Preliminary results of chlorophyll content as a biomarker 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 urban trees we aimed to investigate the impact of anthropogenic activity on chlorophyll content of four tree species (*Acer platanoides* L., *Aesculus hippocastanum* L., *Betula pendula* Roth., *Platanus orientalis* L.) at eight selected sites in the town of Plovdiv (Bulgaria) during spring and autumn seasons of 2010. Results of this preliminary study confirmed that pigment levels in plants varied between species, locations and seasons. In almost all cases the quantity of pigments decreased in the autumn leaves. The reduction of chl *b* was found to be stronger than of chl *a*. Chl *a/b* ratio increased in 73% of autumn samples, compared to the spring ones. In our research we will continue to use the chlorophyll content for monitoring physiological state of these urban tree species, assessment and prognosis included.

Introduction

The impact of atmospheric pollutants on vegetation is a widely documented phenomenon. Plants have a very large surface area and their leaves function as an efficient pollutant-trapping device, therefore, their assimilative organs are directly affected by air pollution.

It is widely known that leaf chlorophyll content is an important parameter for testing plant status. For example, it can be used as an index of the photosynthetic potential as well as of the plant productivity (Carter, 1998). In addition, chlorophyll gives an indirect estimation of the nutrient status (Filella *et al*., 1995) and is closely related to various types of plant stresses and senescence (Gitelson & Merzlyak, 1994).

The present study aimed to investigate the impact of anthropogenic activity on air quality in Plovdiv as reflected by the physiology (chlorophyll content) of the four tree species.

Materials and methods

Study area and sampling sites

The town of Plovdiv (42° 9' N, 24° 45' E) is one of the most densely populated city of Bulgaria (over 365 000 inhabitants on 102 km^2). Eight sampling sites with different type of anthropogenic activity were chosen on the urban landscape, as follows: Site 1 - near the industrial zone, NE district; Site 2 – city park, East district; Site 3 – near the Rail station "Trakiya", SE district; Site 4 (Ruski Bul.) and Site 5 (Nature monument Bunardzhik) – in the real center of Plovdiv; Site 6 – suburban zone, SW district; Site 7 – city park, West district; Site 8 – near the NW end of the town.

Methodology of sampling

First sampling period was 13-14 June 2010, second – 15 September 2010. In all sampling sites were chosen at least two trees of each species (leaves from Oriental plane were sampled only from Sites 1, 4 and 5). The selected trees were of a similar sun expose, age, height and growth form (within the species), growing at 5-10 m away from intense traffic (except Site 4, where trees were on the sidewalk). The samples, each one consisting of 20-40 fully expanded leaves, were taken from the lower part of the tree crown at the 2.5–3 m height, stored in labeled polyethylene bags, and transported to the laboratory.

Chlorophyll analysis

Pigment analysis was performed after extraction with 90% acetone. Wavelength absorbance was measured at 662 nm for chl *a* and 644 nm for chl *b* in a SPECOL 11 absorption spectrophotometer (Faculty of Biology, University of Plovdiv). Concentrations of chl *a*, chl *b* and chl *a+b* were calculated for each sample and expressed on a fresh weight basis – mg g^{-1} fw (Petrova, 2011). We reported our results as mean ± SD, where standard deviations were calculated on the base of triplicate analysis of chlorophyll content.

Results:

Chlorophyll content was significantly different between plant species, sampling sites and seasons. Higher concentrations were determined for chestnut and maple, lower – for plane and birch. As a rule, the leaf samples showed decrease in pigment values in the autumn. Only few exceptions were observed, as follows: for birch in Sites 1, 4 and 8; for maple in Sites 6 and 8; for plane in Site 5.

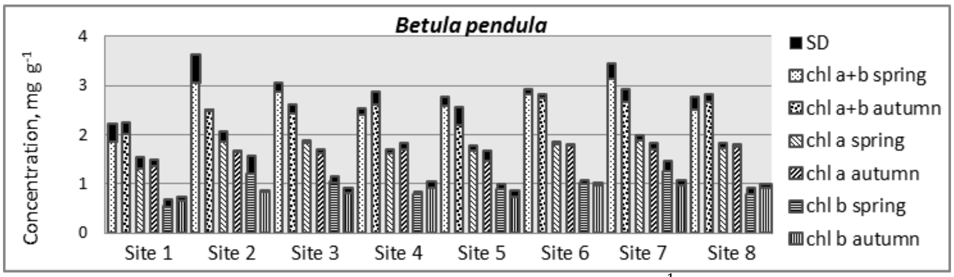

Fig. 1. Chlorophyll content in birch leaves, mg g^{-1} fresh weight

For the birch leaves (Fig. 1) minimal concentrations of chl *a*, chl *b* and chl *a+b* were determined in Site 1 - in the spring (1.324 mg g^{-1}, 0.539 mg g^{-1}, 1.863 mg g^{-1}, respectively) and also in the autumn (1.374 mg g^{-1}, 0.654 mg g^{-1}, 2.028 mg g^{-1}, respectively). Maximal chlorophyll values in the spring were found in Site 7 (chl *a* - 1.891 mg g^{-1}, chl *b* - 1.250 mg g^{-1}, total chl – 3.141 mg g^{-1}), but highest levels in the autumn were measured in Site 6 (chl *a* – 1.770 mg g^{-1}, chl *b* – 0.980 mg g^{-1}, total chl – 2.750 mg g^{-1}).

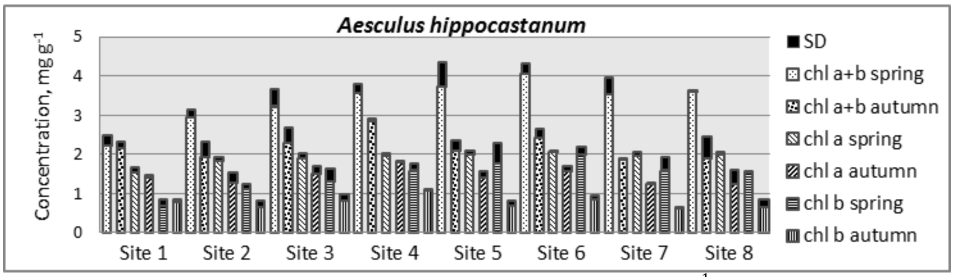

Fig. 2. Chlorophyll content in chestnut leaves, mg g^{-1} fresh weight

Chl *a* in chestnut leaves (Fig. 2) varied in the spring from 1.531 mg g^{-1} at Site 1 to 2.058 mg g^{-1} at Site 6, while in the autumn varied from 1.242 mg g^{-1} at Site 7 to 1.781 mg g^{-1} at Site 4. In the same way chl *b* and total chlorophyll concentrations in the spring were lowest in sample from Site 1 (0.689 mg g^{-1} and 2.219 mg g^{-1}, respectively) and highest in sample from Site 4 (1.062 mg g^{-1} and 2.844 mg g^{-1}, respectively).

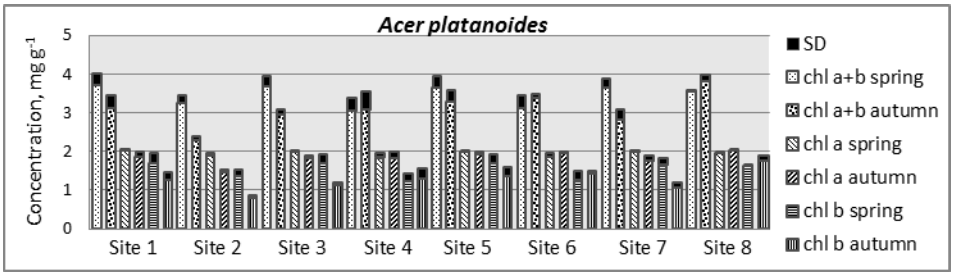

Fig.3. Chlorophyll content in maple leaves, mg g^{-1} fresh weight

In maple leaves (Fig. 3) spring minimum for chl *a* (1.839 mg g^{-1}), chl *b* (1.223 mg g^{-1}) and chl *a+b* (3.061 mg g^{-1}) were observed in Site 4. Maximal values were measured in Site 1: chl *a* - 2.014 mg g^{-1}, chl *b* - 1.699 mg g^{-1} and chl *a+b* – 3.698 mg g^{-1}. Lowest concentrations of chl *a*, chl *b* and chl *a+b* in the autumn were determined in Site 2, as follows: 1.470 mg g^{-1}, 0.809 mg g^{-1} and 2.279 mg g^{-1}, and highest in Site 8 - 2.030 mg g^{-1}, 1.766 mg g^{-1} and 3.796 mg g^{-1}, respectively.

Leaves from Oriental plane were sampled only from Site 1 (the most distant point from center), Site 4 and Site 5 (both situated along one of the major traffic arteries and differed only by the greenbelt which separate Natural monument “Bunardzhik” from the road). Minimal values in spring (chl *a* – 1.629 mg g^{-1}, chl *b* – 0.836 mg g^{-1}, chl *a+b* – 2.466 mg g^{-1}) and maximal in autumn (1.709 mg g^{-1}, 1.001 mg g^{-1}, 2.710 mg g^{-1}, respectively) were measured in Site 5. Spring maximum was found in sample from Site 4: chl *a* – 1.834 mg g^{-1}, chl *b* – 1.158 mg g^{-1}, chl *a+b* – 2.993 mg g^{-1}. Autumn minimum was observed in Site 1: chl *a* - 1.302 mg g^{-1}, chl *b* - 0.647 mg g^{-1}, chl *a+b* – 1.949 mg g^{-1} (Fig. 4).

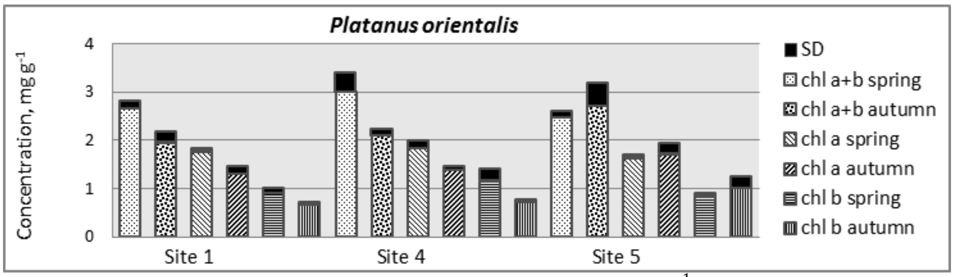

Fig.4. Chlorophyll content in plane leaves, mg g^{-1} fresh weight

Chl *a/b* ratio also varied between plant species, sampling sites and seasons (Fig. 5). For birch leaves maximums in both seasons were determined in Site 1 (2.456 in spring, autumn - 2.101) and minimal values - in Site 7 (1.513 and 1.780, respectively). Spring maximum of chestnut leaves also was detected in Site 1 (2.222), but in the autumn the chl *a/b* ratio was greatest in Site 5 (2.036). Lowest spring ratio for that species was 1.037 (in Site 7) and lowest autumn - 1.677 (in Site 4). Maximal value for chl *a/b* ratio in maple leaves in the spring was observed in Site 4 (1.504), in the autumn – in Site 2 (1.817). In spring it was minimal in Site 3 (1.170), while autumn minimum was found in Site 8 (1.150). In plane leaves, although the small number of sampling sites, it was observed the same tendency to rise the ratio of chl *a/b* in the autumn compared to the spring (from 1.954 to 2.012 at Site 1, from 1.584 to 1.939 at Site 4). Exception was Site 5 where was found a reduction – from 1.948 in June to 1.707 in September.

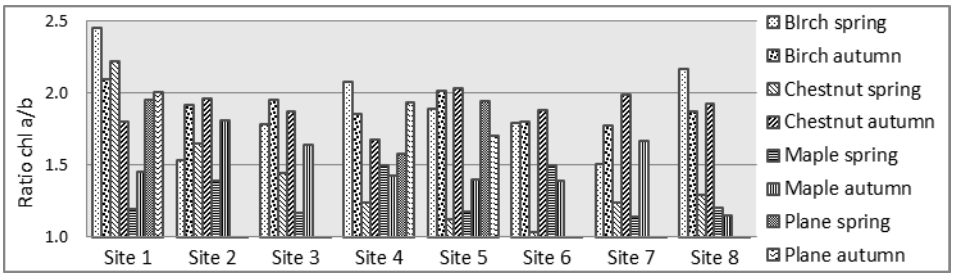

Fig. 5. The dynamics in the chl *a/b* ratio in tree leaves

Discussion

The chestnut leaves from Plovdiv (Fig. 2) showed quite close pigment levels to the values from the urban sites of Istanbul, Turkey (average total chlorophyll concentration in chestnut leaves from 2.418 to 3.308 mg g^{-1} in spring and 1.110 to 2.050 mg g^{-1} fw in autumn) and also the tendency to decrease in the autumn, reported by Baycu *et al.* (2006).

The average chlorophyll *a* and *b* concentrations in June, found in birch leaves from Sofia (Bulgaria), were 1.65 mg g^{-1} and 0.92 mg g^{-1} fw. In the town of Plovdiv (Fig. 1) these values were 4% and 2% higher, respectively. The autumn birch leaves from Sofia also showed a decrease in pigment content, as follows: chl *a* concentration in September was 1.46 mg g^{-1} and chl *b* concentration was 0.78 mg g^{-1} fw (Ivanova & Velikova, 1990).

The change in the chl *a/b* ratio (which had the advantage to be a dimensionless parameter) could be used as more informative indicator in ecological investigations. Typically, decreases in chl *a/b* ratios were observed during senescence. In this study, we found an increase in the ratio of chl *a/b* during autumn in 73% of leaf samples. The major reason for that was the stronger decrease in chl *b* content, than in chl *a*. The decrease of chlorophyll content may be due to an increase of chlorophyll degradation or to a decrease of chlorophyll synthesis. During the process of chlorophyll degradation, chl *b* is converted in chl *a* (Fang *et al.*, 1998) and this may explain the increase of the ratio chl *a/b* together with the depression of chlorophyll content.

Although an extension to the above work is necessary to quantify possible differences between the levels at which chlorophyll components are affected, it is clear that chlorophyll can be a very useful indicator of stress level. Because of the non-specificity in pigment reaction to different type of anthropogenic impact, we recommended to apply a combination among chlorophyll concentration and another parameters (morphological, biochemical, physiological) for the targets of biomonitoring.

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