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MONITORING URBAN HABITAT QUALITY BY DETERMINING CHLOROPHYLL AND ANTHOCYANIN CONCENTRATIONS IN *PRUNUS CERASIFERA VAR. PISSARDII* AND *CATALPA BIGNONIOIDES*, IN URBAN AND URBAN GREEN AREAS OF TIMISOARA

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Abstract. *Urban air pollution has a significant impact on plant health, and biomonitoring is an effective method for assessing these effects. Variations in plant pigment content are sensitive to environmental conditions and serve as a crucial parameter for revealing relevant data on urban habitat quality. This study is based on the non-invasive measurement of chlorophyll and anthocyanin pigment contents in the leaves of the ornamental tree species Prunus cerasifera var. pissardii and Catalpa bignonioides. Data were collected using two portable pigment meters: the OPTI-SCIENCES CCM-300 for chlorophyll content and the OPTI-SCIENCES ACM-200plus for anthocyanin content. The aim was to compare pigment concentrations between individuals located in urban areas exposed to traffic pollution and those in urban green areas, in parks in the city of Timișoara, Romania. Statistical analysis of the data demonstrated that Prunus cerasifera var. pissardii exhibits two adaptive mechanisms in response to urban pollution from road traffic: an increase in chlorophyll pigment concentration and an increase in anthocyanin concentration in foliage, both observed in individuals located in urban areas compared to those in urban green areas. In contrast, Catalpa bignonioides appears to be less resistant, with urban areas individuals showing a tendency to decrease chlorophyll pigment concentrations. This study highlights the importance of using biochemical parameters to monitor the impact of pollution on vegetation and underscores the potential of certain ornamental species as bioindicators of air quality.*

Keywords*: biomonitoring, portable pigment meters, urban pollution, urban green, anthocyanins, chlorophylls*

INTRODUCTION

Air pollutants cause changes in the atmosphere's composition and affect the biotic environment. (NAVNEET & ADAK, 2021). Biomonitoring is a common method for the efficient and inexpensive assessment of urban air quality (ALLAJBEU et al., 2017). Since plants are constantly exposed to air, they are the primary receptors for both gaseous pollutants and atmospheric particles. In terrestrial plant species, the enormous leaf surface acts as a natural filter for pollutants, especially particles (PETROVA et al., 2022). Vegetation traffic barriers along roads can be an effective structure to improve roadside air quality (SHRESTHA et al., 2021)

Leaves are sensitive and highly exposed to air pollution. These vegetative organs of plants can act as persistent absorbers in a polluted environment (PRUSTY et al., 2005). Thus, measuring and monitoring the content of substances in the composition of leaves can reveal relevant data regarding the quality of the environment in the habitat of plants.

Foliar pigments are the basis of the absorption of photosynthetically active radiation by plants, contributing to photosynthesis but also to protection and adaptation to environmental changes. (GITELSON et al., 2020). In most cases, the variation in chlorophyll content is monitored, as this photosynthetic pigment is found in all green cells. There are numerous studies on chlorophyll content in urban pollution studies (DRĂGUCIAN ET AL., 2022). Invasive

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measurement methods based on laboratory procedures are time-consuming, expensive and not suitable for high-throughput phenotyping. (GHOLIZADEH et al., 2017). Non-invasive methods can also be used to monitor chlorophyll content in plant tissues in vivo, such as spectroscopy and portable devices that count the amount of chlorophyll, depending on specific spectral indices. (JIANG et al., 2020).

Anthocyanins are pigments involved in stress responses, protecting leaves against excessive radiation, premature senescence and water loss. (LANDI et al., 2015; PICCOLO et al., 2022). The presence and variation of these pigments in the leaves of urban trees may signal the ability of plants to adapt to pollution and other abiotic stress conditions.

The study of chlorophyll and anthocyanin pigments by rapid and non-invasive methods provides a relevant picture of the impact of abiotic stressors on urban vegetation, constituting a useful resource for environmental monitoring, air quality assessment and supporting green infrastructure in cities.

This study aims to non-invasively measure and compare the content of chlorophyll and anthocyanin pigments in 2 species of ornamental trees from Timișoara, both in polluted urban and urban green environments, to determine the influence of urban habitat quality on the 2 species, *Prunus cerasifera var. pissardii* and *Catalpa bignonioides*.

MATERIAL AND METHODS

The investigation was carried out in the city of Timișoara by measuring the content of chlorophyll and anthocyanin pigments in the leaves of two ornamental tree species, using non-invasive methods.

The study was based on field measurements of the two pigments' content in the leaves of the ornamental tree species *Prunus cerasifera var. pissardii* and *Catalpa bignonioides*, using two portable pigment meters: a chlorophyll content meter, OPTI-SCIENCES CCM-300 and an anthocyanin content meter, OPTI-SCIENCES ACM-200plus. OPTI-SCIENCES ACM-200plus showed ACI units and OPTI-SCIENCES CCM-300 CHL units.

The measurements were performed quickly and non-destructively, without collecting plant material samples, since the readings were taken directly in field conditions. After calibrating each device, the leaf tissue was placed between highly sensitive detectors, and the results were displayed on the instruments' digital screens. The measured pigment contents are expressed in mg/m².

The data were collected with the aim of comparing pigment contents in the leaves of individuals located in urban areas polluted by road traffic and those from individuals in urban green areas, such as parks. The urban environment (U) is represented by heavily trafficked areas in the immediate vicinity of boulevards, while the urban green environment (UG) was selected within parks, at a distance >50 meters from any boulevard. The two species were considered due to their distribution, as they are found both in the traffic-polluted urban environment – alongside streets (in the case of *Prunus cerasifera var. pissardii*) or very close to boulevards (<5 m, in the case of *Catalpa bignonioides*) – and in the urban green environment, within the parks of the studied areas.

The areas of interest for the measurements were Vasile Pârvan Boulevard, Michelangelo Underpass, Roses Park (Parcul Rozelor), "Ion Creangă" Children's Park (Parcul Copiilor „Ion Creangă"), and the park located on Corneliu Coposu Boulevard. The data collection area and each zone are highlighted in Figure 1.

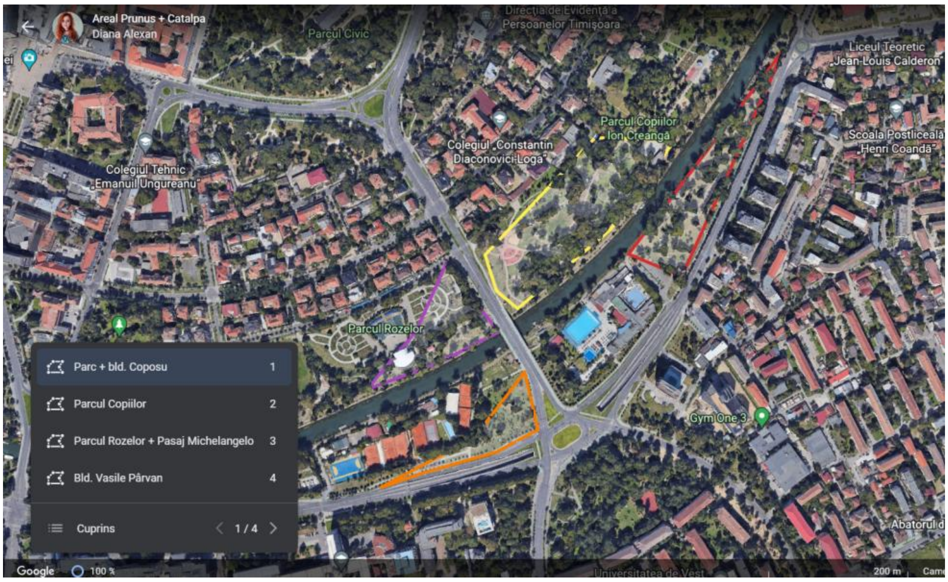

Figure 1. The area where data from the 2 species were collected
(1 – Corneliu Coposu Park and Blvd. (red); 2 – "Parcul Copiilor Ion Creangă" Park (yellow); 3 – Rozelor Park and Michelangelo Passage (purple); 4 – Vasile Pârvan Blvd. (orange))

The data were collected in June 2022, during the summer season, which is characterized by a significant increase in temperatures to which the species were exposed. Thus, the two ornamental tree species in the urban environment were subjected to several abiotic stress conditions, including: high temperatures, very low humidity, intense solar radiation, and pollution caused by heavy road traffic in the highly congested boulevard areas. During the measurement period, *Catalpa bignonioides* was in the flowering stage, while *Prunus cerasifera var. pissardii* was in the fruiting stage.

A total of 480 measurements were conducted. For each of the two ornamental tree species, 240 readings were taken – 120 for each parameter, with 60 measurements taken in U areas and 60 in the UG ones.

The collected data were transcribed into Microsoft Excel, from where they were statistically processed using the Student's t-test (T.TEST function) and the averages of chlorophyll or anthocyanin pigment contents were calculated for each species, depending on the area – U/UG.

As a result of the non-invasive measurement of chlorophyll and anthocyanin pigment content in the leaves of the two ornamental tree species in the two described urban areas the readings were compiled and categorized based on the pigment of interest.

RESULTS AND DISCUSSIONS

Anthocyanin pigment

Catalpa bignonioides

For the foliar anthocyanin pigment (ACI) content, 3 average values were calculated for *Catalpa bignonioides* (average content in U zones, average in UG zones and in the entire monitored area, U+UG).

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The value obtained from the UG environment is considered the reference, because it is the area not influenced by the effects of pollution induced by intense road traffic. The average value obtained from UG is 4.25 ACI, while in the U zone the value varied very little, almost insignificantly, being 5.25 ACI.

Thus, observing this lack of significantly different values, it can be stated that this species has not developed a mechanism for adapting to the polluted environment, based on the level of anthocyanin pigments, which should protect the foliar mesophyll. Regarding the average value in the entire area where the measurements were made, regardless of the area, this is 4.58 ACI.

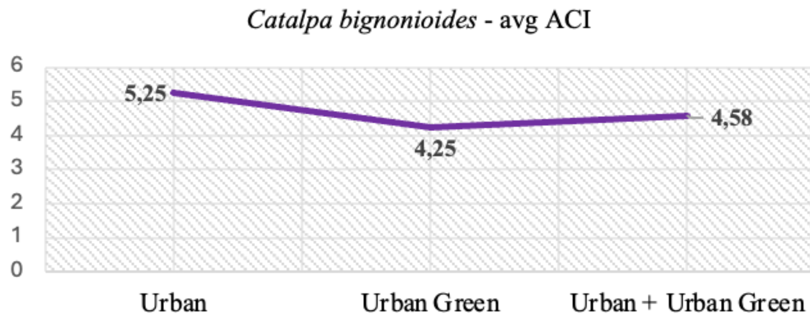

Figure 2. The 3 average values for foliar anthocyanin (ACI) content, measured for *Catalpa bignonioides* (U areas, UG areas, U+UG)

Prunus cerasifera var. pissardii

Regarding the ACI content in the leaves of individuals of the species Prunus cerasifera var. pissardii, the 3 values were significantly different. The average value of the foliar anthocyanin content in U zones was 113.56 ACI, while that in the UG environment, the one considered with values within normal limits, was 79.78 ACI. The average in U+UG was 96.67 ACI.

Thus, in the urban environment subjected to the effects of abiotic stress factors, a significant increase in the anthocyanin content can be observed in the leaves of the trees exposed to unfavorable environmental conditions. The increase in the content of anthocyanin pigments represents, for this species, a way to adapt its tissues exposed to polluting environmental factors.

Due to the increased level of anthocyanins, the leaves in the urban street environment were intensely colored and the adaptation of their phenotypic characteristics could be observed through the much smaller size of the leaves and their impressive thickness (the meters often showed the message "sample too dense" which did not allow the device to display an accurate result).

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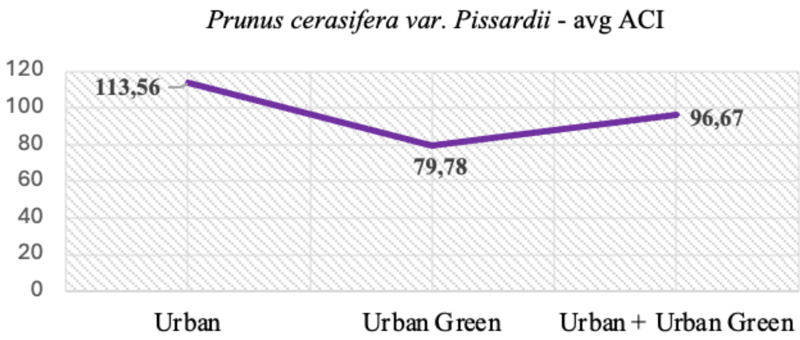

Figure 3. The 3 average values for foliar anthocyanin (ACI) content, measured for *Prunus cerasifera var. pissardii* (U areas, UG areas, U+UG)

Existing studies have shown that there is a relationship between anthocyanin formation and environmental temperature. According to the study by Li and Cheng (2009), high-temperature stress triggers plant protection mechanisms, and their example focused on anthocyanins, which reduce high-temperature damage to the skin of Pypus sp., by increasing pigment concentration. Existing studies have demonstrated that there is a relationship between anthocyanin formation and environmental temperature. According to Li and Cheng (2009), high temperature stress triggers plant protection mechanisms, and their example was based on anthocyanins, which reduce high temperature damage to the bark of Pypus sp., increasing the concentration of pigments.

Chalker-Scot (2000) states that anthocyanin pigments in leaf tissues have a dual function, as absorbers of harmful levels and/or wavelengths of radiation and as osmotic regulators. Anthocyanins in leaves may be protective in preventing damage caused, directly or indirectly, by low temperatures, drought, UV radiation and environmental pollution (Krol et al., 1995).

The Student's t-test (0.05 statistical significance threshold) was applied to compare 2 independent sets of samples, making comparisons between:

• ACI content in the leaves of *Catalpa bignonioides* and in the leaves of the *Prunus cerasifera var. pissardii*; the result, 9.144E-07, is significantly lower than the statistical significance threshold and revealed a strongly significant difference between the 2; anthocyanins are much more present in *Prunus cerasifera var. pissardii* compared to *Catalpa bignonioides*.

• ACI content between the 2 species, both sets of values being those in the U environment; after applying the t-test on these 2 independent sets of values, the result, 0.00029 shows a significant difference between the 2 species in the urban environment, also with a much higher content of anthocyanins in the *Prunus cerasifera var. pissardii* species.

• ACI content between the 2 species, both sets of values being those from the UG environment; the t-test value, 0.0018, shows that these are also significantly different, *Prunus cerasifera var. pissardii* having a significantly higher content in this reference environment for the measured values.

Chlorophyll pigments

Catalpa bignonioides

The same procedure was applied in the case of chlorophyll pigments (CHL), and based on the primary data, three average values were calculated (CHL content in the urban environment – U, in the peri-urban environment – UG, and across the entire monitored area – U+UG) for both Catalpa bignonioides and Prunus cerasifera var. pissardii.

The average value of the UG data is considered the reference. Thus, for *Catalpa bignonioides*, the average chlorophyll content in the UG area was 286.53 CHL, while in the U area, the chlorophyll value decreased moderately due to the influence of pollution factors, reaching 242.34 CHL. If the average was calculated across all the measured locations, regardless of zone, the result is 264.46 CHL.

The high level of pollution due to heavy road traffic and the abiotic conditions typical of the warm season destabilized the foliar chlorophyll content, revealing the lack of adaptive traits in *Catalpa bignonioides* and its low resistance under stress conditions. The reduced chlorophyll pigment levels in street-aligned individuals were also visibly expressed through leaf chlorosis.

The sensitivity of foliar chlorophyll content to poor environmental conditions is supported by Molnar et al. (2018), who focused on assessing the effects of urbanization on 10 tree species by monitoring chlorophyll-a levels. Their study demonstrated that chlorophyll-a content in leaves was a useful indicator for evaluating air pollution levels. Additionally, Sen et al. (2017) and Wagh et al. (2006) also support the idea that, except for a few extremely tolerant species, chlorophyll-a content is generally negatively affected by air pollution.

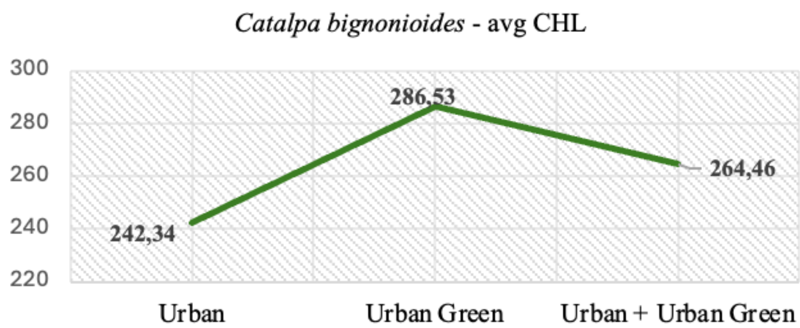

Figure 4. The 3 average values for foliar chlorophyll (CHL) content, measured for *Catalpa bignonioides* (U areas, UG areas, U+UG)

Prunus cerasifera var. pissardii

For chlorophyll pigment content in the leaves of *Prunus cerasifera var. pissardii* species, the values show differences that reflect the adaptive character of the species to unfavorable environmental conditions.

The average foliar CHL value in the urban environment (U) is 288.22 CHL, while in the UG area, considered the reference, it is 223.17 CHL. For this species, an increase in chlorophyll concentration was observed under exposure to environmental conditions such as high solar radiation, elevated temperatures, low humidity, and heavy road traffic. The overall average across both the urban and urban green zones is 255.69 CHL.

This increase in chlorophyll pigment content, also observed in anthocyanin levels in individuals located near boulevards, demonstrates that this variety of the Prunus genus possesses two adaptive mechanisms to poor-quality urban conditions. These mechanisms grant the species notable resilience, thereby justifying its frequent use in street alignments within urban environments.

In a 2022 study, Zhang and collaborators also observed an increase in chlorophyll content in plants subjected to abiotic stress, specifically high heat and drought conditions. The study also described the phenotypic characteristics of plants with high chlorophyll content, traits that were likewise observed in individuals of *Prunus cerasifera var. pissardii* growing in street alignments along boulevards: the leaves of these specimens are smaller and significantly thicker, which contributes to the higher chlorophyll content.

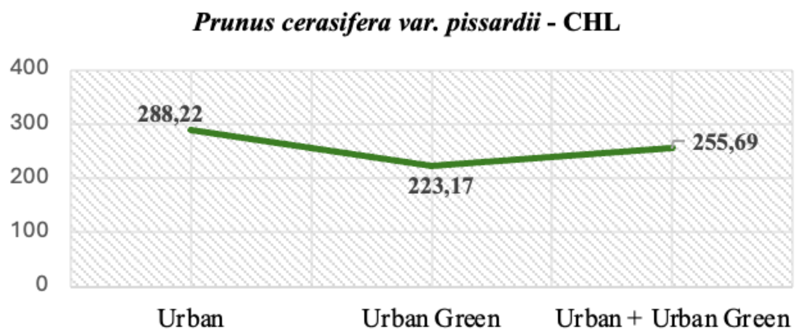

Figure 5. The 3 average values for foliar chlorophyll (CHL) content, measured for *Prunus cerasifera var. pissardii* (U areas, UG areas, U+UG)

In the case of CHL concentrations, the Student's t-test (with a 0.05 threshold for statistical significance) was also applied to compare independent sets of samples, resulting in the following comparisons:

- CHL content in *Catalpa bignonioides* vs. *Prunus cerasifera var. pissardii*: the t-test result was 0.43, which is significantly higher than the threshold for statistical significance. This indicates that the chlorophyll levels in the two species are not significantly different overall.
- CHL values between the two species, with both data sets coming from the U environment: yielded a result of 0.00135, indicating a significant difference between the two species in urban conditions. *Prunus cerasifera var. pissardii* had a higher chlorophyll content compared to the other investigated species.
- CHL values between the two species, both data sets coming from the UG environment: the t-test value was 0.00014, also indicating a significant difference. However, in this case, under normal environmental conditions, *Catalpa bignonioides* had the higher chlorophyll content. This further supports the idea that the elevated chlorophyll pigment concentration observed in *Prunus cerasifera var. pissardii* is an adaptive mechanism in individuals planted along streets in urban settings.

CONCLUSIONS

Catalpa bignonioides is a less resilient species, as the concentration of anthocyanin pigments in its leaves remains almost unchanged under the influence of the polluted urban environment. Regarding the action on chlorophyll, the leaves of individuals in the polluted environment show a tendency toward decreased chlorophyll pigment contents.

Prunus cerasifera var. pissardii demonstrates greater resilience to pollution and abiotic stress conditions in the urban environment, as evidenced by the increase in both anthocyanin and chlorophyll pigment content in its leaves. This species displays two adaptive mechanisms, highlighted by this research, to the polluted urban environment caused by road traffic: an increase in foliar chlorophyll concentration and an increase in foliar anthocyanin concentration. Both effects were observed in individuals located in urban areas compared to those in urban green zones. These findings justify the use of this resilient species for street alignments in urban settings.

The results also indicate that, within the investigated area, there are indeed effects induced by road traffic, high solar radiation, elevated temperatures, and low humidity, all typical of the summer season.

However, more in-depth and complex future studies are needed to monitor the impact of traffic pollution effects on urban vegetation. It is also worth considering the development of standardized protocols for measuring foliar pigment contents using non-invasive methods.

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