Annals of West University of Timişoara, ser. Biology, 2023, vol. 26 (2), pp.115-122

STUDIES REGARDING THE PHYSIOLOGICAL BEHAVIOUR OF *JUGLANS REGIA* AND *ROBINIA PSEUDOACACIA* IN URBAN ENVIRONMENT

Victor-Dimitrie DRĂGUCIAN, Ioana-Maria TOPLICEAN*, Andreea-Raluca MICULESCU, Adina-Daniela DATCU

Biology-Chemistry Department, Faculty of Chemistry, Biology, Geography, West University of Timisoara, Romania

300645, Timisoara, Romania

*Corresponding author's e-mail: *ioana.toplicean03@e-uvt.ro*

Received 26 October 2023; accepted 29 December 2023

ABSTRACT

This paper aim is to present data from an experiment conducted during the autumn of 2022, on two woody species, in the city of Timișoara. The data was collected from two areas in the urban environment, these being: urban and urban green zones. The investigated species were Juglans regia and Robinia pseudoacacia, both of which were commonly found on the roadsides or in the parks that were studied. The investigated indices were both the chlorophyll content as well as the anthocyanin levels in the leaves. Both parameters were analyzed using non-invasive methods. For the chlorophyll content OPTI-SCIENCES CCM-300 Chlorophyll Content Meter was used and the results were presented in mg m^{-2}, and for anthocyanin levels, OPTI-SCICENCES ACM200 Plus Anthocyanin Content Meter was utilized and the results were presented in ACI. The processing of the values was done with the help of Microsoft Office Excel 2016 and for the statistical analysis, the PAST software v3.0 was used. A total of 480 readings were realized, 240 for each parameter investigated. The anthocyanin levels are the ones responsible for the red or blue pigmentation in the leaf, while the chlorophyll content is the one responsible for the green coloration, and its absence is noticeable from the yellow spots that appear on the leaf. Thus, from the collected data, it was noticed that for the urban area, the anthocyanin had a higher concentration, suggesting the adaptation to the emission produced daily by humans, being the same case for chlorophyll. From this, we can say that both the chlorophyll and anthocyanin contents were higher in the urban areas as an adaptation to the environment.

KEY WORDS: *chlorophyll, anthocyanin, urban, biodiversity, air pollution*

INTRODUCTION

Urbanization is and has been a universal and important social as well as economic phenomenon that happens worldwide. This ongoing process, which shows no signs of slowing down, could be the most powerful and visible anthropogenic force that has brought a lot of fundamental changes in land use and landscape patterns everywhere around the globe. Because of this, the rapid growth of urbanization, especially in the developing world, is predicted to remain one of the most crucial issues of the changes happening worldwide during the 21st century (Sui et al. 2001). As a consequence of rapid urbanization and the exploitation of natural resources have resulted in substantial effects on the ecosystem structure, function, and dynamics that have left the urban region really fragile. This can be observed best in the case of the economic-developed regions where the dramatic increase of the urban area and the land use change have led to serious environmental issues (Deng et al. 2009). These qualities and activities give birth to distinct urban climates at the micro and regional levels (Arnfield, 2003; Kalnay and Cai, 2003; Feng et al. 2012; Yan et al. 2016; Chapman et al., 2017a; Oke et al. 2017; Zhou et al. 2017; Chakraborty and Lee, 2019; Sharma et al. 2020).

The cities are represented by nature concentrations of humans, materials and activities, because of this they exhibit both the highest levels of pollution and the largest targets of impact (Fenger, 1999). The most plausible cause for the air quality is the increase of the population in the urban areas, mainly because the increase of the population leads to the growing need for cars, houses, roads and many more that cause the pollution of the air (Mayer, 1999).

Biodiversity serves a multitude of significant roles within the urban environment (McKinney, 2008). Among those roles we can include ecosystem services such as water and air purification (Bolund and Hunhammar, 1999) but also for recreational purposes such as sightseeing and recreation (Miller 2005; 2006). On the other hand, urban biodiversity plays an important role in educating the urban population, which is in a constant growth, about the importance of nature and its conservation (Miller and Hobbs, 2002). The trees in the urban environment are of great value to the city, their performance and aesthetics being beneficial both for the climate but the human population as well (Moser et al. 2016).

Anthocyanin are pigments that provide the red, blue, and violet colors in the flowers, leaves, fruits, and storage organs. In the case of some plants, the anthocyanin are present all time in the leaves, while for other species only at a specific time of the year, or during a particular stage of development (Chalker, 1999). Anthocyanin production is influenced by environmental factors, such as light quantity and quality, mineral and pH imbalances, extreme temperatures, pollution and herbicides.

Juglans regia (commonly known as "Walnut") belongs to the family of Juglandaceae, being used in traditional medicine from ancient times. The main part that has been used in the medicine are the leaves, mainly for treating skin inflammations, different types of infections such as fungal or microbial, for venous insufficiency, for ulcers and hyperhidrosis, and anthelmintic, antiseptic and astringent properties. *J. regia* has also been used in the treatment of diabetes, prostate, and vascular disturbance (Ianovici et al. 2017). The walnut tree, native to central Asia, the western part of the Himalayan chain and Kyrgyzstan, being cultivated in Europe since approximately 1000 BC. Since then, it has disseminated and thrived in various regions featuring Mediterranean-type ecosystems across the globe (Martinez et al. 2010).

Robinia pseudoacacia, also known as "black locust" or "false acacia", is a tree of medium size, approximately 12-18m in height, the radial root extent being about 1-1.5 times the height of the tree. The species is native to North America, but it has spread worldwide thanks to its rapid growth, fast maturity (Wojda et al. 2015), vigorous sprouting and reproduction via underground runners (Wilkaniec et al. 2021). The effective expansion of black locusts is also influenced by the fact that disturbances stimulate the proliferation of clones, resulting in greater number of ramifications. (Cierjacks et al. 2013). The tree has been recommended for the urban areas for a long time, mainly because it facilitates late-successional plant species, this is because the black locust fixes nitrogen and stabilizes the soil (Danso et al. 1995).

The aim of this study is to assess the relation between the anthocyanin and chlorophyll content, the species of plant and the investigated area. There were two main areas studied, these being the urban and urban green, from the Municipality of Timișoara, Romania. Both the chlorophyll and anthocyanin content are an important indicator regarding the leaves health and its physiology.

MATERIALS AND METHODS

The research was conducted out in the Municipality of Timișoara in September 2022. The investigated zones were: Charles Darwin Str., Splaiul Nistrului, Regina Maria Park, Rozelor Park, Karlsruhe Park, Mehadia Str., Student Complex, Begăi bank, Corneliu Miclоși Academic Square. The readings were taken as far away from the heart of the park as feasible for those in the green urban area, and as close to the street as possible for those in the urban region.

The indices tested were anthocyanin content (ANTC), measured with an OPTI-SCICENCES ACM200 Plus Anthocyanin Content Meter and displayed in ACI, and chlorophyll content (Chl), measured with an OPTI-SCICENCES CCM-300 Chlorophyll Content Meter and displayed in mg m^{-2}. Both analyzers are non-invasive and deliver cheap and quick results. The research was conducted on the species *Juglans regia* and

Robinia pseudoacacia. In all, 480 measurements were conducted, 240 for chlorophyll and 240 for anthocyanin levels.

Microsoft Office Excel was used for data processing. PAST 3.0 (Hammer et al. 2001) was used for statistical analysis. Levene's test for variance homogeneity was used. Furthermore, Mann-Whitney U was used to analyze differences between two independent groups where the dependent variable was ordinal or continuous but not normally distributed, and ANOVA was employed when the variances were equal.

RESULTS AND DISCUSSIONS

By collecting samples from various locations, biomonitoring may be performed using the content of two components that reflect the amount of pollution, so their number can grow or decrease. Figure 1 depicts the mean values SE of Chl content for all tested zones. In addition, the outlines are shown. Robinia has the lowest anthocyanin concentration in the green urban region (ANTC = 1.1 ACI), whereas the g Juglans has the highest in the urban area (ANTC = 9.3 ACI). Thus, the minimum point of ANTC for the Juglans in the urban area is 1.3 and the maximum point is 9.3, while the minimum point for the green urban area is 3.6 and the maximum point is 8. Simultaneously, in the urban environment, the minimum point for the Robin is 2.8 and the maximum point is 6.5; in the green urban environment, the minimum point is 1.1 and the maximum point is 6.8.

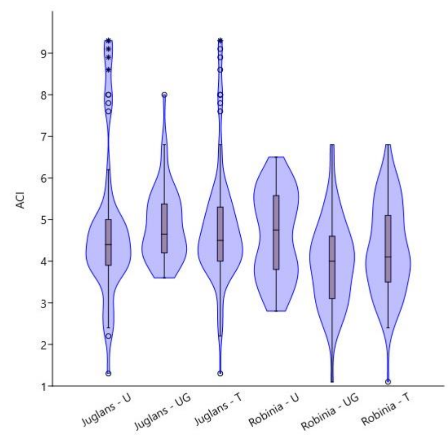

FIG. 1. Anthocyanin mean values ± SE (outlines also showed) for *J. regia* and *R. pseudoacacia* leaves from the three analyzed zones (Urban, urban green and Timisoara, in general)

In Figure 1, three points that are near to the maximum one in the urban environment for Juglans, confirming a maximum of content in the urban area. A kernel density estimation is shown on either side of the violet line to indicate the distribution shape of the data. The violin plot's wider parts reflect a larger possibility that individuals of the population will take on the given value, while the skinnier sections imply a lesser probability. *R. pseudacacia* has a more linear distribution than the *J. regia*, which has a more concentrated distribution between 3.5 and 5.5 ACI for the urban region.

Figure 2 depicts the mean SE values of chlorophyll content for all tested zones. Outlines are also displayed. The statistics demonstrate that the *J. regia* has the lowest chlorophyll level in the urban region (Chl = 180 mg m^{-2}), whereas the *R. pseudacacia* has the highest chlorophyll content in urban zone (Chl = 700 mg m^{-2}). However, the lowest and maximum points for Juglans in the urban environment are 180 and 554, respectively, while the minimum and maximum points for Juglans in the green urban environment are 231 and 497. At the same time, the genus Robinia has a minimum of 193 and a maximum of 700 in the urban environment, and a minimum of 231 and a maximum of 472 in the green urban environment.

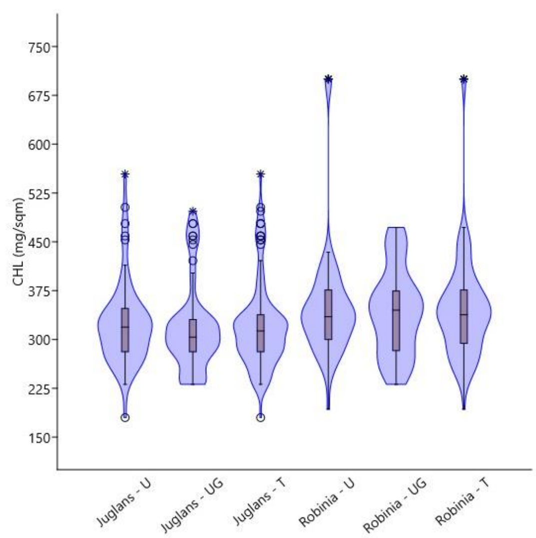

FIG. 2. Chlorophyll mean values ± SE (outlines also showed) for *J. regia* and *R. pseudoacacia* leaves from the three analyzed zones (Urban, urban green and Timisoara, in general)

To demonstrate the distribution form of the data, a kernel density estimation is displayed on either side of the violet line. Wider portions of the violin indicate a higher likelihood that people in the population would choose the given value, whilst thinner sections indicate a lesser probability, resulting in a low density at the lowest and maximum points and a higher density at the graph's midpoints (Figure 2).

TABLE 1. Mann-Whitney test for anthocyanin content

	Juglans - U	Juglans - UG	Juglans - T	Robinia - U	Robinia - UG	Robinia - T
Juglans - U		0.08584	0.3206	0.6763	0.01427	0.2406
Juglans - UG	0.08584		0.3206	0.2899	3.84E-06	0.001025
Juglans - T	0.3206	0.3206		0.7122	4.34E-05	0.00629
Robinia - U	0.6763	0.2899	0.7122		0.001827	0.07151
Robinia - UG	0.01427	3.84E-06	4.34E-05	0.001827		0.07151
Robinia - T	0.2406	0.001025	0.00629	0.07151	0.07151	

The distribution of data in the urban green zone was normal. Then, to evaluate if an ANOVA test was necessary, Levene's test for variance homogeneity was utilized. Significant differences were discovered between the groups studied (p = 3,02E-05, F = 5,838, df = 5). The Mann Whitney test reveals significant variations in anthocyanin content, with four values less than 0.05; these significant differences in anthocyanin occur between: Juglans UG - Robinia UG; Juglans T - Robinia UG; Juglans UG - Robinia T; Robinia U- Robinia UG (Table 1).

TABLE 2. Mann-Whitney test for chlorophyll content

	Juglans - U	Juglans - UG	Juglans - T	Robinia - U	Robinia-UG	Robinia - T
Juglans - U		0.3387	0.5802	0.02435	0.07578	0.01984
Juglans - UG	0.3387		0.58	0.002858	0.0187	0.002041
Juglans - T	0.5802	0.58		0.002467	0.01697	0.000906
Robinia - U	0.02435	0.002858	0.002467		0.9644	0.9794
Robinia - UG	0.07578	0.0187	0.01697	0.9644		0.9794
Robinia - T	0.01984	0.002041	0.000906	0.9794	0.9794	

Data in the urban green zone exhibited a normal distribution. Following that, Levene's test for variance homogeneity was used to determine whether an ANOVA test was required. Significant differences were found between the examined groups (p = 0,01078, F = 3,019, df = 5). The Mann Whitney test reveals substantial variations in chlorophyll content, with seven values less than 0.05; these significant differences in chlorophyll occur between: Juglans UG - Robinia U; Juglans T - Robinia U; Juglans UG - Robinia UG; Juglans T - Robinia UG; Juglans U - Robinia T; Juglans UG - Robinia T; Juglans T - Robinia T (Table 2).

Following these tests and data, it appears that both the anthocyanin and chlorophyll content is homogeneously distributed in the habitats; nevertheless, there are considerable variances in both contents when comparing species and habitats.

CONCLUSIONS

This study presented data from an experiment in which the capability of two plant species *Juglans regia* and *Robinia pseudoacacia*, from the city of Timisoara, Romania. was investigated. The main areas that were studied were the urban and urban green zones. The investigated parameters were the chlorophyll and anthocyanin contents, measured through a non-invasive, fast, and affordable method.

In general, for both species there was a difference between the urban and urban green areas, the content of both parameters being higher in the urban zone than it was in the other one. For both *Juglans regia* and *Robinia pseudoacacia*, the values had a normal distribution in the urban green areas.

ACKNOWLEDGEMENTS

The authors wish to thank Institute for Advanced Environmental Research Timișoara for the equipment.

REFERENCES

- Arnfield A.J. 2003. Two decades of urban climate research: A review of turbulence, exchanges of energy and water, and the urban heat island. *International Journal of Climatology*, 23, 1−26, https://doi.org/10.1002/joc.859
- Bolund P., Hunhammar S. 1999. Ecosystem services in urban areas. *Ecological economics*, 29(2): 293-301. https://doi.org/10.1016/S0921-8009(99)00013-0
- Chakraborty T., Lee X. 2019. A simplified urban-extent algorithm to characterize surface urban heat islands on a global scale and examine vegetation control on their spatiotemporal variability. *Int. J. Appl. Earth Obs. Geoinf.*, 74: 269−280, https://doi.org/10.1016/j.jag.2018.09.015
- Chalker-Scott L. 1999. Environmental significance of anthocyanins in plant stress responses. *Photochemistry and photobiology*, *70*(1): 1-9. https://doi.org/10.1111/j.1751-1097.1999.tb01944.x
- Chapman S., Watson J.E.W., Salazar A., Thatcher M., McAlpine C.A. 2017. The impact of urbanization and climate change on urban temperatures: A systematic review. *Landscape Ecology*, 32:1921−1935, https://doi.org/10.1007/s10980-017-0561-4.
- Cierjacks A., Kowarik I., Joshi J., Hempel S., Ristow M., von der Lippe M., Weber E. 2013. Biological flora of the British Isles: *Robinia pseudoacacia*. *Journal of Ecology*, 101(6):1623-1640. https://doi.org/10.1111/1365-2745.12162
- Danso S.K.A., Zapata F., Awonaike K.O. 1995. Measurement of biological N_2 fixation in field-grown *Robinia pseudoacacia* L. *Soil Biology and Biochemistry*, 27(4-5):415-419. https://doi.org/10.1016/0038-0717(95)98612-R
- Deng J.S., Wang K., Hong Y., Qi J.G. 2009. Spatio-temporal dynamics and evolution of land use change and

landscape pattern in response to rapid urbanization. *Landscape and urban planning*, 92(3-4):187-198. https://doi.org/10.1016/j.landurbplan.2009.05.001

- Feng J.-M., Wang Y.-L., Ma Z.-G. and Liu Y.-H. 2012. Simulating the regional impacts of urbanization and anthropogenic heat release on climate across China. J. Climate, 25, 7187−7203, https://doi.org/10.1175/JCLI-D-11-00333.1
- Fenger J. 1999. Urban air quality. *Atmospheric environment*, 33(29):4877-4900.
- Hammer Ø. Harper D.A.T., Ryan P.D. 2001. PAST: Paleontological Satistics software package for education and data analysis. Paleontologica Electronica 4(1): 1-9.
- Ianovici N., Latis A.A., Radac A.I. 2017. Foliar traits of *Juglans regia*, *Aesculus hippocastanum* and *Tilia platyphyllos* in urban habitat. *Romanian Biotechnological Letters*, 22(2), 12400-12408.
- Kalnay E., Cai M. 2003. Impact of urbanization and landuse change on climate. *Nature*, 423, 528−531, https://doi.org/10.1038/nature01675
- Martínez M.L., Labuckas D.O., Lamarque A.L., Maestri D.M. 2010. Walnut (*Juglans regia* L.): genetic resources, chemistry, by-products. *Journal of the Science of Food and Agriculture*, 90(12): 1959-1967. https://doi.org/10.1002/jsfa.4059
- Mayer H. 1999. Air pollution in cities. *Atmospheric environment*, 33(24-25): 4029-4037.
- McKinney M.L. 2008. Effects of urbanization on species richness: a review of plants and animals. *Urban ecosystems*, 11:161-176. https://doi.org/10.1007/s11252-007-0045-4
- Miller J.R. 2005. Biodiversity conservation and the extinction of experience. *Trends in ecology & evolution*, 20(8): 430-434. https://doi.org/10.1016/j.tree.2005.05.013
- Miller J.R. 2006. Restoration, reconciliation, and reconnecting with nature nearby. *Biological conservation*, 127(3): 356-361. https://doi.org/10.1016/j.biocon.2005.07.021
- Miller J.R., Hobbs R.J. 2002. Conservation where people live and work. *Conservation biology*, 16(2): 330-337. https://doi.org/10.1046/j.1523-1739.2002.00420.x
- Moser A., Rötzer T., Pauleit S., Pretzsch H. 2016. The urban environment can modify drought stress of small-leaved lime (*Tilia cordata* Mill.) and black locust (*Robinia pseudoacacia* L.). *Forests*, *7*(3): 71. https://doi.org/10.3390/f7030071
- Oke T.R., Mills G., Christen A., Voogt J. A. 2017.Urban Climates. *Cambridge University Press*, https://doi.org/10.1017/9781139016476
- Sharma A., Wuebbles D.J., Kotamarthi R., Calvin K., Drewniak B., Catlett C.E., Jacob R. 2020. Urban-scale processes in high-spatial-resolution earth system models. *Bull. Amer. Meteor. Soc.*, 101,:E1555−E1561, https://doi.org/10.1175/BAMS-D-20-0114.1
- Sui D.Z., Zeng H. 2001. Modeling the dynamics of landscape structure in Asia's emerging desakota regions: a case study in Shenzhen. *Landscape and urban planning*, 53(1-4): 37-52. https://doi.org/10.1016/S0169-2046(00)00136-5
- Wilkaniec A., Borowiak-Sobkowiak B., Irzykowska L., Breś W., Świerk D., Pardela Ł., ... & Wielgus K. 2021. Biotic and abiotic factors causing the collapse of Robinia pseudoacacia L. veteran trees in urban environments. *PLoS One*, 16(1): e0245398. https://doi.org/10.1371/journal.pone.0245398
- Wojda T., Klisz M., Jastrzębowski S., Mionskowski M., Szyp-Borowska I., Szczygieł K. 2015. The geographical distributionof the black locust (*Robinia pseudoacacia* L.) in Poland and its role on non-forest land. *Papers on Global Change IGBP*, 22(1): 101-113. https://doi.org/10.1515/igbp-2015-0018
- Yan Z.-W., Wang J., Xia J.-J., Feng J.M. 2016. Review of recent studies of the climatic effects of urbanization in China. *Adv. Clim. Chang. Res.*7: 154−168, https://doi.org/10.1016/j.accre.2016.09.003
- Zhou B., Rybski D., Kropp J. P. 2017. The role of city size and urban form in the surface urban heat island. *Scientific Reports*, 7: 4791, https://doi.org/10.1038/s41598-017-04242-2