NON-INVASIVE CHLOROPHYLL INVESTIGATION ON ORNAMENTAL PLANTS FROM URBAN HABITATS

V.-D. DRĂGUCIAN, Alexandra-Samira LĂPĂDAT, Daniela-Georgiana CIOBANU, Nicoleta IANOVICI, Adina-Daniela DATCU

West University of Timișoara, Faculty of Chemistry, Biology, Geography, Biology-Chemistry Department

Corresponding author: dana_datcu19@yahoo.com

Abstract. *This paper aim is to present data from an experiment realized during the summer of 2022, on four ornamental species in the city of Timisoara. The data were taken from two urban habitats: urban and urban green. The investigated species were Acer negundo, Acer platanoides, Liquidambar styraciflua and Cotinus coggygria. These plant species are frequent on the roadsides or in parks, in the studied areas. The investigated index was chlorophyll content. This parameter was analyzed using a non-invasive approach, using OPTI-SCIENCES CCM-300 Chlorophyll Content Meter and the results were presented in mg* m^{-2}*. Data processing was done using Microsoft Office Excel 2016 and statistical analysis was realized using PAST software v4.03. A total of 480 chlorophyll content samples were taken from all the species researched. Readings were taken from multiple types of leaves, from both those that appeared healthy and those that showed signs of chlorosis, from both research areas. It is a known fact that chlorosis is one of the signs that a leaf is not healthy, probably due to a physiological deficiency. This affection is mainly present in areas with a higher level of traffic such as urban areas. A symptom that can help identify the infected leaves was the appearance of small yellow spots that can spread over the entire surface of the leaf. From the collected data, a lack of chlorophyll was noticed within both investigated zones, but mainly in the urban area, where the levels of exhaust gas are higher compared to the ones in the urban green areas, which is in general a park. Thus, after the completion of Welch F test, it was observed that in urban area the level of chlorophyll was significantly reduced, when compared to the level from the urban green zone.*

Keywords: *urban zone, chlorophyll content, urban green, adaptation, physiological indices*

INTRODUCTION

Urbanization comes as a natural consequence of the global population growth that we have faced in recent decades. According to a report published in 2022 by United Nation, Department of Economic and Social Affairs, Population Division, Earth's population tends to be very close to 10 billion people by the year 2050 (approximately 8.5 billion in 2030 and 9.7 billion, respectively, in 2050.). Due to this series of events, we are witnessing excessive pollution. Air pollution is a natural consequence of urbanization, especially in cities where environmental management is very low (TEREKHINA AND UFIMTSEVA, 2020). According to the annual report regarding air quality issued by the European Environment Agency (EAA) in 2021, approximately 97% of the urban population is exposed to fine particulate matter (PM 2.5) above the World Health Organization limit. The main air pollutants identified by the EAA are PM 10, PM 2.5, nitrogen dioxide (NO_2), ozone, benzo(a)pyrene and other pollutants (such as sulfur dioxide). Although an improvement in air quality is observed, in the sense of decreasing trends for PM 10, PM 2.5 and NO_2, these changes seem to be due to the measures imposed by the quarantine of 2020. Furthermore, pollution remains a subject of great concern, fundamentally important being here the pollution monitoring and the various methods by which it can be done.

Air quality monitoring can be done by invasive and non-invasive methods, the last, being, of course, preferable. MIROWSKY AND GORDON (2015) summarize some non-invasive methods of studying air quality in human populations, such as: blood pressure measurement, spirometric measurements, heart rate measurement, inflammatory biomarkers, quantification of exhaled nitric oxide and carbon dioxide, etc. In humans, air pollutants can enter directly into the systemic circulation or via the respiratory tract, regardless of the route, causing the production of pro-inflammatory factors: pro-inflammatory cytokines, reactive oxigen species, proteinases, proteins of the complement pathway, which can ultimately lead to the establishment of systemic or localized inflammation at the level of different organs, such as the brain (MANISALIDIS et al., 2020). Other examples of non-invasive methods for studying air quality can be palynology studies (IANOVICI, 2007) or studies on bird feathers (LODENIUS and SOLONEN, 2013). There are also invasive methods of studying air quality using bioindicators, but non-invasive methods that do not involve slaughter or direct exposure of individuals to pollutants are much more preferable. For example, a comparative study on invasive methods vs. non-invasive methods to study the exposure to metals found in the environment, concludes that the slaughter of animals does not represent an extremely necessary method of air quality monitoring because there is no organ that can be considered a true representative of direct exposure to metals, thus being necessary the development of new monitoring methods (SIDRA et al., 2022). At present, the non-destructive detection measurements are applied widely due their advantage of rapid assessment. These methods are: near infrared spectroscopy (HUANG et al., 2008), ultrasonic detection (MIZRACH, 2008), machine vision (CUBERO et al., 2011), biosensor technology, nuclear magnetic resonance technique (CLARK et al., 1997), mechanics detection (GARCÍA-RAMOS et al., 2005).

With regard to phytoindicators, ornamental species, specific to urban and green urban areas, are generally used. Some examples of species used in air quality monitoring are: *Tilia cordata, Populus sp., Quercus robur, Ulmus laevis, Syringa vulgaris, Cotoneaster lucidus, Berberis vulgaris, Syringa josikaea, Rosa rugosa* (TEREKHINA & UFIMTSEVA, 2020); *Robinia pseudoacacia, Acer saccharinum, Tilia × europaea, Acer platanoides, Fraxinus excelsior, Betula pendula, Celtis occidentalis, Platanus × acerifolia* (SIMON et al., 2021); *Platanus orientalis, Pinus nigra* (SAWIDIS et al., 2011) or *Alnus glutinosa* (SALA et al., 2020). Depending on the species, MUTHU et al. (2021) summarize the following negative effects that pollutants can have on plants, thus justifying the reasons why plants can be used as bioindicators of air quality: reduction of growth and number of leaves, variations in anatomy and leaf morphology, decreasing the amount of chlorophyll, stomata blocking, reducing total chlorophyll and protein content in leaves, affecting leaf morphology, etc. Phenolic substances, such as anthocyanins and tannins, are also capable of acting as nonenzymatic antioxidants (NIJVELDT et al. 2001; POLLASTRI AND TATTINI 2011). Anthocyanins are a kind of flavonoid natural water-soluble pigments widely existing in vacuoles of plant flowers, fruit, stems, and leaves (ALVAREZ-SUAREZ et al., 2021). There are numerous studies on chlorophyll content in urban pollution studies. The invasive approach of chlorophyll measurement is laborious, but are some non-invasive approaches that are quick and cheap (UDDLING et al., 2007).

As for trees, they are shown to be very effective in air monitoring because, on the one hand, they are present in most urban ecosystems (DATCU et al., 2020) and, on the other hand, their morphology and physiology allow them to sequester particles in their stomata and waxy cuticles (SIMON et al., 2020). Another way to measure the level of pollution in trees is by analyzing the chemical composition of the trees, basically by testing the cellulose and lignin content. The aforementioned polymers represent basic indicators of wood quality (TEACĂ, 2021).

The aim of this study is to assess the relation between chlorophyll content, plant species and studied area. There were two main areas studied: urban and urban green, from the city of Timisoara, Romania. The chlorophyll content is an important indicator of leaves health and its physiology.

MATERIALS AND METHODS

The investigation was conducted in the summer of 2022, on June and July. Readings were done in Timisoara, Romania, in two areas: urban and urban green. The urban area is considered the area located immediately near an intense circulated street (for example, Daliei or Mihail Kogălniceanu streets) or boulevard (for example, Calea Aradului) and urban green areas are within parks (Karlsruhe park). A total of 480 readings were done, 60 for each species per investigated area. The studied individuals had mainly a healthy foliage and internervary parts were analyzed, where the photosynthetic tissue is present.

The studied species were: *Acer platanoides*, *Acer negundo, Cotinus coggygria* and *Liquidambar styraciflua*. These can be found in both investigated areas.

The chlorophyll content was determined using OPTI-SCIENCES CCM-300 Chlorophyll Content Meter. This index is expressed in mg m^{-2}.

Data processing was done using Microsoft Office Excel 2016 and statistical analysis with PAST v4.03 (HAMMER et al. 2001). Shapiro-Wilk test was used to test the normality of data. Spearman correlations between all data sets were realized. Levene's test for homogeneity of the data and Welch F Test for unequal variances.

RESULTS AND DISCUSSIONS

This research aimed to compare the chlorophyll content (mg m^{-2}) for some ornamental species, from urban and urban green areas, located in Timisoara.

Minimum and maximum values of Chl content for all the studied species from both areas can be observed in Table 1.

Table 1

Minimum and maximum values of Chl content for all studied species, from both areas

Plant species	Area	Min value (mg m^{-2})	Max value (mg m^{-2})
A. negundo	U	193	389
	UG	97	389
A. platanoides	U	42	440
	UG	307	414
L. styraciflua	U	53	478
	UG	180	560
C. coggygria	U	319	706
	UG	250	554

From the analysis of Table 1, it can be observed that for *A. negundo*, *A. platanoides* and *L. styraciflua* are differences regarding minimum values of the analyzed index. The fourth studied species had both, minimum and maximum values obtained for Chl content, bigger then the values obtained for the other studied species.

Mean values, ± SE can be observed in Figure 1. Also, the outlines were represented. In the case of *A. negundo*, mean Chl content was bigger in UG area (Chlc = 317.7 mg m^{-2}), than in U area (Chlc = 304.1 mg m^{-2}).

For *A. platanoides*, same trend was observed, with a mean value of Chl content in UG area of 361.28 mg m^{-2}, bigger than the average obtained for U area (Chlc = 356.06 mg m^{-2}). Liquidambar had a mean Chl content of 294.38 mg m^{-2} in U area, lower than 309.4 mg m^{-2}, obtained for UG area.

Cotinus, on the other hand, presented bigger mean contents of chlorophyll for the individuals from U area (Chlc = 392.38 mg m^{-2}), when compared to those from UG area (Chlc = 357.41 mg m^{-2}). Thus, three of the studied species had a bigger chlorophyll content for the individuals from parks. Thus, urban pollution affects this index. Cotinus presented the highest mean values of the studied index in the urban area.

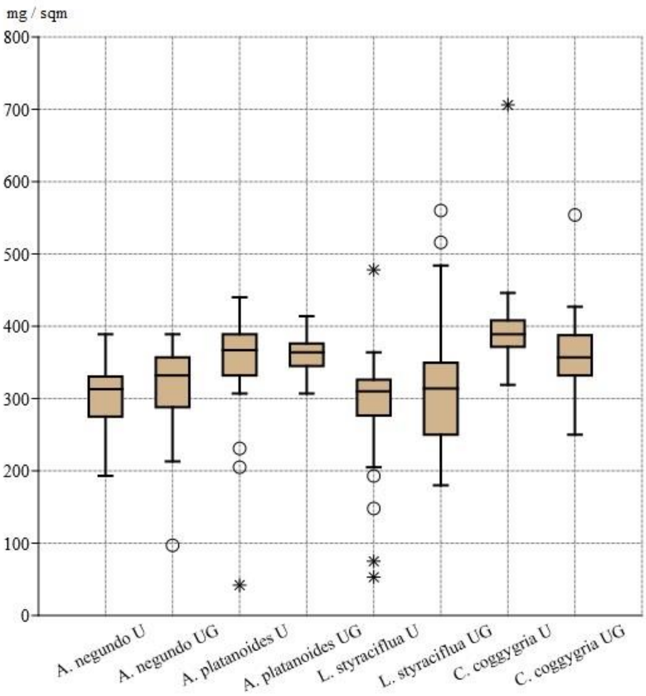

Figure 1. Chlorophyll content (Average ± SE) in mg m^{-2} for all species and areas (outlines also showed).

After the completion of Shapiro-Wilk test, the only data set with a normal distribution was for *Acer platanoides* from UG area (p = 0.592). The majority of data sets had an abnormal distribution. Thus, Spearman correlation tests were realized, and the results of this analysis can be observed in Table 2.

The correlation matrix between the chlorophyll content index values, for the studied species, can be observed in Table 2. A strong positive correlation was observed between *A. negundo* from both studied zones (R = 0.86749). For the other species studied, there were also positive correlation between the zones.

After the completion of Levene´s test for homogeneity of variance, from means, the obtained p was p = 0.00000155. In this case, for unequal variances, Welch F test was applied. The results (F=27.81, df=198.3, p=1.934E-26) is that the Chl content between the analyzed groups are significantly different.

Excepting *Cotinus coggygria*, all the analyzed species had a bigger amount of chlorophyll in the case of individuals from urban green area. It is a known fact that the chlorophyll content is directly linked to photosynthetic efficiency, but also with other principal physiological processes. Thus, urban green area seems a suitable zone for the health of urban habitat.

Table 2

Correlation (Spearman) matrix for all the studied species, from both zones

Species	*A. negundo U*	*A. negundo UG*	*A. platanoides U*	*A. platanoides UG*	*L. styraciflua U*	*L. styraciflua UG*	*C. coggygria U*	*C. coggygria UG*
A. negundo U		0.86749	2.14E-06	6.23E-01	0.30591	0.32999	8.50E-01	6.75E-01
A. negundo UG	35039		0.010245	0.8968	0.013642	0.0076259	4.08E-01	0.039441
A. platanoides U	57883	47835		0.42845	5.24E-01	0.93915	0.64365	0.56908
A. platanoides UG	33425	36372	32061		8.61E-01	3.48E-02	0.007253	0.5307
L. styraciflua U	40556	24333	32850	34922		0.10233	6.14E-01	2.08E-02
L. styraciflua UG	31293	48363	35516	25955	43491		8.97E-01	4.67E-03
C. coggygria U	34826	39616	37894	48121	33387	35181		0.89162
C. coggygria UG	37748	45463	38485	38662	46599	22661	35106	

CONCLUSIONS

This study presented data from an experiment conducted on four plant species in the city of Timisoara, Romania. There were two studied areas: urban and urban green. The investigated index was chlorophyll content, measured through a non-invasive, quick and cheap method: a chlorophyll metter.

Generally, *Cotinus coggygria* and *Acer platanoides* presented the highest Chl values. Data had not a normal distribution, excepting *Acer platanoides* samples from UG zone. The Chl values from both studied areas in the case of *A. platanoides*, *A. negundo* and *C. coggygria* were strongly correlated. Both Acer species and *Liquidambar styraciflua* presented bigger values of this index in urban green area, while *Cotinus coggygria* seemed that had the ability to adapt to urban area.

ACKNOWLEDGEMENTS

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

BIBLIOGRAPHY

ALVAREZ-SUAREZ J.M., CUADRADO C., BALLESTEROS I., GIAMPIERI F., BUELGA C.S. 2021. Novel approaches in anthocyanin research-plant fortification and bioavailability issues. Trends Food Sci. Technol. 117: 92–105.

CUBERO S., ALEIXOS N., MOLTÓ E., GÓMEZ-SANCHIS J., BLASCO, J. 2011. Advances in machine vision applications for automatic inspection and quality evaluation of fruits and vegetables. Food Bioproc. Tech. 4(4): 487-504.

DATCU A.-D., SALA F., IANOVICI N. 2017. Studies regarding some morphometric and biomass allocation parameters in the urban habitat on *Plantago major*. Research Journal of Agricultural Science 49 (4): 96-102.

EUROPEAN ENVIRONMENT AGENCY. 2021. Air quality in Europe 2021. Report no. 15/2021. doi: 10.2800/549289.

García-Ramos F., Valero C., Homer I., Ortiz-Cañavate J., Ruiz-Altisent, M. 2005. Non-destructive fruit firmness sensors: a review. Span. J. Agric. Res. 3(1): 61-73.

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.

Huang H., Yu H., Xu H., Ying Y. 2008. Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: A review. J. Food Eng. 87(3): 303-313.

Ianovici N. 2007. Quantitative aeropalinology in the atmosphere of Timisoara City, Romania. Bull. Univ. Agric. Sci. Vet. Med. Cluj Napoca, Agriculture 63: 417-423.

Ianovici N., Vereș M., Catrina R.G., Pîrvulescu A.-M., Tănase R.M., Datcu A.D. 2015. Methods of biomonitoring in urban environment: leaf area and fractal dimension. Annals of West University of Timişoara, ser. Biology, 18 (2): 169-178.

Lodenius M., Solonen T. 2013. The use of feathers of birds of prey as indicators of metal pollution. Ecotoxicology 22(9): 1319-1334.

Manisalidis I., Stavropoulou E., Stavropoulos A., Bezirtzoglou, E. 2020. Environmental and health impacts of air pollution: a review. Front. public health. 8(14).

Mirowsky J., Gordon T. 2015. Noninvasive effects measurements for air pollution human studies: methods, analysis, and implications. J Expo Sci Environ Epidemiol. 25(4): 354-80.

Mizrach A. 2008. Ultrasonic technology for quality evaluation of fresh fruit and vegetables in pre-and postharvest processes. Postharvest Biol. Technol. 48(3): 315-330.

Muthu M., Gopal J., Kim D.H., Sivanesan I. Reviewing the impact of vehicular pollution on road-side plants—future perspectives. Sustainability. 13(9): 5114.

Nijveldt R. J., Van Nood E. L. S., Van Hoorn D. E., Boelens P. G., Van Norren K., & Van Leeuwen P. A. 2001. Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. 74(4): 418-425.

Pollastri S., Tattini M. 2011 Flavonoids: old compounds for old roles. Ann. Bot. 108: 1225–1233.

Sala F., Datcu A.D., Rujescu C. 2020. Leaf area determination for Alnus glutionosa (L.) Gaertn using nondestructive methods. LUCRĂRI ȘTIINȚIFICE MANAGEMENT AGRICOL 22(2): 131-139.

Sawidis T., Breuste J., Mitrovic M., Pavlovic P., Tsigaridas K. 2011. Trees as bioindicator of heavy metal pollution in three European cities. Environ. Pollut. 159(12): 3560-3570.

Sidra S., Rashid M., Akmal M., Arooj F., Khan B. N., Javid A., Nimra A., Jamil M., Hussain, A. 2022. Comparative assessment of invasive and non-invasive approaches for estimating metallic pollutants in the environment. J. King. Saud Univ. Sci. 102146.

Simon E., Molnár V.É., Lajtos D., Bibi D., Tóthmérész B., Szabó S. 2021. Usefulness of tree species as urban health indicators. Plants 10(12): 2797.

Simon E., Molnár V.É., Tóthmérész B., Szabó S. 2020. Ecological assessment of particulate material (PM5 and PM10) in urban habitats. Atmosphere. 11(6):559.

Teaca C.A. 2021. Trees as bioindicators of environmental pollution and its impact on wood chemical composition. BioResources 16(2): 2184-2187.

Terekhina N.V, Ufimtseva M.D. 2020. Leaves of trees and shrubs as bioindicators of air pollution by particulate matter in Saint Petersburg. Geogr. Environ. Sustain., 13(1): 224- 232.

Uddling, J., Gelang-Alfredsson, J., Piikki, K., Pleijel, H. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth. Res. 91(1): 37-46.

United Nations. Department of Economic and Social Affairs. 2022. World Population Prospects 2022. Summary of Results. UN DESA/POP/2022/TR/NO. 3.