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USE OF BIOCHEMICAL METHODS FOR ASSESING OXIDATIVE STRESS IN TREES IN URBAN AREA DURING GROWING SEASON

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Abstract

Due to increased urbanization and industrialization, the emission of toxic material into the atmosphere is in expansion, which has a negative impact on the environment and human health. In this research, we monitored the effect of air pollution on the peroxidase (POD) activity and total antioxidant capacity of different tree species during the growing season. The main goal was to determine which tree species developed the highest tolerance to unfavorable environmental conditions at the end of growing season, based on the response of their antioxidative metabolism. The greatest change in POD activity was observed in the Fagus sylvatica L. leaves, where enzyme activity was more than doubled in the autumn, in comparison to spring. On the other hand, decrease in POD activity was the greatest in Cedrus atlantica (Endl.) Manetti ex Carrière needles. Moreover, total antioxidative capacity was altered during growing season in almost all examined tree species. The Magnolia spp. showed the most consistent response to the given environmental pollution with both portrayed parameters induced during growing season. In general, we can conclude that the tree species investigated in this research possess distinctive tolerance potential to air pollutants.

Keywords: urban area, street trees, oxidative stress, peroxidase, antioxidative capacity.

INTRODUCTION

Environmental pollution has a severe ecological and social impact, particularly in industrialized and urban regions. Significant pollutant sources are industry, traffic, households, consumption of low-quality fuels and agricultural production [1]. Also, unorganized urbanization and industrialization have detrimental effect on ecosystems dynamics, especially in developing countries [2]. Among different air pollutants, particulate matter (PM_{10}, and $PM_{2.5}$), heavy metals (predominantly Pb and Cd), nitric oxides, ozone and carbon monoxide have the most negative impact on human health and the environment. In that regard, in urban densely populated areas, planting vegetation (i.e. trees) is one of the main factors in ameliorating the consequences of air pollution [3]. Numerous research papers have shown that trees have the highest pollution removal capacity, and thus a crucial contribution to air quality improvements in comparison to green roofs and green walls. Also, the important benefit of trees in urban areas lies in their capacity to reduce air temperature, which is very important during hot summer months [5]. On the other hand, trees from urban areas are constantly exposed to negative environmental conditions, like pathogen attacks and different abiotic stress factors. Almost all types of stressors cause oxidative damage of biomolecules (proteins, lipids, carbohydrates, and nucleic acids) by reactive oxygen species (ROS) [6]. On the other hand, ROS can act as secondary messengers involved in the regulation of plant defence by different mechanisms (apoptoses, induction of antioxidants, and retardation of growth) [7]. Plants possess an efficient antioxidant system involved in neutralization of the harmful effects of the ROS excess, via activating various antioxidative enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase, peroxidase class III, ascorbate peroxidase) or nonenzymatic antioxidants (ascorbate, glutathione, α-tocopherol, flavonoids and carotenoids).

In the present study we investigated the antioxidative capacity as well as POD activity extracted from the leaves of chosen tree species grown in different municipalities of the City of Belgrade (Čukarica, Vračar, Stari grad and Savski venac). All measurements were performed on leaves sampled at the beginning and the end of the growing season, to observe species specific acclimation to harmful environmental conditions [8]. Our goal was to determine which of the commonly grown trees in the urban area of Belgrade can be considered for future planting based on their response to oxidative stress caused by pollution and severe conditions [1].

MATERIALS AND METHODS

Plant material

Trees from four Belgrade municipalities were used for monitoring plant's response to oxidative stress during growing season. Location of all trees were given as GPS coordinates in Table 1. Sampling for biochemical measurements was performed twice, at the beginning and at the end of the growing season.

Table 1 *Location (GPS coordinates) of all trees used for monitoring seasonal variation in biochemical parameters*

Tree species	GPS coordinates	Tree species	GPS coordinates
1. *Ginkgo biloba* L.	44.798430, 20.465322	10. *Taxus baccata* L.	44.782968, 20.420341
2. *Ginkgo biloba* L.	44.790244, 20.440110	11. *Taxus baccata* L.	44.817950, 20.452024
3. *Cupressus arizonica* Greene	44.787843, 20.454914	12. *Taxus baccata* L.	44.817950, 20.452024
4. *Fagus sylvatica* L.	44.785460, 20.449492	13. *Cedrus atlantica* (Endl.) Manetti ex Carrière	44.786608, 20.447019
5. *Fagus sylvatica* L.	44.822933, 20.454354	14. *Quercus robur* L.	44.819501, 20.451536
6. *Pinus wallichiana A.B.Jacks.*	44.791531, 20.432272	15. *Platanus acerifolia* (Aiton) Willd.	44.798904, 20.472900
7. *Magnolia x soulangeana* Soul.-Bod f. *Lennei*	44.787416, 20.454242	16. *Aesculus hippocastanum* L.	44.821523, 20.464327
8. *Magnolia x soulangeana* Soul.-Bod.	44.790242, 20.439805	17. *Corylus colurna* L.	44.821275, 20.450662
9. *Taxus baccata* L.	44.787561, 20.454018		

Five samples of twenty fully developed leaves per tree for biochemical measurements were immediately frozen in liquid nitrogen and stored at -80°C. For both antioxidative capacity and POD activity, leaf samples were finely ground to powder in liquid nitrogen.

Determination of POD activity

Class III peroxidase (PODs, EC 1.11.1.7) was measured according to Kukavica *et al*. [9], with some modifications. Homogenized leaves were extracted in 100 mM sodium phosphate buffer (pH 6.5) with 2 mM EDTA, 2 mM PMSF (phenylmethanesulfonyl fluoride) and 5% (*w*/*v*) insoluble polyvinylpyrrolidine (PVP). Obtained homogenate was centrifuged at 10 000 *g* for 10 min at 4°C. The POD activity was measured spectrophotometrically in a reaction mixture consisting of 100 mM K-phosphate buffer pH 6.5, 20 mM guiacol and aliquots of plant fraction. The reaction was started by addition of 5 mM H_2O_2, and an increase in absorbance at 470 nm was followed. Activity of POD was calculated using the extinction coefficient for guiacol ($\varepsilon = 26.6$ mM^{-1}cm^{-1}).

Antioxidative capacity

The total antioxidative capacity of leaf samples was analyzed according to the protocol from Cano *et al*. [11]. The reaction mixture contained 2 mM 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 0.015 mM H_2O_2 and 0.25 μM horseradish peroxidase (HRP) in 50 mM potassium phosphate buffer (pH 7.5) and 50 μl of methanol plant extract. The decrease in absorbance due to the depletion of radical was measured at 730 nm. Ascorbic acid was used as a standard to form a standard curve in order to determine the relative antioxidative capacity of samples.

Statistical analyses

Significant differences in POD and total antioxidative activity between spring and autumn leaf samples were determined using a Student t-test. The significance threshold value was set at $P \leq 0.05$. This test was conducted with IBM SPSS statistics software (Version 268 20.0, SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Monitoring of POD level is an indicative way of determining the plant health status long before the damage becomes visible. Therefore, in this research special attention was brought to measuring activity of PODs in the trees' leaves during growing season [8].

It has been shown that plant species, like *Magnolia spp.* accumulate high levels of heavy metals (Pb, Cd and Cu) in urban areas compared to the suburbs, which implies their tolerant potential to air polluted environment. Consequently, it is recommended to be planted in industrialized urban areas and city parts with heavy traffic conditions [11,12]. In our research, POD activity was elevated by more than 25% in *Magnolia spp.* samples from the autumn in comparison to spring, while the total antioxidant capacity was increased by more than half, Figure 1. Also, increased POD level was also observed in *Fagus sylvatica* L. leaves in the end of the experiment, which is in accordance with induced antioxidative defence [13]. The opposite trend was noticed in *Cedrus atlantica* (Endl.) Manetti ex Carrière needles, where decreased POD activity in autumn was accompanied with decreased total antioxidative activity [14]. In our study an accumulation of antioxidative components in the leaves of

Platanus acerifolia (Aiton) Willd. found in autumn is linked to elevated POD activity in a study which deals with the heavy metal’s influence on trees ecophysiological parameters in urban area [15].

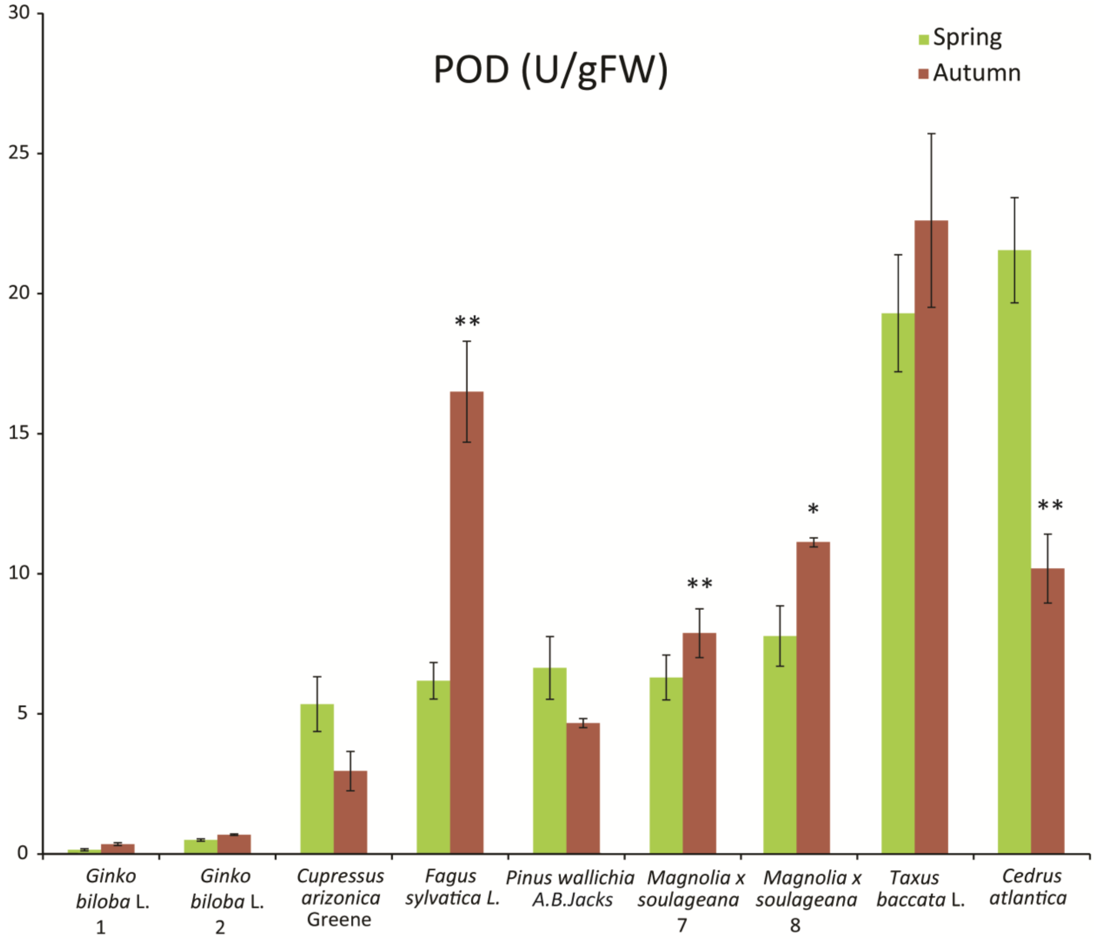

Figure 1 *POD activity in different plant species during growing season. Results represent mean ± SE ($n \geq 5$). Significant differences between Spring and Autumn according to the Student’s t-test are indicated ($^{*}P < 0.05$, $^{**}P < 0.01$)*

Seasonal variations in the antioxidant capacity were investigated in the leaves of *Quercus robur* L. in Hungarian forests during vegetation period [16]. Similarly, in our study, the antioxidative capacity was also reduced by 30% by the end of the growing season, Table 2. Also, decreased antioxidative capacity was noticed in *Aesculus hippocastanum* L. leaves in the end of the experiment [16]. Besides its well-known medicinal, food and pharmaceutical properties, *Ginkgo biloba* L, a living fossil tree, possesses a high potential for absorbing air pollutants. Therefore, in comparison with *Magnolia spp.* and *Cedrus atlantica (Endl.) Manetti ex CarrièreMan.*, *Ginkgo biloba* L. has medium value for metal accumulation index [12]. Moreover, in our research, antioxidative capacity of *Ginkgo biloba* L. leaves in the autumn was doubled.

Table 2 *Total antioxidant capacity (TAC) in extracts of the leaves of the examined plant species shown in equivalents of ascorbic acid in period from April to October. Results represent mean ± SE (n ≥ 5). Significant differences between Spring and Autumn according to the Student's t-test are indicated (*P < 0.05, **P < 0.01, ***P < 0.001)*

Tree species	**Spring**	**Autumn**
	TAC [ekq mmol Asc]	
1. *Ginkgo biloba* L.	0.77 ± 0.04	1.21 ± 0.1*
2. *Ginkgo biloba* L.	0.44 ± 0.02	0.99 ± 0.07***
3. *Cupressus arizonica* Greene	3.22 ± 0.08	2.91 ± 0.06***
4. *Fagus sylvatica* L.	1.76 ± 0.05	2.25 ± 0.09***
5. *Fagus sylvatica* L.	1.22 ± 0.31	/
6. *Pinus wallichiana A.B.Jacks.*	2.53 ± 0.07	1.47 ± 0.04***
7. *Magnolia x soulangeana Soul.-Bod forma f. Lennei*	1.07 ± 0.04	1.83 ± 0.08***
8. *Magnolia x soulangeana Soul.-Bod.*	0.61 ± 0.05	0.98 ± 0.09*
9. *Taxus baccata* L.	2.47 ± 0.03	2.74 ± 0.07
10. *Taxus baccata* L.	1.01 ± 0.04	0.92 ± 0.02**
11. *Taxus baccata* L.	0.74 ± 0.04	0.64 ± 0.02**
12. *Taxus baccata* L.	0.79 ± 0.06	0.72 ± 0.10
13. *Cedrus atlantica (Endl.) Manetti ex CarrièreMan.*	2.38 ± 0.07	1.37 ± 0.08***
14. *Quercus robur* L.	1.06 ± 0.04	0.70 ± 0.01***
15. *Platanus acerifolia* (Aiton) Willd.	1.35 ± 0.03	1.42 ± 0.02**
16. *Aesculus hippocastanum* L.	1.05 ± 0.08	0.53 ± 0.06**
17. *Corylus colurna* L.	0.68 ± 0.06	0.76 ± 0.03*

CONCLUSION

Proposed measuring of biochemical parameters is an efficient approach to pinpoint tree species which are more suitable for planting in industrialized urban areas. Leaves of trees are suitable to be used as indicators of the overall fitness of the tree, via these two suggested methods, even before any symptoms of disease or stress are visible. Overall, we can conclude that *Magnolia spp.* and *Fagus sylvatica L.* species are particularly suitable trees for planning urban landscapes, as they proved to be resistant to hazardous environmental conditions.

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