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Photosynthetic Pigments as Parameters/Indicators of Tree Tolerance to Urban Environment (Plovdiv, Bulgaria)

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Abstract. As polluted air is a stress factor that contributes to the decline of trees in urban areas, we aimed to investigate the complex impact of anthropogenic activity on chlorophylls and carotenoids content of four tree species (*Acer heldreichii* Boiss., *Tilia tomentosa* Moench, *Fraxinus excelsior* L. and *Pinus nigra* L.). Seedlings were purchased from certified greenery and planted by us at four selected sites in the city of Plovdiv (Bulgaria) during spring of 2015. Leaf samples were taken monthly and photosynthetic pigments content was measured immediately after sampling. Results of this preliminary study confirmed that pigment levels in plants varied between species, locations and seasons. Although an extension to the above work is necessary to quantify possible differences between the levels at which photosynthetic components are affected, it is obvious that both ratios chlorophyll a/b and chlorophylls/carotenoids could serve as very useful indicators of stress level. Because of the non-specificity in pigment reaction to different type of anthropogenic impact, we recommended to apply a combination among pigments concentration and another parameters (morphological, biochemical, physiological) for the targets of biomonitoring.

Key words: photosynthetic pigments, air pollution, urban environment, *Acer heldreichii, Tilia tomentosa, Fraxinus excelsior, Pinus nigra*.

Introduction

Over billions of years, green plants have formed as a perfect system for absorbing and transforming solar radiation. Radiation absorbed by plant communities is included as a driving force of all life processes or remains stored in the accumulated biomass, defining the overall productivity of plants. Productivity is a complex and multi-step process that involved many different interconnected and interdependent processes. In the ontogenetic development plants are subjected to the continuous impact of environmental factors, including the atmospheric anthropogenic pollution. Exceeding values above the permissible level, and the continued operation of the average concentrations of toxic gases, aerosols and dust negatively affect the status and operation of the plant organism. Green plant as a complex integrated system operating on the principle of feedback and self-regulation is particularly vulnerable to any extreme influence.

Some authors have discussed that the plants have growing under conditions of constant "re-adaptation" to the dynamic presence of various contaminants in the environment. According to LEVITT (1972) sustainability of the plant organism to the impact of extreme factors is determined by two basic reactions - "ability to avoid stress" and "resistance to stress". Stress effects on plants have three stages: 1) primary stress reactions; 2) adaptive responses; 3) weight loss and death. In a strong but short-term stress exhibit the nonspecific adaptive responses while in a prolonged impact – the specific mechanisms. It has been shown that repeated high doses of stress cause a kind of "hardening" of the plant to stress impact and that "curing" to one stress factor may lead to adjustments of the body and to other stress factors.

Chlorophyll content is an important parameter to evaluate the effect of air pollutants on plants as it plays an important role in plant metabolism. For example, it can be used as an index of the photosynthetic potential as well as of the plant productivity (CARTER, 1998) and is closely related to various types of plant stresses and senescence (GITELSON & MERZLYAK, 1994). Carotenoids are accessory pigments and essential structural components of the photosynthetic antenna and reaction centers in higher plants. As non-enzymatic antioxidants, their main function is to protect the photosynthetic apparatus, dissipating energy to avoid harmful photooxidative processes.

Aim of this study is to make field evaluation of air pollution tolerances of three deciduous (*Acer heldreichii, Tilia tomentosa, Fraxinus excelsior*) and one coniferous (*Pinus nigra*) trees as reflected by their photosynthetic pigments content.

Material and Methods

Sampling plots. For the purposes of our study, four sampling plots were selected on the basis of typology of urban environment they represent and the type of anthropogenic impact (Fig. 1). Seedlings of selected tree species were purchased from certified greenery and planted in the plots during the spring of 2015.

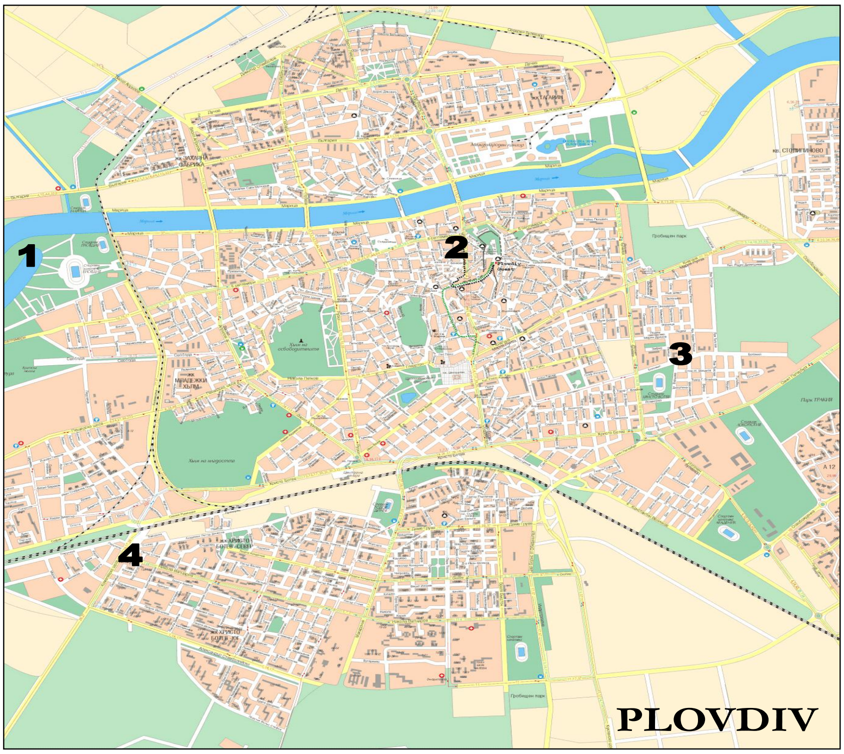

Fig. 1. Map of Plovdiv and locations of the four selected plots: Plot 1 – at City park "Recreation and Culture" (Control); Plot 2 – near Automatic station for environmental monitoring "Banya Starinna; Plot 3 – at Automatic station for environmental monitoring "Kamenitza"; Plot 4 – at Komatevo Road Junction.

Plot 1 was chosen in the West part of the city (NW direction), in the big city park "Recreation and Culture", subjected to a low anthropogenic impact. Trees were planted in a large green area, close to the city outskirts and we aimed to use them as a "provisional control" of urban environment.

Plot 2 was chosen in the Central part of the city (NE direction), along to a busy road junction and close to the Automatic station for environmental monitoring, named "Banya Starinna". Trees were planted in a large green area, located at a distance of about 5 m of the junction.

Plot 3 was also situated in the Central part of the city (SE direction), close to the Automatic station for environmental monitoring, named "Kamenitza" and was characterized by moderate household pressure. Trees were planted in small green patches, 8–10 m away from tall buildings.

Plot 4 was chosen in the West part of the city (SW direction), "Smirnenski"suburb and the trees were planted in a large green area, situated between two railroad tracks and the "Komatevo" road junction.

Plant species. Based on our previous researches (PETROVA *et al.*, 2014) and on the literature review (PICZAK *et al.*, 2001; ROMANIC & KRAUTHACKER, 2004; AKSOY & DEMIREZEN, 2006; SERBULA *et al.*, 2013), three deciduous and one coniferous tree species were selected.

Acer heldreichii, common names Heldreich's maple, Greek maple and Balkan maple, is a European species of maple. It is native to Greece, Albania, Bulgaria, Macedonia, Montenegro, Kosovo, Serbia, and Bosnia-Herzegovina. Tree is up to 20 m, with broad, spherical crown. Leaves are 5–14 cm long, deeply 5-lobed with middle lobe free nearly to base; lobes acute, with 2 or 3 large teeth on each side, dark green, glabrous above, light green and hairy on veins beneath. Fruits are glabrous, with arcuate wings usually diverging at an obtuse angle. *Acer platanoides* is well known biomonitor of air pollution (SAEBO *et al.*, 2012; PETROVA *et al.*, 2014), but we could not find any information about *Acer heldreichii* as an object of biomonitoring studies. That was the reason for choosing this species.

Tilia tomentosa, common names Silver lime or Silver linden, is native to southeastern Europe and southwestern Asia, from Hungary and the Balkans east to western Turkey, occurring at moderate altitudes. *T. tomentosa* is a deciduous tree growing to 20–35 m tall, with a trunk up to 2 m in diameter. Leaves are alternately arranged, rounded to triangular-ovate, 4–13 cm long and broad with a 2.5–4 cm petiole, green and mostly hairless above, covered by densely white tomentose with white hairs below and with a coarsely toothed margin. This species is approved as efficient biomonitor in many studies because of its bioaccumulation capability and sensitivity to air pollution (BRAUN *et al.*, 2007; ANICIC *et al.*, 2011; PETROVA *et al.*, 2014).

Fraxinus excelsior, known as European ash or Common ash, is native to most of Europe from Portugal to Russia, with the exception of northern Scandinavia and southern Iberia. It is also considered native in southwestern Asia from northern Turkey east to the Caucasus and Alborz mountains. It is a large deciduous tree, growing to 20–35 m tall with a trunk up to 2 m diameter, with a tall, domed crown. Bark is smooth and pale grey on young trees, becoming thick and vertically fissured on old trees. Shoots are stout, greenish-grey, with jet black buds (which distinguish it from most other ash species, which have grey or brown buds). Leaves are 20–35 cm long, pinnate compound, with 7-13 leaflets, the leaflets 3–12 cm long and 0.8–3 cm broad, sessile on the leaf rachis and with a serrated margin. Leaves are often among the last to open in spring and the first to fall in autumn if an early frost strikes; they have no marked autumn colour, often falling dull green. This species have not received very attention as potential biomonitor, only few studies were conducted (AKSOY & DEMIREZEN, 2006).

Pinus nigra, common names Austrian pine or Black pine, is a moderately variable species of pine, occurring across southern Mediterranean Europe from Spain to the eastern Mediterranean on Anatolian peninsula of Turkey and on Corsica/Cyprus, including Crimea. It is a large coniferous evergreen tree, growing to 20–55 m tall at maturity. Bark is grey to yellow-brown, and is widely split by flaking fissures into scaly plates, becoming increasingly fissured with age. It is also well known biomonitor of air pollution by organic substances and toxic elements (TSIKRITZIS *et al.*, 2002; ROMANIC & KRAUTHACKER, 2004; LEHNDORFF & SCHWARK, 2008; SAWIDIS *et al.*, 2011).

Leaf sampling and pigment analysis. Samples were taken monthly through the vegetation period when the leaf petiole was fully developed (July, August and September) and photosynthetic pigments content was measured immediately after sampling. In order to obtain a homogeneous sample, a large number of one–year–old leaves, comparable in size and shape, were taken by hand from the branches, taking care to minimize contact with the leaf surface. Usually 80–100 fully expanded leaves per tree were collected and a composite sample was prepared for analyses. All the samples were stored in clean, labeled, polyethylene bags, closed tightly to avoid contamination during transport.

Pigment analysis followed SHLYK (1965). Spectrophotometric reading of photosynthetic pigments was performed after extraction with 90% acetone at 440.5 nm for carotenoids, 644 nm for chlorophyll b and 662 nm for chlorophyll a. Concentrations of chlorophyll a, chlorophyll b, total chlorophylls and carotenoids were calculated for each sample and presented in mg g^{-1} fresh weight and then were calculated the ratios chlorophyll a/b and total chlorophylls/ carotenoids (PETROVA, 2011).

Data processing. All data presented in the present study were an average of triplicate analysis of three separate subsamples. The concentrations were expressed as arithmetic means and standard deviations (mean±SD). For the statistical evaluation of the data obtained, the raw values of 3 subsamples per species per site were used. T-test was performed for detecting significant differences between sampling plots and between studied plant species ($p<0.05$). All statistical analyses were made with the STATISTICA 7.0 statistical package (STATSOFT, 2004).

Results and Discussion

Our studies on determining the tree tolerance to the impact of urban environment were focused on the quantities of the main components of the pigment complex – chlorophyll a, chlorophyll b, total chlorophylls and carotenoids as chlorophylls and carotenoids are the main photosynthetic pigments in plants.

Total chlorophylls amount normally decreases from June to September and together with leaf ageing the decomposition of chlorophyll a is faster than chlorophyll b (WAGH *et al.*, 2006). Our results showed a complex situation in this respect when a comparison was made between selected plant species and sampling sites. Chlorophyll b was found as more susceptible to pollution compared to chlorophyll a, corresponding to the findings of PAVLOVIC *et al.* (2017). A similar trend was found by GAJIĆ *et al.* (2009) at polluted sites (urban parks of Belgrade), where higher content of chlorophyll b in *Ligustrum ovalifolium* Hassk. was measured in June than in October. In our study, the highest concentrations of chl b were determined in July in leaves of *Fraxinus excelsior* and *Tilia tomentosa*, followed by *Acer heldreichii* and *Pinus nigra*. However, the different responses of chlorophyll a and chlorophyll b are not unexpected, since different types of pollution exert different effects on the pigment content, hence the response of a plant can be attributed to the interaction between various types of pollutants as well as abiotic factors (high temperature, drought, intense insolation, etc.) (BRUGNOLI *et al.*, 1994; PAVLOVIC *et al.*, 2017).

In July and August, a general tendency towards increasing the amount of total chlorophylls and chlorophylls/carotenoids ratio in the three deciduous tree species was established in Plot 3 (moderate household pressure). Tendency to reduce pigments content and chlorophyll a/b ratio was observed in Plot 4 (heavy traffic). According to many authors (WAGH *et al.*, 2006; JOSHI & SWAMI, 2009; PAVLOVIC *et al.*, 2017) the changes in the pigment content are one of the first signs of the harmful effect on the plants caused by atmospheric pollutants. Toxic gases penetrate in leaves, accumulate in the chloroplasts and damage their structure leading to reduce of the pigment content. Although, many authors have shown that an increased content in polluting agent leads to chlorophyll inhibition either through direct inhibition of several enzymatic steps (JOSHI & SWAMI, 2009) or as a result of substitution of the central Mg ion (GAJIĆ *et al.*, 2009), several studies have shown that exposure to heavy metals induces oxidative stress which is accompanied by an increase in the chlorophyll content (PETROVA, 2011; PAVLOVIC *et al.*, 2017). Our results obtained by the studied coniferous species are in good agreement with these findings as in the same period, maximum content of photosynthetic pigments and ratio values in *Pinus nigra* were observed in the leaf samples from Plot 4 (heavy motor and railroad traffic) (Fig. 5, 9, 13).

In September, we found very different changes in the four studied plant species, as explained below. Maximum total chlorophylls (2.08 mg kg^{-1}) and carotenoids (0.32 mg kg^{-1}) content in *A. heldreichhi* leaves was measured in Plot 1 (control), while minimum content was obtained in Plot 3 (1.26 mg kg^{-1} and 0.27 mg kg^{-1}, respectively) (Fig. 2). Highest chlorophyll a/b ratio (1.81) was calculated for Plot 3 and lowest (1.37) – for Plot 4 (Fig. 6). When comparing with the previous months, it is obvious that the chlorophyll a/b ratio has decreased by 25%, probably due to the fast decomposition of chlorophyll b and its transform to chlorophyll a. Highest chlorophylls / carotenoid ratio was obtained in Plot 2 (7.65) and lowest (3.78) – in Plot 3 (Fig. 10). As a whole, we found an increment of this ratio which could be explained by both the increment of total chlorophylls content accompanied by a decrement of carotenoid content.

Tendency to maintain highest pigment content and ratios values in Plot 3 and lowest ones in Plot 4 during the all studied period was found in *T. tomentosa* leaves (Fig. 3, 7, 11). Data showed almost a twice increase in chlorophylls/carotenoids ratio (from 4.04-4.83 to 7.21-8.45) which is probably due to the reduced carotenoid synthesis (Fig. 3) in September.

F. excelsior showed similar pigment content in both 3 months of the study: chlorophyll a varied from 0.63 to 1.29 mg kg^{-1}; chlorophyll b from 0.53 to 0.94 mg kg^{-1}; carotenoids varied from 0.17 to 0.52 mg kg^{-1} (Fig. 4). Results from September revealed a significant increment of chlorophylls/ carotenoids ratio in Plot 3 (28%) and Plot 1 (261%) ($p<0.05$) (Fig. 12).

In *P. nigra* leaf samples the most pronounced change was the decrease of carotenoids within the vegetation period in all sampling plots, as follows: Plot 1 – 2.0 fold; Plot 2 – 2.66 fold; Plot 3 – 1.75 fold; Plot 4 – 2.42 fold ($p<0.05$) (Fig. 5). This reducing has led to the increased chlorophylls/ carotenoids ratio values reaching up to 13.57 in Plot 2 (Fig. 13).

SILLANPÄÄ *et al.* (2008) measured elevated carotenoid level in *B. pendula* leaves sampled from pollutes area in comparison with samples from unpolluted one. They indicated that carotenoids perform many important physiological functions in plants like influencing development and adaptation mechanisms, suggesting coordination of their synthesis in different physiological processes, but mostly serve as antioxidants against endogenous and exogenous oxidative stress. Oxidative stress caused by pollutants occurs when the amount of oxidants in the cell exceeds that of antioxidants. In our study, we found an increment in carotenoids content in September only in *F. excelsior* leaves from Plot 4 (Fig. 4), which was in agreement with the findings of SILLANPÄÄ *et al.* (2008) as this plot was characterized as most polluted. The low carotenoids values in the other plots and their dynamics could be explained by JOSHI *et al.* (2009), WAGH *et al.* (2006), JOSHI & SWAMI (2009) findings that the changes in pigment content can characterize the resistance and the degree of adaptation of plants to constant and high level of atmospheric pollution in the environment.

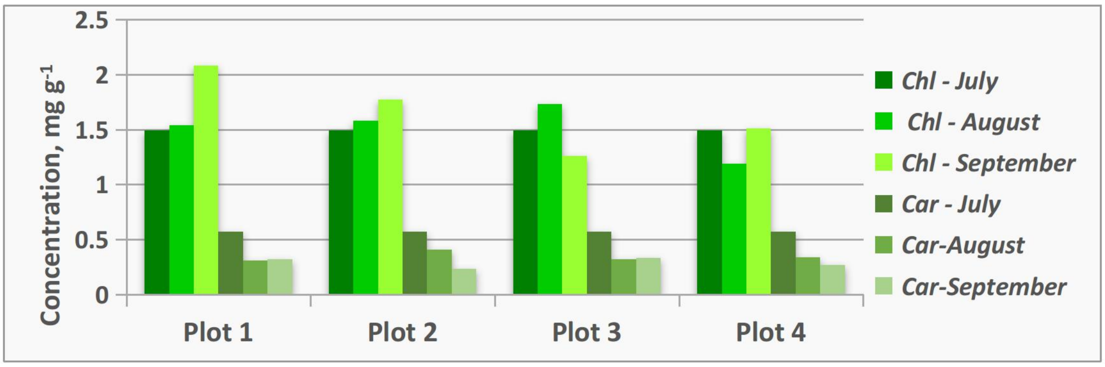

Fig. 2. Total chlorophylls and carotenoids content in *Acer heldreichii* leaf samples.

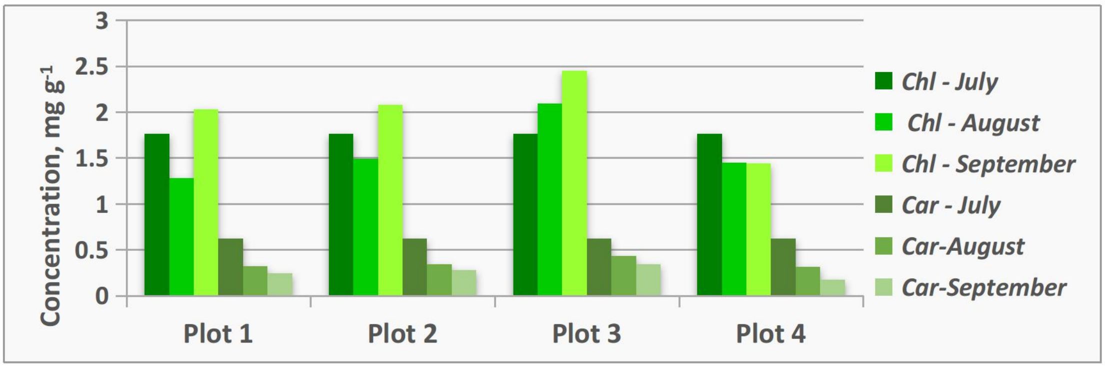

Fig. 3. Total chlorophylls and carotenoids content in *Tilia tomentosa* leaf samples.

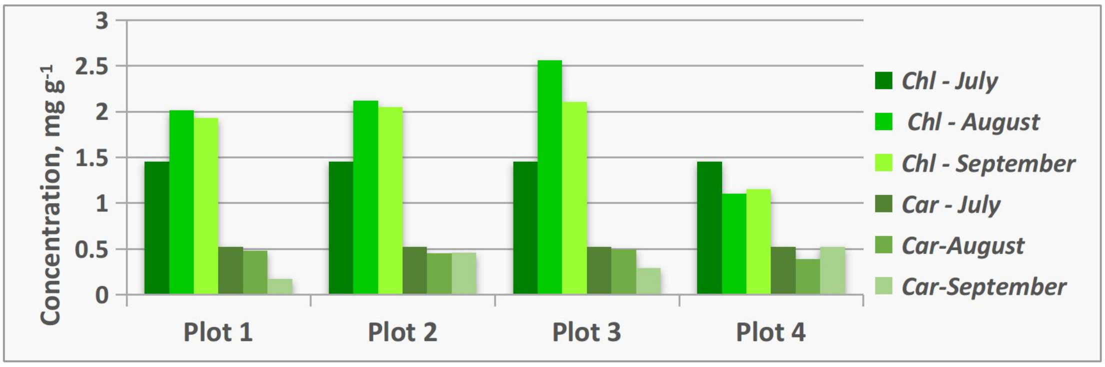

Fig. 4. Total chlorophylls and carotenoids content in *Fraxinus excelsior* leaf samples.

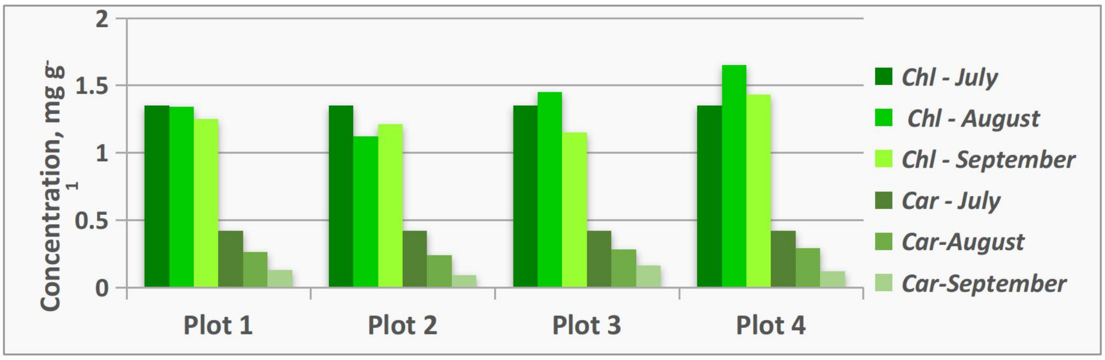

Fig. 5. Total chlorophylls and carotenoids content in *Pinus nigra* leaf samples.

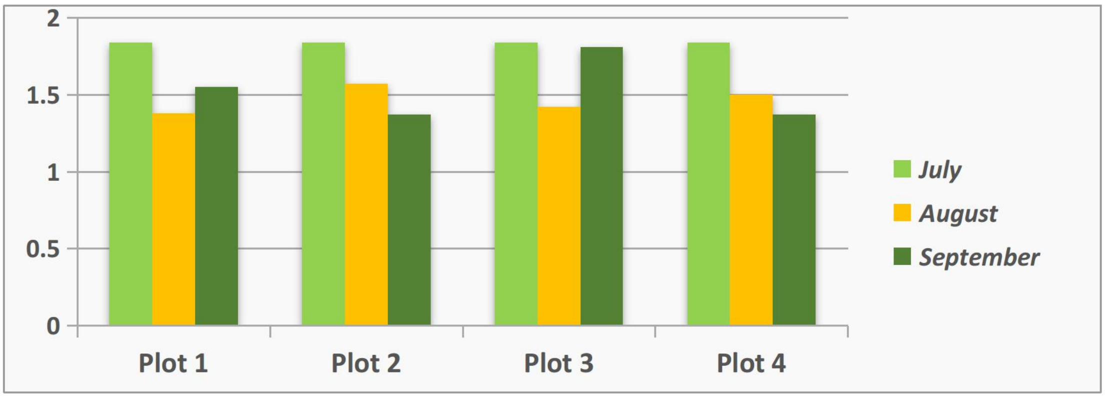

Fig. 6. Chlorophyll a/b ratio in *Acer heldreichii* leaf samples.

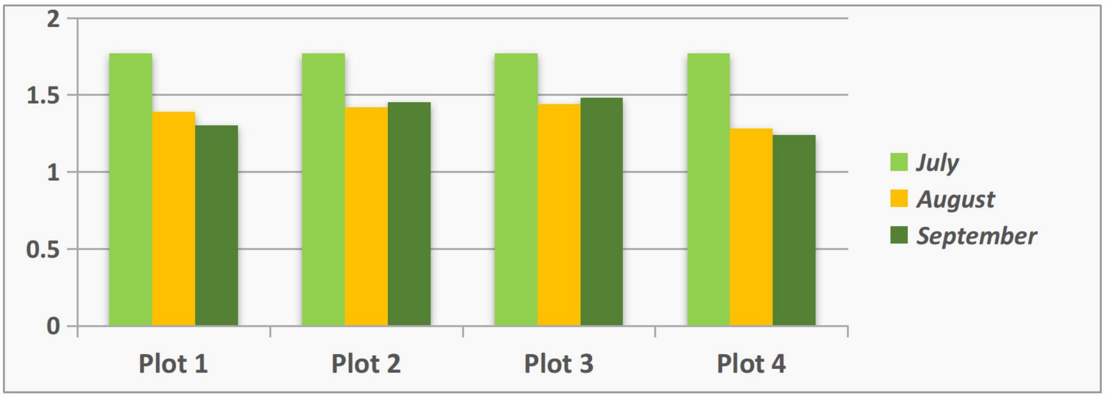

Fig. 7. Chlorophyll a/b ratio in *Tilia tomentosa* leaf samples.

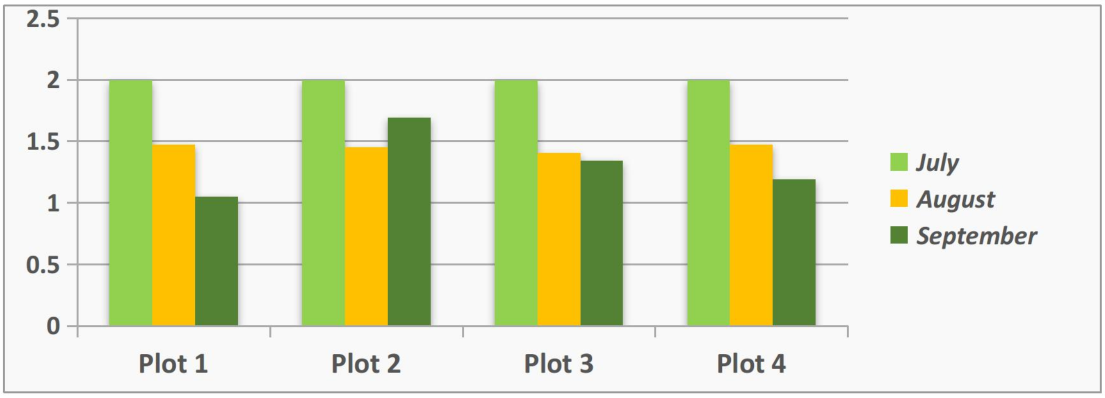

Fig. 8. Chlorophyll a/b ratio in *Fraxinus excelsior* leaf samples.

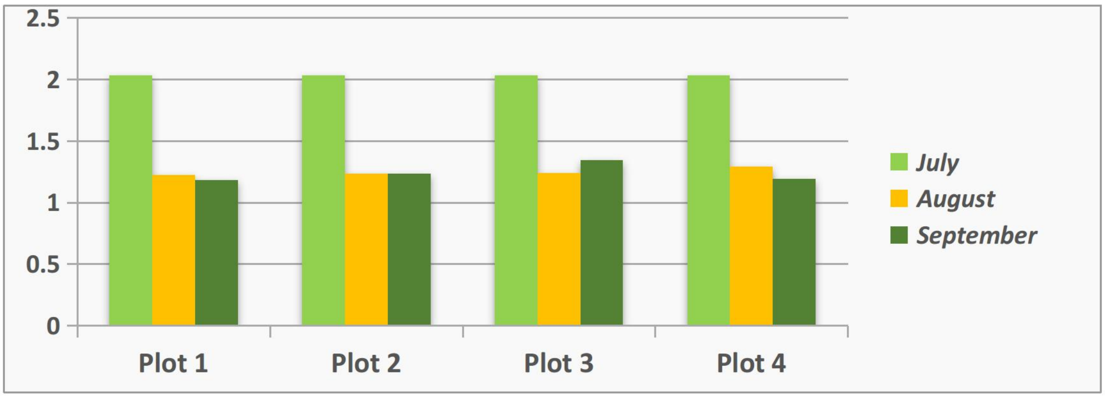

Fig. 9. Chlorophyll a/b ratio in *Pinus nigra* leaf samples.

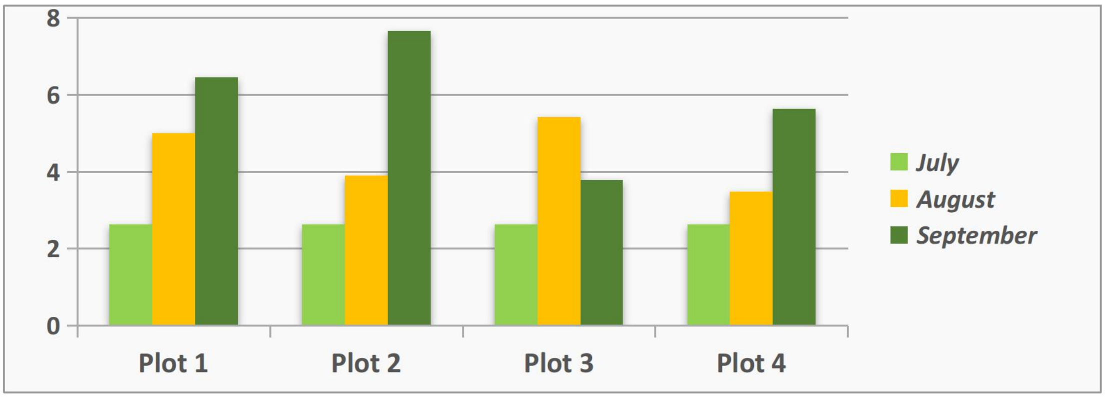

Fig. 10. Chlorophylls/carotenoids ratio in *Acer heldreichii* leaf samples.

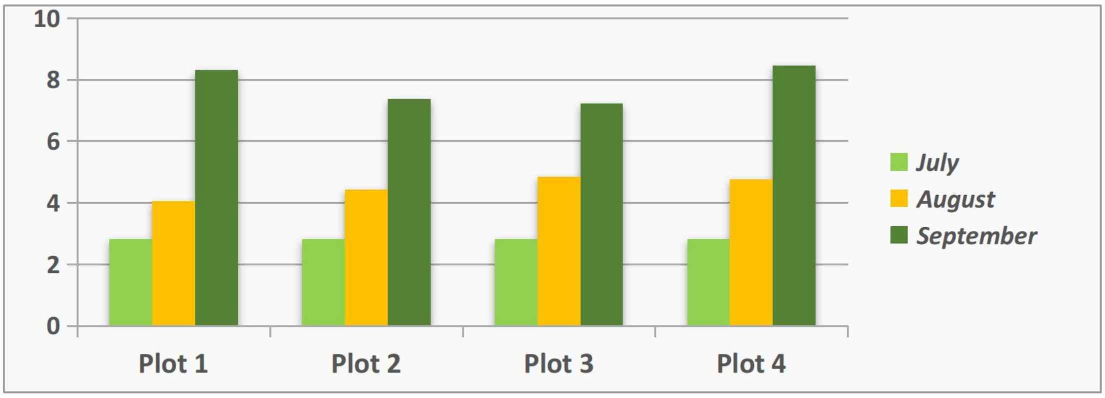

Fig. 11. Chlorophylls/carotenoids ratio in *Tilia tomentosa* leaf samples.

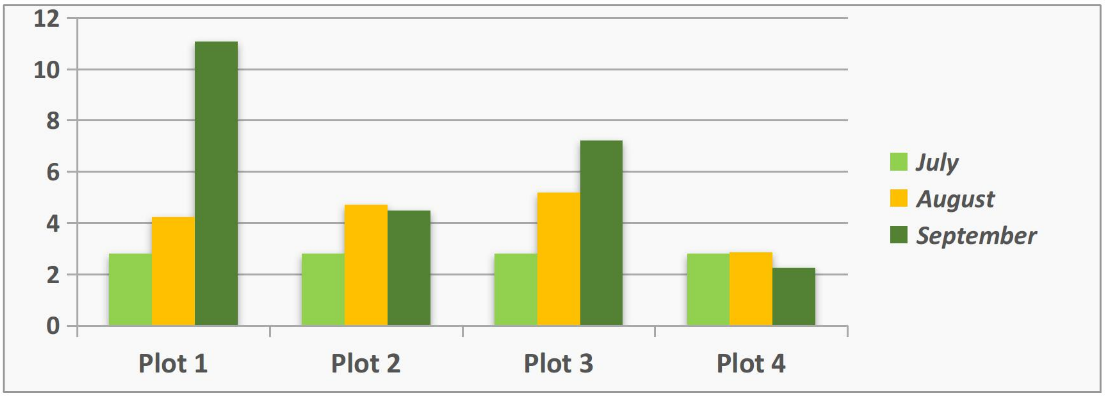

Fig. 12. Chlorophylls/carotenoids ratio in *Fraxinus excelsior* leaf samples.

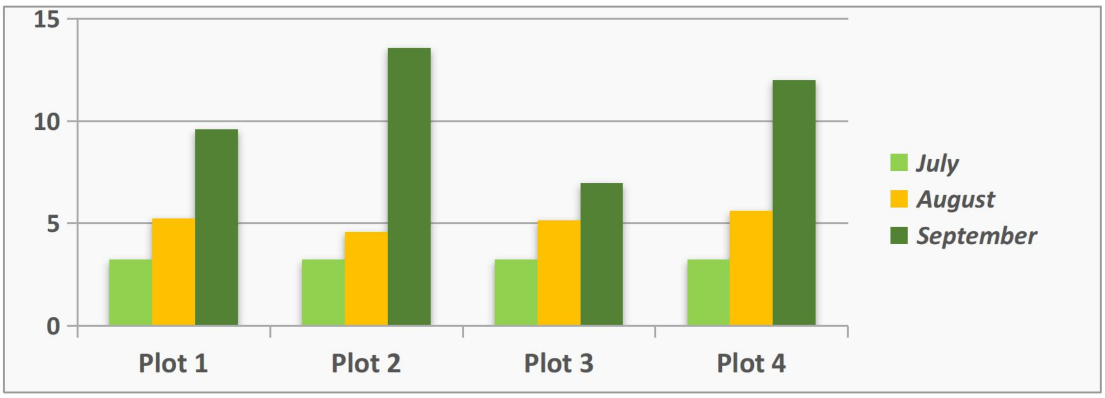

Fig. 13. Chlorophylls/carotenoids ratio in *Pinus nigra* leaf samples.

Conclusions

Results of this preliminary study confirmed that pigment levels in plants varied between species, locations and seasons. We could not found some significant differences between the pigment content of all studied trees from Plot 1, selected as a "provisional control" of urban environment and the trees in the other three plots ($p<0.05$). As more efficient parameters in this aspect were proven the chlorophyll a/b ratio and the chlorophylls/carotenoids ratio, where we found a general trend towards lower values at all studied plots and tree species compared to the control due to the anthropogenic impact on trees. These ratios could be used as more informative indicators in ecological investigations than pigment content. They have also the advantage to be dimensionless parameters, so they could be calculated and used for evaluation of plant status in different studies, regardless the extraction procedure of pigments and the analytical methods.

This study clearly indicates the great relevance of the problem of the connection of the host plant with the environment, both theoretically and in practice. This actuality is necessary not only to the requirements for environmental protection, but also in relation to the establishment of species specificity in the response of different plant species and their tolerance to pollution. This is crucial to forecasting the green system in urban areas and the selection of appropriate species for landscaping activities.

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