33*
SO_2 (µg/m^3)	17.17*	14.49*
O_3 (µg/m^3)	38.66	48.51
NO_2 (µg/m^3)	31.91*	31.96*
NO (µg/m^3)	13.97	13.71
NO_x (µg/m^3)	45.88	45.67
Char	12.80	13.26

** indicates that air quality is in the first class quality category in comparison with the given parameter.*

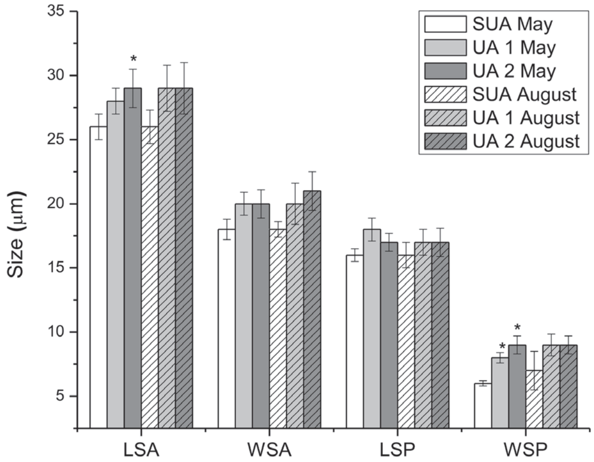

Fig. 6. The size of stomatal apparatus and pore in *Tilia cordata* leaves sampled in May and August, 2014. SUA- control samples collected in suburban area; UA 1- samples collected in urban area from crown parts not directly exposed to roads; UA 2- samples collected in urban area from crown parts directly exposed to roads; LSA- stomatal apparatus length; WSA- stomatal apparatus width; LSP- stomatal pore length; WSP- stomatal pore width; Control samples (SUA) were compared with samples of urban trees (UA 1 and UA 2) by statistical data processing, * indicates statistically significant difference.

were not significantly different between urban and suburban trees; although wider stomatal pores were observed in urban samples. Although it is possible that gaseous atmospheric pollutants' are responsible for the stomata characteristics we observed, one must also consider other environmental factors as well, since the sampled plants were not under controlled conditions. In addition to temperature, light levels and relative humidity, microclimate conditions in the habitat must be taken into consideration, since all of these factors affect the number and characteristics of stomata (Taiz and Zeiger 2002). The absence of a stomatal response in *T. cordata* leaves from urban areas with a high concentration of atmospheric pollutants might suggest other mechanism(s) neutralizing toxic substances play a role in this species. Some authors have reported that *T. cordata* has relatively high tolerance for sulphur oxides and ozone due to inherent peroxidase activity, which is greatly increased in response to high concentrations of these gases (Jager et al. 1985). Also, literature data indicate that proline, a reliable biochemical marker of this type of stress, plays a significant role in the adaptation of *T. cordata* to increased concentrations of sulphur and nitrogen oxides (Supuka 1996). Contrary to *T. cordata*, *B. pendula* displayed a stronger stomatal response, given that stomata number was significantly smaller in statistical terms in comparison with controls during both seasons. Apart from visual reduction of stomata number in the leaves of urban trees, greater width and length of stomatal pores was noticed. This effect was particularly pronounced in leaves directly exposed to roads. The higher sensitivity of *B. pendula* to increased ozone concentrations was confirmed in many studies (Matyssek et al. 1991; Pääkkönen et al. 1997). Also, it has been shown that birch trees exposed to increased ozone content display an increase in guard cell length in addition to a decrease in stomata density (Riikonen et al. 2008). The author Wright (1988) states that nitrogen and sulphur oxides lead to microanatomic damage in the structure of guard cells that prevents guard cell closing. Thus, opening stomatal pores in response to nitrogen and sulphur oxide pollution increases the sensitivity of birch trees, due to increased water loss via transpiration. That is why birch leaves can reduce

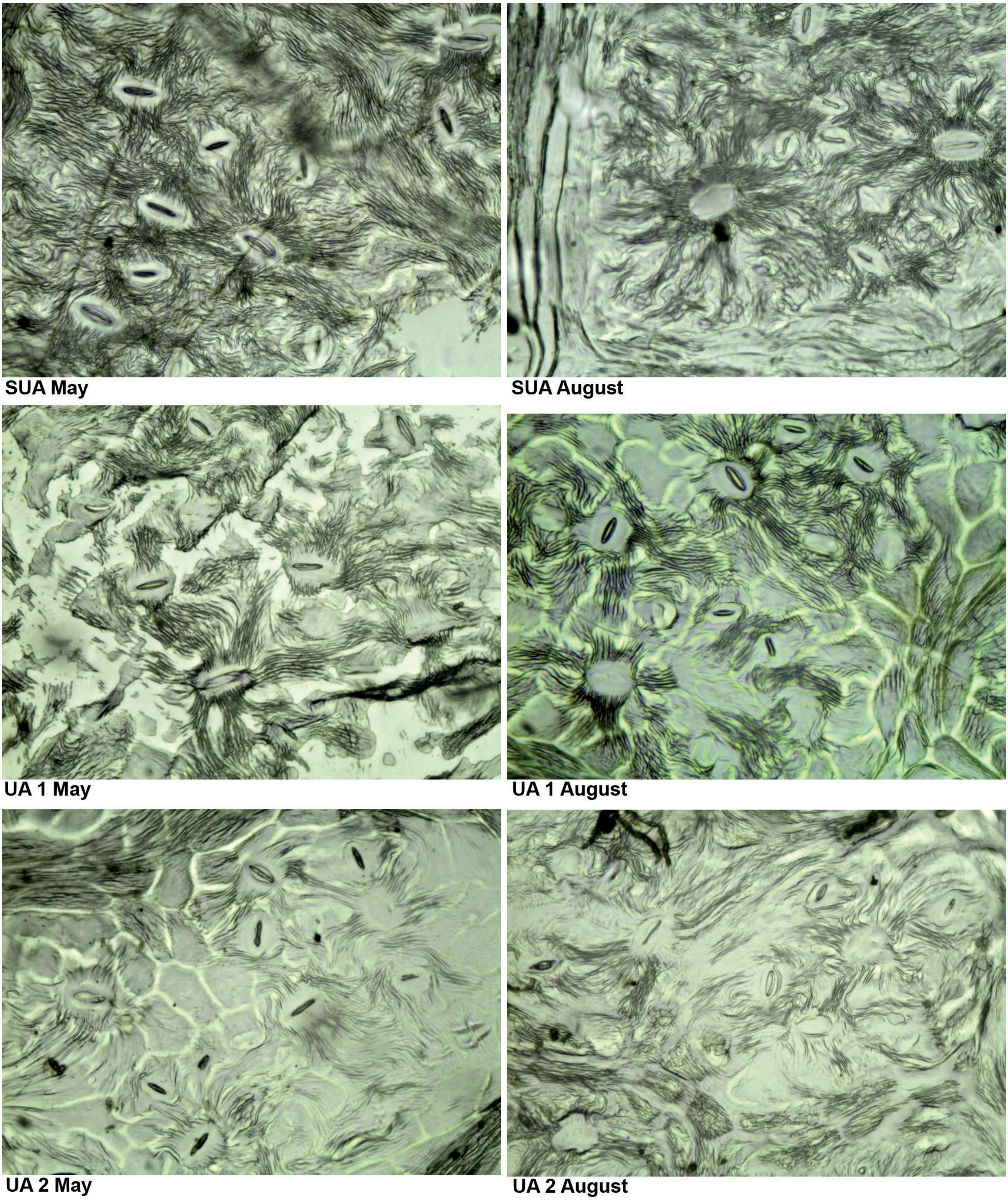

Fig. 7. Stomata on abaxial epidermis of *Tilia cordata* leaves from city and suburban trees sampled in May and August, 2014. SUA-control samples collected in suburban area; UA 1- samples collected in urban area from the crown parts not directly exposed to roads; UA 2- samples collected in urban area from crown parts directly exposed to roads.

cuticular transpiration in response to increased concentrations of sulphur and nitrogen oxides (Neighbour et al. 1988).

CONCLUSIONS

Based on analysis of stomata characteristics in the examined species, we conclude that *T. cordata* and *B. pendula* possess different strategies for adaptation to urban conditions with increased concentrations of atmospheric pollutants. *Betula pendula* has shown to be a more sensitive species, since a significant reduction in stomata number was observed in leaves taken from the urban area in comparison with control samples from the suburban area. Also, microanatomic changes in the size of stomatal apparatus were noticed in the examined species on samples taken from the urban zone. On the other hand, no significant changes were noticed in *T. cordata* in the majority of analysed stomata characteristics, indicating that this species possesses higher degree of resistance to atmospheric pollutants common in urban areas.

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REFERENCES

Abeyratne VDK, Ileperuma OA. 2006. Impact of ambient air pollutants on the stomatal aperture of *Argyreia polulifolia*. Ceylon Journal of Science. 35(1):9–15.

Antipov WG. 1979. Ustojchiwosc dreviesnykh rastcenijj k promyshlennym gazam. Minsk: Nauka i Technika.

Balasooriya BLWK, Samson R, Mbikwa F, Vitharana UWA, Boeckx P, Van Meirvenne M. 2009. Biomonitoring of urban habitat by anatomical and chemical leaf characteristics. Environmental and Experimental Botany. 65:386–394.

Dimitrova I, Yurukova L. 2005. Bioindication of anthropogenic pollution with *Plantago lanceolata* (Plantaginaceae): metal accumulation, morphological and stomatal leaf characteristics. Phytologia Balcanica. 11(1):89–96.

Duranni, GF, Hassan M, Baloch MK, Hameed G. 2004. Effect of traffic pollution on plant photosynthesis. Journal of Chemical Society of Pakistan. 26(2):176–179.

Ghorbanli B, Bakand Z, Bakhkshi Khaniki G, Bakand S. 2007. Air pollution effects on the activity of antioxidant enzymes in *Nerium oleander* and *Robinia pseudoaccacia* plants in Teheran. Iranian Journal of Environmental Health Science and Engineering. 4(3):157–162.

Gostin I, Ivanescu L. 2007. Structural and micromorphological changes in leaves of *Salix alba* under air pollution effect. International Journal of Energyand Environment. 4(1):219–226.

Govindapyari H, Leleeka M, Nivedita M, Uniyal PL. 2010. Bryophytes: indicators and monitoring agents of pollution. NeBIO. 1(1):35–41.

Govindaraju K, Tamilselvan S, Kiruthiga V, Singaravelu G. 2010. Biogenic silver nanoparticles by *Solanum torvum* and their promising antimicrobial activity of silver nanoparticles. Journal of Biopesticides. 3(1):394–399.

Hagen-Thorn A, Callesen I, Armolaitis K, Nihlgård B. 2004. The impact of six European tree species on the chemistry of mineral topsoil in forest plantations on former agricultural land. Forest Ecology and Management. 195(3):373–384.

Jablanović M, Jakšić P, Kosanović K. 2003. Uvod u ekotoksikologiju. Univerzitet u Prištini, Prirodno-matematički fakultet Kosovska Mitrovica, Kosovska Mitrovica

Jaffe DA, Hafner W, Chand D, Westerling A, Spracklen D. 2008. Influence of fires on O3 concentrations in the western U.S. Environmental Science and Technology. 42(16):5885–5891.

Jager HJ, Bender J, Grunhage L. 1985. Metabolic responses of plants differing in SO_2 sensitivity towards SO_2 fumigation. Environmental Pollution. 39(4):317–335.

Kardel F, Wuyts K, Babanezhad M, Vitharana UWA, Wuytack T, Potters G, Samson R 2010. Assessing urban habitat quality based on specific leaf area and stomatal characteristics of *Plantago lanceolata* L. Environmental Pollution. 158(3):788–794.

Kley D. 1997. Tropospheric chemistry and transport. Science. 276(5315):1043–1045.

Kosiba P. 2008. Variability of morphometric leaf traits in small-leaved linden (*Tilia cordata* Mill) under the influence of air pollution. Acta Societatis Botanorum Poloniae. 77(2):125–137.

Kurteva M. 2008. Bioindication ability of tree and shrub species under industrial environmental pollution. Natura Montenegrina. 7(2):485–503.

Lindsay WS, Brown JS, Stanek J, Gift J, Costa DL. 2010. Air Pollution Toxicology - A brief review of the role of the science in shaping the current understanding of air pollution health risks. Toxicological Sciences. 120:8–27.

Majernik O, Mansfield TA 1972. Stomatal response to raised atmospheric CO_2 concentrations during exposure of plants to SO_2 pollution. Environmental Pollution. 3(1):1–7.

Matyssek R, Günthardt-Gorge M, Sauer M, Keller T. 1991. Seasonal growth δ^{13}in leaves and stem, and phloem structure of birch (*Betula pendula*) under low ozone concentration. Trees. 6(2):9–76.

McAinsh MR, Evans NH, Montgomery LT, North KA 2002. Calcium signalling in stomatal responses to pollutants. New Phytologist. 153(3):441–447.

Miszalski Z, Mydlarz J. 1990. SO_2 influence on photosynthesis of tomato plants at different CO_2 concentrations. Photosynthetic. 24:2–8.

Moldau H, Vahisalu T, Kollist H. 2011. Rapid stomatal closure triggered by a short ozone pulse is followed by reopening to overshooting values. Plant Signalling and Behaviour. 6(2):311–313.

Mudd JB, Kozlowski TT. 1975. Responses of plants to air pollution. New York: Academic Press.

Mulgrew A, Williams P. 2000. Biomonitoring of air quality using plants. WHO Collaborating Centre for the Air Quality Management and Air Pollution Control, Berlin. Air Hygiene Report No. 10.

Musselman WJ, Massman WJ 1999. Ozone flux to vegetation and its relationship to plant response and ambient air quality standards. Atmospheric Environment. 33(1):65–73.

Nash TH, Gries C. 2002. Lichens as bio-indicators of sulphur dioxide. Symbiosis. 33(1):1–21.

Neighbour EA, Cottam DA, Mansfield TA 1988. Effects of sulphur dioxide on the control of water loss by birch *(Betula* spp.*)*. New Phytologyst. 108(2):149–157.

Pääkkönen E, Holopainen T, Kärenlampi L. 1997.Variation in ozone sensitivity among clones of *Betula pendula* and *Betula pubescens*. Environmental Pollution. 95(1):37–44.

Petrović M, Štrbac D. 1996. Fiziologija biljaka – praktikum. Poljoprivredni fakultet, Univerzitet u Banjaluci.

Pearson M, Mansfield, TA. 1993. Interacting effects of ozone and water stress on the stomatal resistance of beech (*Fagus sylvatica* L.). New Phytologist. 123(2):351–358.

Riikonen J, Syrjää L, Tulva I, Mänd P, Oksanen E, Poteri M, Vapaavuori E. 2008. Stomatal characteristics and infection biology of *Pyrenopeziza betulicola* in *Betula pendula* trees grown under elevated CO_2 and O_3. Environmental Pollution. 156(2):536–543.

Shweta T. 2012. Air Pollution Induced changes in Foliar Morphology of two shrub species at Indore city, India. Research Journal of Recent

Sciences. 2(3):195–199.

Somers CM, McBarry BE, Malek F, Quin JS. 2004. Reduction of particulate air pollution lowers the risk of heritable mutations in mice. Science. 304(5673):1008–1010.

Supuka J. 1998. Free proline dynamics in leaves of *Tilia cordata* Mill. in changed conditions of environment (1996). Acta Horticulturae et Regioculturae. 1:47–58.

Taiz L, Zeiger E. 2002. Plant Physiology, 3rd edition. Sunderland (MA): Sinauer Associates.

Tretyakova IN, Noskova NE. 2004. Scotch pine pollen under conditions of environmental stress. Russian Journal of Ecology. 35(1):20–26.

Velissariou D, Davison A, Barnes JD, Pfirrmann T, Madean De Holevas CD. 1992. Effects of air pollution on *Pinus halepensis* (Mill.): Pollution levels in Attica, Greece. Atmospheric Environment. 26(3):373–380.

WHO. 2006. Air quality guidelines, Global update 2005, particulate matter, ozone, nitrogen dioxide and sulphur dioxide. Copenhagen (Denmark): WHO Regional Office for Europe. http://www.euro.who.int/__data/assets/pdf_file/0005/78638/E90038.pdf.

Wright EA. 1988. Some effects of low levels of sulphur dioxide and nitrogen oxide on the control of water-loss by *Betula* ssp. In: Mathy P, editor. Air Pollution and Ecosystems. Dodrecht: Reidel Publishing Company. p. 760–765.

Yilmaz S, Zengin M. 2004. Monitoring environmental pollution in Erzurum by chemical analysis of Scots pine (*Pinus sylvestris* L.) needles. Environment International. 29(8):1041–1047.

Zelitch I. 1961. Biochemical control of stomatal opening in leaves. Proceedings of the National Academy of Sciences of the United States of America. 47(9):1423–1433.