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PRELIMINARY STUDY REGARDING PHYSIOLOGICAL BEHAVIOUR OF *PLANTAGO MAJOR* IN URBAN HABITAT USING DIFFERENT METHODS. CASE STUDY: TIMIȘOARA

Maria-Isabela PLEȘU, Ioana-Maria TOPLICEAN*, Alexandru PAHOMI, Daniela-Georgiana CIOBANU, Adina-Daniela DATCU
Biology-Chemistry Department, Faculty of Chemistry, Biology, Geography, West University of Timisoara, Romania
Corresponding author's e-mail: ioana.toplicean03@e-uvt.ro
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ABSTRACT
Plantago major is a known and widely used bioindicator of air quality, being common in urban habitats. This species responds pretty well to different variations in numerous environments. Thus, the aim of this study was to comparatively analyze Plantago major behavior in an urban habitat, using different methods. Non-invasive methods permit a quick and cheap assessment and can be used repeatedly for same vegetal individual. The research was conducted during Octomber 2022, in two different environments: urban and urban green zones, in the city of Timisoara, Romania. The urban zone was represented by a population from Cuvin streets and urban green zone was within Copiilor Park. The analyzed indices were chlorophyll and anthocyanin contents. The chlorophyll content was determined using a non-invasive approach, with OPTI-SCIENCES CCM-300 Chlorophyll Content Meter and the results were presented in mg m^{-2}*. The anthocyanin content was determined with OPTI-SCICENCES ACM-200 Plus Anthocyanin Content Meter and the results were presented in ACI. Also, spectrophotometric qualitative determination of chlorophyll was realized. Data analysis was done using Microsoft Office Excel 2016 and statistical processing was realized with PAST software v4.03. The results of the study confirm its capacity for being an indicator of urban habitat health. Plantago major has a bigger chlorophyll content in urban green zone. Also, this plant adapts to urban green areas through a deposition of anthocyanin at foliar level, but chlorophyll in the leaves from this environment is lower.*
KEY WORDS: *Plantago major, chlorophyll content, anthocyanin content, spectrophotometric determinations*

INTRODUCTION

Plantago major L. (greater or common plantain), as member of the Plantaginaceae family, is a perennial herbaceous plant, having the leaves in a rosette. It is a really common herb and in cities is found on waste areas, roadsides, in parks or cultivated zones (Galal & Shehata, 2014; Lacey & Herr, 2000). Woody or herbaceous plants could be used in habitat quality assessment due to their wide distribution and high accessibility (Kardel et al. 2009; Ianovici et al. 2015; Ianovici, 2016; Ianovici et al. 2017).

Although criteria like the specific leaf area have been acknowledged to fluctuate based on microclimatic circumstances, morphological and plant biomass characteristics have received less attention as indications of long-term urban habitat change (Balasooriyaa et al. 2009). Emissions from all sources of combustion have an ongoing impact on urban air quality. Air pollution monitoring with accumulation surfaces of green elements is frequently employed as a quick, yet dependable method to investigate habitat quality in cities and detect contamination hot areas, mostly in terms of particle matter (PM) pollution (Sawidis et al. 2011; Castanheiro et al. 2016; Dzierżanowski et al. 2011; Kardel et al. 2011; Mo et al. 2015; Popek et al. 2013; Tomašević et al. 2005; Wang et al. 2013). Particulate matter and gaseous contaminants, produced by a variety of dynamic physical and chemical processes, can have a deleterious influence on plant life, human or animal health, and on the ecosystem at large through atmospheric change (Ghorbanli et al. 2007). Industrial pollutants are generally recognized to have a harmful impact, particularly on leaves (Manning & Feder 1980; Lacasse & Treshow, 1976; Schubert 1985). It is quite simple and affordable to collect samples of plants and analyze their morphological and anatomical characteristics to look for long-term responses to changing urban habitat quality (Balasooriya et al. 2009).

For ages, various regions of the world have employed the *Plantago* species as a traditional medicine (Abd El-Gawad et al. 2015; Gomes de Andrade et al. 2018). Both in urban and rural environments, *Plantago lanceolata* and *P. major* are mainly spread and simple to identify. Both species are known to contain high amounts of trace elements, according to previous investigations (Tinkov et al. 2016; Nadgórska-Socha et al. 2017; Skrynetska et al. 2018).

On the other hand, the capacity to measure leaf pigment concentration and composition using remotely sensed data offers a distinctive nondestructive capability to evaluate photosynthetic activities in plants as well as monitor and diagnose foliar conditions and various environmental challenges (Anatoly et al. 2009). It is generally acknowledged that anthocyanin perform vital biological processes involved in stress reduction and adaptability (Chalker-Scott 1999; Neill & Gould 1999; Hoch et al. 2001; Steyn et al. 2002; Close & Beadle 2003; Hughes & Smith 2007; Gould et al. 2008). Chlorophyll pigments are necessary for converting light energy into chemical energy which is stored. Traditional methods for determining chlorophyll and anthocyanin require the extraction of pigment in organic solvents for subsequent spectrophotometric measurements (Anatoly et al. 2009).

Moreover, a type of spectroscopy called spectrophotometry measures a material's reflectance or transmission qualities numerically as a function of wavelength. Measurements of spectral reflectance should be used for nondestructive remote estimation of pigment concentration in situ under field conditions. Researchers have created several reflectance modifications to help them identify the principal leaf pigments, called vegetation indices (Chappelle et al. 1992; Buschmann & Nagel 1993; Gitelson & Merzlyak, 1994).

The goal of this study was to analyze *Plantago major* behavior through some cheap and quick indices, in urban habitat. In addition, we have tested the chlorophyll amount obtained from spectrophotometric analyzer from two zones, an urban and an urban green one.

MATERIAL AND METHODS

The studied species was *Plantago major*, a known bioindicator of air quality in urban environments. The study was done within Timișoara, Romania, in two different urban habitats: urban and urban green zones. Also, the samples were analyzed together, as Timisoara habitat. Data were collected during the autumn of 2022. The urban zone was represented by Cuvin street and the urban green zone was in Copiilor Park, far from any highly circulated area. Only healthy and intact leaves were investigated. The analyzed indices were: anthocyanin content (ANTC), using OPTI-SCICENCES ACM-200 Plus Anthocyanin Content Meter and the results were presented in ACI and chlorophyll content (chl) was determined with OPTI-SCIENCES CCM-300 Chlorophyll Content Meter and the results were presented in mg m^{-2}. Both analyzers are non-invasive and permit cheap and quick determinations. 240 readings were done: 120 in urban zone and 120 in urban green zone. Data from both areas were compared with the total. Also, some preliminary spectrophotometric chlorophyll determinations were realized. There were several types of materials used to determine chlorophyll: Plantago leaves, a watch glass, a mortar and pestle, filter paper, a filter funnel, a test tube, and spectrophotometer cuvettes. A balance, a centrifuge, and a spectrophotometer are also required. The substance used to extract the chlorophyll from the leaves is acetone, with a concentration of 80% (Lichtenthaler et al. 1987).

The leaves were weighted first since only 125 mg of leaves per samples are necessary. These were macerated in a pestle with mortar with 10 mL of acetone at an 80% concentration. After shredding the leaves, the liquid from the solid were separated using a filter funnel and filter paper. Following this process, the cuvette was introduced into the spectrophotometer and the intensity of the green color of the pigment was measured at 645 nm for chlorophyll a, 633 nm for chlorophyll b, and 652 nm for total chlorophyll content (Arnon, 1949). In this case T90+ UV/VIS Spectrophotometer was used.

The processing of data was done using Microsoft Office Excel 2016 and statistical analysis was realized using Past v 4.03 (Hammer et al. 2001). The homogenous or heterogeneous distribution of data was analyzed using Shapiro-Wilk test. Next, Levene's test for homogeneity of variance was done. In addition, Welch test for unequal variances and ANOVA in the case of equal variances were applied.

RESULTS AND DISCUSSION

In figure 1, the mean values±SE of chl content for all studied zones, can be observed. Moreover, the outlines are also presented. The biggest mean value for chl content was observed in urban green zone (Chl = 385.6667 mg m^{-2}) and the lowest value in urban zone (Chl = 323 mg m^{-2}). The readings from urban green zone has the minimum point of Chl = 256 mg m^{-2}, and the maximum point of 472 mg m^{-2}. In the case of urban zone, the minimum point of Chl from Plantago leaves was Chl = 205 mg m^{-2}, and the maximum point was Chl = 402 mg m^{-2}.

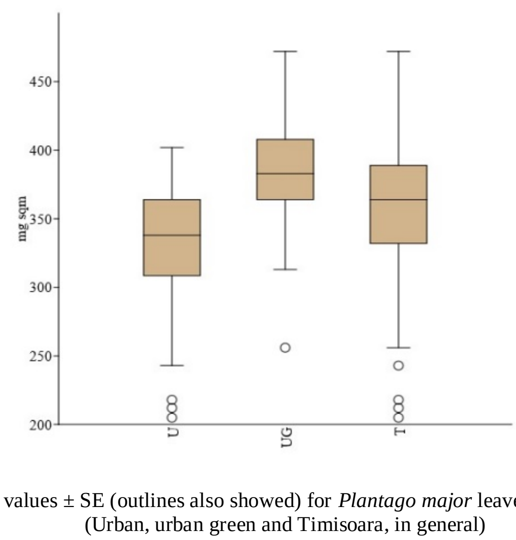

FIG 1. Chlorophyll mean values ± SE (outlines also showed) for *Plantago major* leaves from the three analyzed zones (Urban, urban green and Timisoara, in general)

Same trend was observed for other species, Chl content being bigger in urban green zones for *Liquidambar styraciflua, Acer negundo* and *A. platanoides* (Drăgucian et al. 2022). Other authors also showed that chlorophyll content is affected by the urbanization (Dezhban et al. 2015; Duan et al. 2019). It can be appreciated that the investments in mesophyll are better for the plants from urban green zone.

After the completion of Shapiro-Wilk test for testing the distribution of the data, it was observed that per total and in urban zone, the data did not have a normal distribution. In urban green zone, data had a normal distribution. Next, Levene´s test for homogeneity of variance conducted to the need for ANOVA test. Significant differences between the analyzed group were obtained ($p = 0.000000008093$, $F = 20.18$, $df = 2$). Thus, the chl content of the samples from urban green zone was significantly bigger, when compared to the rest of the readings. So, photosynthesis efficiency is bigger in urban green zone.

In figure 2, mean±SE values of anthocyanin content for all studied zones can be observed. Also, outlines are showed.

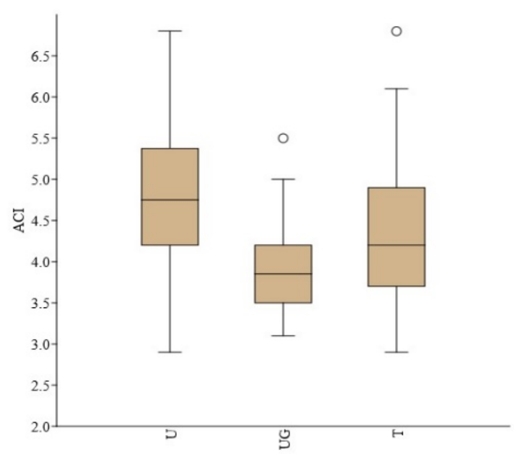

FIG 2. Anthocyanin mean values ± SE (outlines also showed) for *P. major* leaves from the three analyzed zones (Urban, urban green and Timisoara, in general)

Mean ANTC in urban zone was bigger (ANTC = 4.765 ACI), when compared with all samples (ANTC = 4.325 ACI) and urban green zone (ANTC = 3.885 ACI). Regarding urban zone, the minimum point of ANTC was 2.9 and the maximum point red was 6.8. In the case of urban green zone, the minimum point of anthocyanin content was 3.1, and the maximum point was 5.5.

Same trends were observed for other species, like *Cotinus coggygria*, *Liquidambar styraciflua*, *Acer negundo* and *A. platanoides* (Lăpădat et al. 2022). Thus, plants from urban zones tend to accumulate more anthocyanin.

After the completion of Shapiro-Wilk test for testing the distribution of the data, it was observed that per total and in urban green zone, the data did not have a normal distribution. In urban zone, the data had a normal distribution. Next, Levene´s test for homogeneity of variance conducted to the need for Welch F test. The results of this test showed that there are significant differences between the analyzed data ($p = 0.000000000102$, $F = 27.42$, $df = 134.9$).

Also, chlorophyll types from both zones were presented through spectrophotometric analysis. The chlorophyll spectrum for the extracts from urban zone can be observed in figure 3 and that for the extracts from the urban green zone can be observed in figure 4.

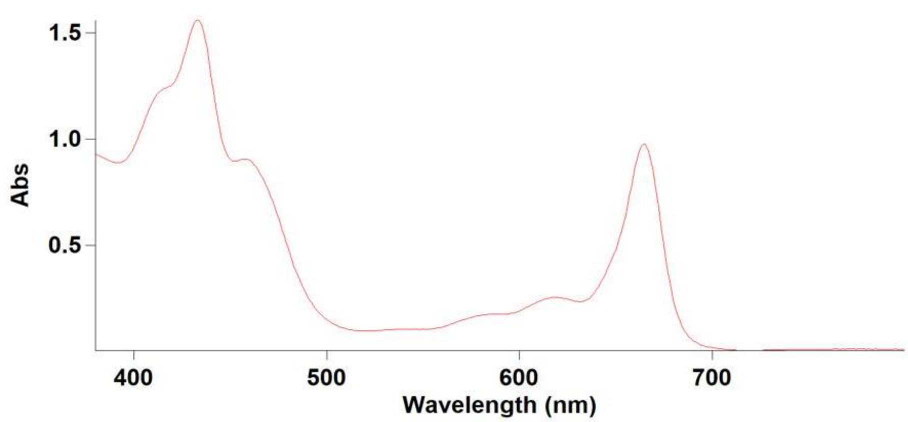

FIG 3. Chlorophyll spectrum for *P. major* leaves from urban zone

The readings were done for 645 nm, 652 nm and 633 nm. For the samples from urban green zone, chl content was bigger that the chl content for the samples from urban area. This is in accordance with the results obtained using the non-invasive method.

According to Kumar et al. (2012), the concentration of photosynthetic pigments is affected by traffic load, and chlorophyll content may fluctuate in all of the selected plant species during the sampling period based on their habitat, climatic condition, pollution level, and so on.

Several researchers have observed that the total chlorophyll content of polluted leaves is lower than that of control leaves (Somashekar et al. 1999; Mandal & Mukherji, 2000).

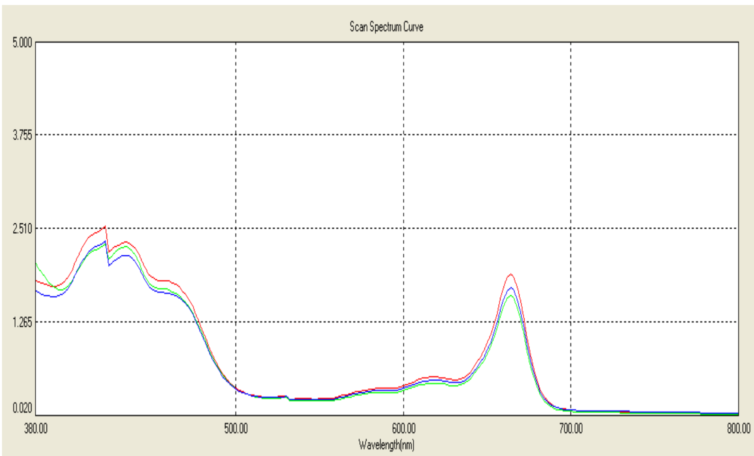

FIG. 4. Chlorophyll spectra for *P. major* leaves from urban green zone

The readings show a similarity between the three samples subjected to the spectrophotometer. Also, it was obeserved that the chlorophyll content is higher in healthy plants as compared to contaminated plants, where the chlorophyll "a" content was lower (Kumar et al. 2012).

The chlorophyll a level of polluted leaves varied, indicating that the chlorophyll content was drastically reduced in comparison to the measured in chosen plant leaves (Kumar et al. 2012). All plant species had a significant decrease in total chlorophyll content in the traffic area. *Pongamia pinnata(L)* had a much lower total chlorophyll content than the control (Somashekar et al. 1999).

CONCLUSIONS

Plantago major has been widely used in biomonitoring studies and is known as a good bioindicators of air quality in various habitats, including urban zones. In this study, two content meters were used in order to obtain values of chlorophyll and anthocyanin contents. Using this meters, further readings can be done for same individuals. The results show that chlorophyll content is significantly bigger in urban green zone, when compared to urban zone. In urban green zone, it can be appreciated that photosynthesis and other physiological processes are more efficient.

In the urban green zone, this plant has a bigger level of anthocyanin. These compounds help the plant to adapt to a zone with heavy traffic and industry. Further studies can be done to observe the behavior of these plant species regarding chl content and ANTCH in seasonal dynamics or quantitative spectrophotometric analysis for chlorophyll and other compounds.

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