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LEAF NITROGEN BALANCE INDEX USED TO MONITOR STRESS RESPONSE TO AIR POLLUTION OF DECIDUOUS TREE SPECIES GROWN IN URBAN ZONE OF BELGRADE

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Abstract

Street trees are important component of urban forest presenting a first barrier between air pollution originated from vehicle traffic and pedestrians. It implies that an improvement of air quality in urban areas greatly depends on green biomass, in short, the bigger and greener tree crown, better for human health and wellbeing. Determination of Leaf Nitrogen Balance Index (LNBI) and chlorophyll concentration (ChlC) by a non-invasive methodology and a user friendly instrument (Dualex 4, Force), widely used in agronomy and horticulture, was tested here for the assessment of tree fitness in urban zones. Investment of energy and resources either in growth or defence according to the trade-off strategy of plants may be indicated by LNBI, which approximately presents a Nitrogen/Carbon ratio. We selected few tree species from Belgrade's streets to determine those two parameters during summer. We also presented the changes in those parameters of the introduced bamboo species within ten years at several urban locations in Belgrade differing in air pollution aiming to evaluate usefulness the LNBI parameter in access of multiyear exposure to the effect of intense vehicle traffic. Numerous limiting factors for development of healthy tree crowns in urban ecosystem, such as low capacity to cope with toxic pollutant, sensitivity to diseases, early senescence and etc., greatly depends on tree species. We propose this methodology may also contribute in the process of choice of the adequate tree species to be planted along streets.

Keywords: LNBI, chlorophyll, epidermal flavonoids, street trees, bamboo, air pollution.

INTRODUCTION

Taking into account that urban areas (city, megalopolis) become everyday environment of the majority of global population, about 55%, and even 80% in EU countries [1], urban forest research received a considerable attention from scientific community with an involvement of both, natural and social science disciplines. Environmental benefits of urban trees for human health and environmental health have been thoroughly analysed elsewhere [2]. Authors of this review emphasized that urban forest planning and management should strategically promote trees as a social determinant of public health. Street trees contribute significantly to urban air quality by forming the closest buffer zone between sources of air pollution originated from vehicles traffic and residential areas. Main benefits provided by rich tree crowns related to environmental protection are amelioration of the effects of greenhouse gasses, adsorption of particulate matters, microclimate change mitigation. Though a releasing oxygen via photosynthesis by trees – "the lungs of the earth", is the first argument used in urban forest management, the actual effect of urban trees on the oxygen concentration in cities are to be scientifically supported [3,4]. However, gas-exchange measurements of tree canopy are very complex as photosynthetic activity of tree leaves are highly variable depending on leaf genetically heterogeneity, position within crown, which mainly determine their light exposure and hydration, tree age, morphology etc. [5]. In searching for the methodology to best suit study of street trees, we tried to test whether a non-invasive optical measurements of leaf transmittance (375) using a commercial Chl and flavonoid (Flav) meter – Dualex 4 Scientific, proposed to be used in crops surveys and nutrition management [6,7], might be also used for the assessment of tree's physiological status in their natural (urban) environment. Though determination of Chl is widely used as a stress meter (SPAD), this device measures Chl and Flav using leaf clip on the same leaf spot, allowing a generation of nitrogen balance index (NBI) which approximately presents a Nitrogen/Carbon ratio. Based on the hypothesis – the trade-off strategy of plants – that energy and resources are invested either in growth (primary metabolism, proteins, and chlorophyll) or defence (secondary metabolism, phenolic compounds), under the same nitrogen availability LNBI could be used as an indicator of plant metabolic status. Thus, monitoring of LNBI might be good indicator of plant sensitivity to pollutants, high temperature and sunlight or drought, commonly occurring abiotic stressors during summer in urban areas. Aim of this report is to encourage using this optical methodology as an early diagnostic tool of either air pollution or early senescence or species specific response occurring in urban trees as one of the contribution to fostering tree monitoring technologies.

MATERIALS AND METHODS

Experimental design

Various tree species (*Tilia sp.*, *Platanus acerifolia*, *Aesculus hippocastanum* L., *Corylus colurna* L., *Fagus sylvatica* L., *Ginkgo biloba* L., *Magnolia x soulangeana* Soul.-Bod., *Pinus wallichiana A.B.Jacks*, *Platanus acerifolia* (Aiton) Willd., *Quercus robur* L.) grown in urban zones in Belgrade municipalities (Arboretum and along main streets) were used to monitor physiological plant status by determining several optically derived parameters: 1) leaf nitrogen balance index – LNBI, 2) chlorophyll concentration – ChlC, and 3) epidermal flavonoids – EFlav. Measurements of Chl fluorescence were obtained from 20 to 30 mature, sun-exposed leaves of each tree or bamboo, about 5 to 7 hours after sunrise. The location of the monitored trees was given by names of city districts and streets. Leaves of bamboo, all exposed to sun and either close to traffic pollution source (Despota Stefana street) or from Košutnjak (Arboretum) were similarly analyzed.

Determination of ChlC, LNBI and EFlav

Total chlorophyll concentration (ChlC), epidermal Flavonoids (EFlav), and the leaf nitrogen balance index (LNBI) were obtained from the same leaf spot with the Dualex (Dx4, FORCE-A, Orsay, France; see Cerović *et al*. [6] for more details). Two laser beams, at 375 and 650 nm, were applied on leaf surface using leaf clip, to excite Chl in the leaf mesophyll. By equalizing Chl fluorescence under visible (650 nm) and UV (375 nm) light excitation, an electronic feedback loop, variable Chl fluorescence is avoided and a precise measurement of the absorbance of Flav in the UV-A is secured [8,9]. As a non-destructively measured leaf Chl content was used as indicator of N nutrition of crops [e.g. 10,11]. The new index called NBI (nitrogen balance index) derived from the Chl/Flav ratio is an indicator of C/N allocation changes [11]. Also the Chl/Flav ratio would correspond to an LMA-corrected Chl, i.e. Chl on mass basis.

Statistical Analysis

Significant differences in bamboo leaf ChlC, EFlav and LNBI ratio between locations in four years were determined using a two-way ANOVA test. The significance threshold value was set at $P \leq 0.05$. The homogeneity of variance was checked with Levene's test. Following two-way ANOVA analysis, the Tukey post hoc test was used for specific comparisons among experimental groups ($P \leq 0.05$). The experimental data were analysed using the software package Statistica 8.0. To test for significant differences in chlorophyll content and LNBI index between June and August, the Mann–Whitney U/t-test was used, and the significance threshold value was set at 0.05. The experimental data were analysed using software package Statistica 8.0.

RESULTS AND DISCUSSION

Effects of traffic pollution on LNBI

Traffic pollution can have various effects on the LNBI, which is being an indicator of the nitrogen content implies the status of stress impact on plant based on the metabolic strategy defence int leaves. Particulate matter (PM) from vehicle emissions ($PM_{2.5}$, PM_{10}) can settle on leaves and obstruct the metabolism either by closing stomata which can reduce photosynthesis and transpiration or by importing adsorbed toxic heavy metals (12), leading to a decline in the LNBI (Table 1).

Table 1 *Effects of traffic pollution on Leaf Nitrogen Index (LNBI) of the examined plant species. White fields represent trees at unpolluted locations (Košutnjak, Ušće); dark fields represent trees exposed to pollution (Kneza Miloša street, King Aleksander boulevard, Makenzijeva street. Results represent the mean value from 30 leaves ± SE*

Species	LNBI	Species	LNBI
Tilia platyphyllos	46.5 ± 4.9	*Platanus acerifolia* 1	27.8 ± 0.9
Tilia europea 1	46.7 ± 4.2	*Platanus acerifolia* 2	20.9 ± 8.5
Tilia europea 2	40.4 ± 7.1	*Platanus acerifolia* 3	20.1 ± 4.3
Tilia europea 3	64.7 ± 9.9	*Platanus acerifolia* 1	14.5 ± 1.4
Tilia europea 4	68.8 ± 15.7	*Platanus acerifolia* 2	19.8 ± 1.6
Tilia platyphyllos	29.7 ± 0.6	*Platanus acerifolia* 3	18.4 ± 2.4
Tilia cordata 1	29.4 ± 3.5	*Platanus acerifolia* 4	20.4 ± 3.1
Tilia cordata 2	20.4 ± 1.9	*Platanus acerifolia* 5	15.6 ± 2.6
Tilia grandiflora 1	24.5 ± 2.0	*Platanus acerifolia* 6	21.8 ± 2.9
Tilia grandiflora 2	21.2 ± 4.8	*Platanus acerifolia* 7	15.3 ± 2.1
Tilia grandiflora 3	38.1 ± 2.7	*Platanus acerifolia* 8	20.1 ± 1.0
Tilia grandiflora 4	21.1 ± 1.9	*Platanus acerifolia* 9	15.2 ± 1.6
Tilia europea 1	26.2 ± 4.7	*Platanus acerifolia* 10	15.6 ± 1.7
Tilia europea 2	33.9 ± 8.4	*Platanus acerifolia* 11	16.3 ± 1.4
Tilia europea 3	35.1 ± 6.7	*Ginkgo biloba L.*	51.4 ± 10.3
Tilia europea 4	24.1 ± 4.3	*Ginkgo biloba L.*	31.2 ± 4.1

Traffic pollution can also contribute to leaf damage and premature senescence. Traffic pollution often includes emissions of nitrogen dioxide (NO_2) and nitrogen oxides (NO_x). These pollutants can deposit onto plant leaves and negatively impact their nitrogen metabolism, which can affect plant growth and productivity (13). Leaves exposed to high levels of pollutants may exhibit symptoms such as smaller leaf size, abnormal shape, or chlorosis (yellowing). Traffic pollution is often accompanied by other air pollutants, such as sulfur dioxide (SO_2) and ozone (O_3) (14). Elevated ozone levels can increase oxidative stress in plants, leading to nitrogen imbalances and reduced LNBI.

Species specific variation of ChlC, EFlav and LNBI

Total chlorophyll concentration (ChlC) levels varied in different tree species depending on the month during summer season with the greatest effect observed in *P. wallichiana*, *A. hippocastanum*, *M. soulangeana*, *P. acerifolia*, and *Q. robur*, while magnolia had the lowest ChlC. Unique pattern was observed in ginkgo, where the ChlC first increases in June and then gradually decreases until October (Table 2, and data not shown).

Table 2 *Total chlorophyll concentration (ChlC, μg/cm^2) and concentration of epidermal flavonoids (EFlav,* μg/cm^2*) in the leaves of the examined plant species in summer season, measured by Dualex. Results represent the mean value from 30 leaves ± SE. Significant differences in ChlC between June and August according to Mann–Whitney U/t-test in each experiment are indicated (* $^{*}P < 0.05$, $^{**}P < 0.005$*)*

Species	**ChlC (μg/cm^2)**		**EFlav (μg/cm^2)**	
	June	**August**	**June**	**August**
Aesculus hippocastanum L.	27.4 ± 0.6	33.8 ± 1.5*	1.09 ± 0.07	0.96 ± 0.06
Corylus colurna L.	24.8 ± 0.4	21.3 ± 0.5	1.03 ± 0.02	1.30 ± 0.05*
Fagus sylvatica L.	57.8 ± 10.9	26.5 ± 5.3*	1.56 ± 0.19	1.60 ± 0.15
Ginkgo biloba L.	47.0 ± 2.0	38.3 ± 4.0*	0.98 ± 0.24	1.21 ± 0.20*
Magnolia x soulangeana Soul.-Bod. 1	25.9 ± 3.9	28.7 ± 6.3	1.46 ± 0.29	1.32 ± 0.30
Magnolia x soulangeana Soul.-Bod. 2	25.7 ± 3.7	27.9 ± 4.0	1.02 ± 0.43	1.43 ± 0.29*
Pinus wallichiana A.B.Jacks	57.8 ± 10.9	47.1 ± 10.9*	1.73 ± 0.11	1.42 ± 0.11*
Platanus acerifolia (Aiton) Willd.	35.3 ± 1.5	31.7 ± 1.1*	1.51 ± 0.03	1.66 ± 0.01*
Quercus robur L.	18.2 ± 0.6	22.7 ± 0.9*	1.43 ± 0.04	1.46 ± 0.04

The highest accumulation of epidermal flavonoids (EFlav) in April was recorded in *P. wallichiana*, and it was twice less in *A. hippocastanum*, *G. biloba*, and *C. colurna* (data not shown). Furthermore, it was observed that there was a significant accumulation of EFlav in *F. sylvatica*, ginkgo, and *P. acerifolia* during the summer, indicating that these tree species may have an active mechanism for biosynthesis and distribution of flavonoid *sunscreeners* in leaf epidermal layer throughout the growing season (Table 2). In contrast, while the ChlC in magnolia increases during the growing season, there was no change in the level of EFlav. On the other hand, with *A. hippocastanum* and *P. wallichiana*, a linear decrease in EFlav content was observed from spring to autumn, with *P. wallichiana* showing a more significant decrease than *A. hippocastanum* (Table 2).

The accumulation of flavonoids in the epidermal layer of leaf cells is an important mechanism that protects the leaf from photo-oxidation caused by high doses of ultraviolet radiation, which is especially increased in the summer [15]. The results suggest that plants synthesize flavonoids in the spring, and the level is maintained during the summer months [16]. The accumulation of flavonoids is influenced by a variety of factors, mostly by the level of exposure to sunlight, besides species of the plant, and the metabolic priorities of the plant during different phases of its growth cycle [17]. Accordingly, sun-exposed leaves tend to have higher levels of epidermal flavonoids than shaded leaves, indicating that this protective mechanism against harmful UV radiation.

It was noted that the lowest value for the leaf nitrogen index (LNBI) was observed in beech tree and magnolia, while the highest value was observed in Himalayan pine, indicating tickness of leaf is an important factor to take into account using Dualex4. The biggest initial increase in the LNBI index was seen in beech tree, ginkgo, and magnolia, with values increasing by 2–3 times (Table 3).

Table 3 *Leaf Nitrogen Index (LNBI) of the examined plant species in the period from April to October, measured by Dualex. Results represent the mean value from 30 leaves ± SE. Significant differences in LNBI between June and August according to Mann–Whitney U/t-test in each experiment are indicated (* $P < 0.05$, ** $P < 0.005$)*

Species	**LNBI**	
	June	**August**
Aesculus hippocastanum L.	28.3 ± 1.7	36.1 ± 1.1*
Corylus colurna L.	24.5 ± 0.8	17.3 ± 0.9*
Fagus sylvatica L.	36.8 ± 5.4	17.0 ± 4.9**
Ginkgo biloba L.	51.4 ± 10.3	32.6 ± 5.5**
Magnolia x soulangeana Soul.-Bod. 1	18.3 ± 4.5	23.2 ± 8.2*
Magnolia x soulangeana Soul.-Bod. 2	30.5 ± 5.5	20.3 ± 4.6**
Pinus wallichiana A.B.Jacks.	36.8 ± 5.4	29.3 ± 6.8*
Platanus acerifolia (Aiton) Willd.	23.3 ± 1.0	19.4 ± 0.9*
Quercus robur L.	21.6 ± 0.5	29.1 ± 1.1*

The results indicate that in 2012, the ChlC was significantly higher in bamboo leaves from Košutnjak compared to the more polluted location at Despota Stefana street (Figure 1). However, over the next two years, the ChlC became relatively similar at both locations and remained so even after 10 years in 2023. Additionally, there was a significant decrease in ChlC at both locations after 11 years compared to the beginning of the measurement in 2012.

The study also found that the content of EFlav decreased over the four years of the study in bamboo leaves at both locations, with a lower accumulation of EFlav observed in bamboo leaves from the more polluted location (Figure 1). The changes in the content of EFlav were followed by a change in the level of the LNBI. A significantly lower LNBI was measured in bamboo leaves grown in Despota Stefana street, while a decrease in LNBI over the years was recorded only in bamboo leaves from Košutnjak (Figure 1). The more polluted location at Despota Stefana street showed a lower accumulation of EFlav and a lower LNBI compared to the less polluted location at Košutnjak. Overall, these results suggest that both locations experienced a decrease in the health of the bamboo plants over time, as indicated by the decreasing chlorophyll content, decreasing epidermal flavonoids, and decreasing NBI index.

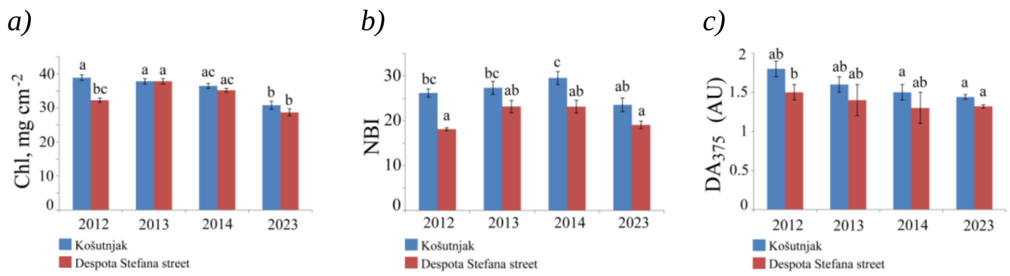

Figure 1 *a) Total chlorophyll concentration (ChlC), b) leaf nitrogen index (LNBI) and c) concentration of epidermal flavonoids (EFlav) of bamboo leaves measured in 2012, 2013, 2014 and 2023 by Dualex. Values are presented as means ± SE (n = 20). Different letters denote significant differences between means according to Tukey HSD post*

CONCLUSION

The coordination of continuous activities by municipality in synergy with academic and social organizations including a continuous monitoring of urban forestry as well as a proper nourishment and selection of the best adaptable tree seedlings in nurseries to these specific environmental conditions, is of crucial importance for further development of green city and maintenance of healthy urban forest. To ascertain the actual human health benefits derived from urban forest, a future research requires an inevitable multidisciplinary approach implemented in the long-termed ecological projects. We present here that use of described technology, adapted measurement of Chl fluorescence from leaf by the Dualex (Dx4, FORCE-A might contribute to the monitoring of plant sensitivity to pollutants, high temperature and sunlight or drought, commonly occurring abiotic stressors during summer in urban areas. Results encourage using this optical methodology as an early diagnostic tool of either air pollution or early senescence or species specific response occurring in urban trees as one of the contribution to fostering tree monitoring technologies.

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