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NON-INVASIVE ANTHOCYANIN INVESTIGATION ON SOME PLANT SPECIES FROM URBAN HABITATS

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Abstract. *This study purpose is to present data from an experiment realized in the city of Timisoara, during 2022. There were two investigated zones: urban and urban green and the studied species were Acer negundo, Acer platanoides, Liquidambar styraciflua and Cotinus coggygria. In the city of Timisoara, these species are quite commonly met on the boulevards or in parks. The investigated parameter was anthocyanin content. This parameter was analyzed using a non-invasive approach, OPTI-SCICENCES ACM-200 Plus Anthocyanin Content Meter and the results were presented in ACI. Data analysis was realized using Microsoft Office Excel 2016 and statistical processing was realized with PAST software v4.03. A total of 480 anthocyanins content samples were taken from all the species researched. In the end, each of the species researched (Acer negundo, Acer platanoides, Cotinus coggygria and Liquidambar styraciflua) had a total of 120 samples that were collected from both the Urban and Urban green zones. Readings were taken from multiple types of leaves, from both studied areas. The anthocyanin levels are the ones responsible for the red coloration of the plant tissue. Usually, a high level of anthocyanin accumulated in the leaf is a good indicator that the plant is under some environmental stress such as strong light, low temperature, certain herbicides, and pollutants and many more. If the leaves don't present any kind of red coloration on them then the anthocyanin levels are between normal parameters, and we can say that the plant isn't under any kind of environmental stress. From the collected data, it was observed that only in the urban green zone the distribution is normal, when compared to the urban zone, where the levels of anthocyanin are below average. In conclusion, it can be said that the anthocyanin content found in the leaves is influenced by the environment where the species is located, having lower levels in the urban area compared to the urban green one.*

Keywords*: urban area, anthocyanin content, urban green, adaptation, physiological indices*

INTRODUCTION

Air pollution is a real problem that we face (ESCOBEDO et al., 2011; AYTURAN et al., 2018). Urbanization, the oil refining industry, factories, over-used cars, are just some of the sources that are damaging nature in very serious ways, including the health of trees (NANDY et al. 2014; NOWAK et al., 2014). Air pollution is one of the forms of pollution with major, disastrous effects on the environment (SIROMLYA, 2011), especially in the highly populated areas such as big cities (KUMAR et al., 2019, 2022; OTTOSEN AND KUMAR, 2020). Technically, any physical, chemical or biological change in the atmosphere can be referred as air pollution and occurs when any harmful gas, smoke or dust, enters the atmosphere and affects plants, animals and human beings (SEMEENA et al. 2006; UNECE, 2010). Implicitly, air pollution means pollution of the atmosphere and thus any gas or substance entering the atmosphere can create undesirable imbalances in the environment for long term. Therefore, the thinning of the ozone layer in the atmosphere caused by air pollution is a major threat to the existence of ecosystems on the planet (JANKOWSKI AND CADER, 1997; HÄDER et al., 2011).

Despite the effects of light or nutrients on plant development (PORTSMUTH and NIINEMETS, 2007), there are numerous studies that investigated how plant growth and development are highly influenced by vehicle gases also. All things considered, there is a naturally increase in interest when it comes to the fact that roadside vegetation may provide an opportunity to reduce near-road pollutant concentrations in urban areas (PETROVA et al., 2012; GALAL and SHEHATA, 2015; DATCU et al., 2017; KOSTIĆ et al., 2019; IANOVICI et al., 2020). Also, cheap and quick methods for the analyses of plant health were developed (SALA et al., 2015). Some methods recommended for the preservation of a good habitat health include a protective attitude on the existing trees and bushes, as well as planting vegetation, which may be some of the few near-term mitigation strategies available for urban developers and facilities already subject to high pollution levels near roads. If these attenuating methods are conduct to succes, they can complement already existing pollution control regulations and programs, as well as providing another way of reducing impacts from sources that are harder to control such as tire wear (BALDAUF, 2017). Different types of vegetation such as trees, bushes, but also herbaceous species have been shown to reduce the air pollution levels by intercepting airborne particles or through the uptake of polluting gasses through the surface of the leaves (JANHALL, 2015, GALLAGHER et al., 2015; VALENCIA et al., 2017).

Human activity, in order to maintain the species through adaptive processes, generates energy flows, additional to and often different from natural ones, which are introduced into the environment. This statement is now accepted as the definition of the pollution phenomenon (ALLOWAY, 1993). In the protocols for determining air pollutant concentrations (National Air Pollution monitoring, EC Directives) some stationary and sampling methods are given for these measurements (CĂLDĂRARU AND CĂLDĂRARU, 2010). The analysis of sulphur dioxide can be done using the protocol from ISO/FDIS 10498, through fluorescence method. For the analysis of nitrogen dioxide and oxides of nitrogen the method is that laid down in ISO 7996/1985. For Ambient air - determination of mass concentration of nitrogen oxides, a chemiluminescent method is used. For the analysis of nitrogen dioxide and oxides of nitrogen the method is that laid down in ISO 7996/1985 and for the Ambient air - determination of mass concentration of nitrogen oxides, chemiluminescent method is also used (CĂLDĂRARU AND CĂLDĂRARU, 2010). Anthocyanins are water-soluble and are found in the vacuoles of plant cells (HUGHES et al., 2007). They are indicators of the level of pollution to which trees are exposed, especially in urban areas, where the air is highly charged with elements that affect trees (MATE AND DESHMUKH, 2016). Anthocyanin levels increase in trees, especially those exposed to high levels of pollution. These plants, which are constantly exposed to pollutants that affect their normal functioning, can become bioindicators of air quality. Clearly, in urbanized areas, air pollution is much higher (IANOVICI et al., 2020), and there are also differences between trees in urban areas and those in green urban areas. Trees with much higher levels of anthocyanins than other trees around them, of the same or different species, show that they are trying to adapt quickly to the polluted air they are exposed to.

The aim of this study is to assess the relation between anthocyanin content, plant species and studied area. Two main habitats were studied: urban and urban green, both located within the city of Timisoara, Romania. The anthocyanin content is an important index of leaves health.

MATERIAL AND METHODS

The study was conducted in the summer of 2022, on June and July. Samples were taken from Timisoara, Romania. Two main habitats were studied: urban and urban green. Urban area it’s considered the area located immediately near an intense circulated street. In this case, the samples were taken from: Calea Aradului, Daliei and Socrate streets. Urban green areas are located within parks, the studied parks are the following: Copiilor and Karlsruhe parks.

The anthocyanin content was analyzed using a non-invasive method, with OPTI-SCIENCES ACM-200 Plus Anthocyanin Content Meter. Anthocyanin content is in ACI units.

The studied species were: *Acer platanoides, Acer negundo, Cotinus coggygria* and *Liquidambar styraciflua*. These were found in both studied zones. A total of 480 readings were done with anthocyanin content meter. 60 reads were done for each plant species, for both zones. The readings were realized from internervary zones of healthy leaves.

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. Levene's test for homogeneity of the data and Welch F Test for the unequal variances.

RESULTS AND DISCUSSIONS

This research aimed to determine the values of anthocyanins content for four plant species, from two habitats, within Timisoara.

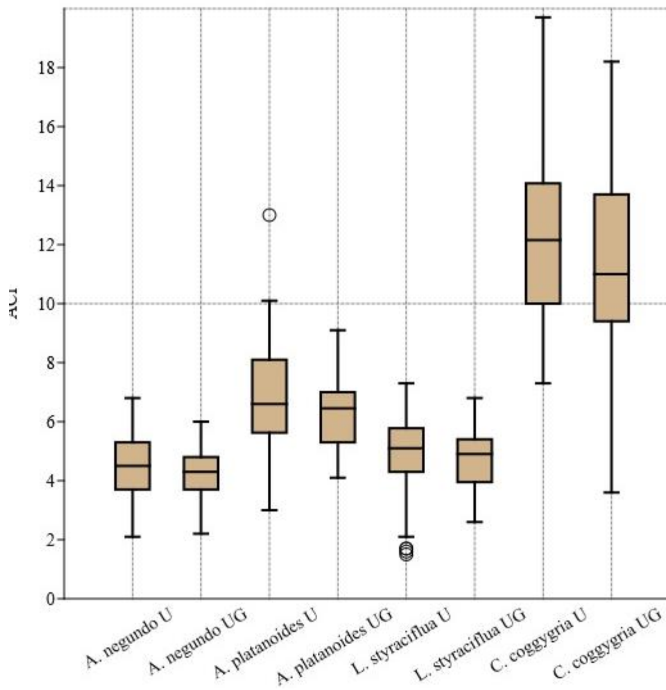

Figure 1. Leaves anthocyanin levels (mean ± SE) depending on species and studied area

Mean values of anthocyanin content in the investigated species are presented in Figure 1.

The highest mean values were observed in *Cotinus coggygria,* both in urban (Anthocyanin content = 12.32 ACI) and urban green (Anthocyanin content = 11.45 ACI) areas. The lowest mean values were recorded for *Acer negundo*, in the urban green zone (Anthocyanin content = 4.17 ACI).

Acer platanoides also presented a high level of anthocyanins in the urban area (Anthocyanin content = 6.84 ACI), when compared to the level of anthocyanins it had in the urban green area (Anthocyanin content = 6.28 ACI).

Minimum and maximum levels of anthocyanins content for all the studied species, from both studied zones, can be observed in Table 1.

Table 1

Minimum and maximum levels of anthocyanins found in the studied plants species, on both areas

Plant species	Area	Min value	Max value
A. negundo	U	2.1	6.8
	UG	2.2	6
A. platanoides	U	3	13
	UG	4.1	9.1
L. styraciflua	U	1.5	7.3
	UG	2.6	6.8
C. coggygria	U	7.3	19.7
	UG	3.6	18.2

The highest values of anthocyanins recorded in urban and green urban areas were in *Cotinus coggygria* species.

The lowest value of anthocyanins in the Urban area was recorded in the species *Liquidambar styraciflua*. In the urban green zone, *Acer negundo* showed the lowest anthocyanin level.

Table 2

Shapiro-Wilk results for all investigated species, from both areas

	A. negundo		*A. platanoides*		*Liquidambar styraciflua*		*Cotinus coggygria*	
	U	UG	U	UG	U	UG	U	UG
N	0.9792	0.9816	0.9548	0.9773	0.9353	0.9746	0.9666	0.9802
Shapiro-Wilk W	0.3955	0.5004	0.0263	0.3254	0.003349	0.2425	0.09879	0.436

According to the Shapiro-Wilk test (Table 2), normal anthocyanin contents were recorded in the urban and urban green zone for *Acer negundo*.

Acer platanoides with *Liquidambar stuyraciflua* showed normal values in the test only for the readings done in the urban green area.

All three species had values greater than 0.05 in the areas mentioned above.

According to Levene's test, the data are not homogeneous because the result was <<0.01. Welch F test in the case of unequal variances: F=123.5, df=200.1, p=4.618E-69, p-value being much smaller than 0.05, which shows that the data are significantly different.

CONCLUSIONS

This study presented data from an experiment conducted on four species in the city of Timisoara, Romania. There were two studied areas: urban and urban green. The investigated index was anthocyanin content. The anthocyanin content was analyzed using a non invasive method, with OPTI-SCIENCES ACM-200 Plus Anthocyanin Content Meter. *Cotinus coggygria* showed the highest anthocyanin value among the four species studied, both in the urban and urban green zones, showing that the species is trying to adapt to the environment.

Acer platanoides, was the second species with the highest anthocyanin values in both areas studied. *Acer negundo* and *Liquidambar styraciflua* showed fairly similar maximum anthocyanin values. Three of the four species studied (*A. negundo, A. platanoides, L. styraciflua*) showed close minimum values, the lowest value being measured in *Lyquidambar styraciflua*, whereas *Cotinus coggygria showed* a higher minimum anthocyanin value than the other three species in the urban area, which again shows that the species is trying to adapt to the environment. The values taken show that the anthocyanin content is higher in urban areas than in green urban areas. Bioindicators are organisms that, by their presence, development, growth or numbers, help us identify certain problem related to environmental conditions, the degree of pollution or the presence of certain substances in the air. From the data collected for this study and the results of the tests carried out, we can clearly see that the increased level of anthocyanins is the result of the exposure of the four species studied to a problematic, polluted environment that does not help the normal development and functioning of the trees.

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