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ASSESSMENT AND PREDICTION OF VIABILITY AND METABOLIC ACTIVITY OF *TILIA PLATYPHYLLOS* IN ARID STEPPE CLIMATE OF UKRAINE

ABSTRACT

Urban phytocenoses preserving under the warming climate becomes especially urgent problem in the arid areas, including the steppe zone of Ukraine. Expected elimination of the susceptible woody plants defines necessity of species composition enrichment by introduction. In order to estimate *Tilia platyphyllos* adaptive capacity, leaves growth and metabolic features were determined for both shaded and lighted trees grew at the plots polluted with transport exhausts in Dnipro city. Reducing leaf surface area by 29 – 60% compared to conventional control (the Botanical Garden) was associated with level of pollution and lighting as well. Leaf weight (per cm2) exceeded the control (4 – 25%) at the most contaminated plots, but diminished with increasing distance from the pollution source. Compared to control, stomata density increased in all leaves, especially at the most polluted and shaded plots (50% above control).

Total chlorophyll content was below the control value (maximum 16%) at almost all polluted plots, while Chl a/Chl b ratio exceeded the control. Redox state of glutathione reached the maximum in leaves at the Botanical Garden, decreasing notably at contaminated plots, whereas the total accumulation of glutathione was enhanced.

Activity of glutathione-S-transferase was the highest in the most lighted leaf even on contaminated plot, while was inhibited (11-32% below control) by pollutants in shaded leaves. Results showed variability of morphometric characteristics and metabolic properties of large-leaved linden, depending on local environmental conditions. Phenotypic plasticity of urban *T. platyphyllos* trees is quite capable play a key role in adaptation to climate change allowing survival of the species.

Keywords: large-leaved linden, climate change, adaptability, morphometric and metabolic traits

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INTRODUCTION

In recent decades, many studies have been focused on the identification of climate change trends and prediction of the consequences of their effects on ecosystems. With regard to Europe, the basic climate shifts can be expressed as higher mean summer temperatures and prolonged summer drought periods (Scherrer et al., 2011), and lower summer precipitation as well (Frei et al., 2006). It was suggested that changes in rainfall, increasing temperatures and drought risks may affect plant species distribution and community composition (Fraser et al., 2008). More dramatic prognosis focuses on complication of woody plants survival (Talbi et al., 2015), and even on extinction of European forests populations with low ecological plasticity (Bussotti et al., 2015).

In steppe zone of Ukraine, the urgent need to preserve and expand planted woody communities is dictated by extremely small natural forests area. The role of urban trees can not be overemphasized in the industrial cities given the numerous environmental, social, economic, aesthetic, and health benefits (Gillner et al., 2015). However, tree community composition is limited in the phytocenoses of Dnipro city because of inappropriate climate and anthropogenic load. Herein planted wood communities were created involving both the indigenous and successfully introduced species. In particular, genus *Tilia* is currently represented by *T. cordata* Mill, which is an autochthonous species in arboreal flora of Steppe zone, and by introduced species *T. amurensis, T. platyphyllos* Scop. (large-leaved linden), *T. tomentosa* Moench, and *T. x europea* L. as well..

All species of *Tilia* genus are remarkable ornamental trees with a dense crown and broad leaf surface; linden trees are able to provide the aesthetic aspect and a comfortable microclimate in places of recreation of people. Moreover, *T. platyphyllos* is able to accumulate some heavy metals (Marković et al., 2013) and mercury (Kowalski et al., 2016). *T. platyphyllos* is widely planted throughout the temperate areas as an ornamental tree in parks and city streets. Large-leaved linden was characterized as a species with the moderate drought sensitivity (Scherrer et al., 2011) and rate of net photosynthesis (Gillner et al., 2015) in the urban conditions of Central Europe

The expected consequences of climate change can be particularly severe for tree species in arid regions (Talbi et al., 2015). In this context, the predictive assessment of viability of woody species in the face of increasing aridity is justified. According to Bussotti et al. (2015), useful tree genotypes should have features of adaptation to drought first of all, which can be reflected in variation at the morphological, physiological and phenological level. It was established, that plants have the thermal sensors to program metabolic level and provide acclimation to short-term fluctuations or adaptation to gradual temperature change (Bahuguna, Jagadish, 2015). Therefore, the study of intraspecific morphological and metabolic variations influenced by local adverse environmental conditions can indicate the possible ways of adapting of tree species. The objective of our study was to reveal variability of morphological and metabolic features of *T. platyphyllos* in order to assess the species adaptation capacity to environment adverse impacts and to predict the probability of survival in the urban plant communities when climate changes.

MATERIAL AND METHODS

Study area

The study was conducted in urban phytocenoces of Dnipro city (steppe zone of Ukraine). Steppe climate is continental with sharp fluctuations in temperature, unstable moisture, and seasonal drought periods, which accompanied by high temperature and dry winds. The annual amount of evaporation exceeds precipitation by 2–3 times, because an average annual rainfall is 472 mm, while it could fall to 250 mm in dry years. In addition, the successful development of urban trees is complicated due to city environment pollution with the motor exhausts and industrial emissions.

Study sites were located in four planted woody plant communities, in which *T. platyphyllos* Scop. (large-leaved linden) was introduced 50 – 55 years ago (Figure 1).

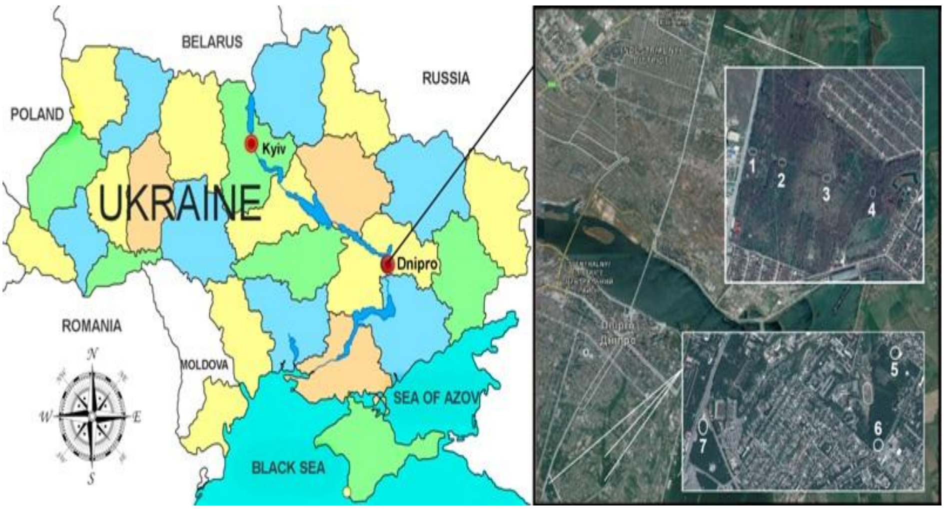

Figure 1: Study areas of the urban phytocenoces in city of Dnipro, Ukraine

Plot 1, plot 2, plot 3, and plot 4 were located at the territory of "Peoples' Friendship Park" (48°32'04.22" – 48°31'56.13"N, 35°05'15.91" – 35°06'00.45"E), which lies on the north-eastern outskirt of Dnipro city, and occupies area of 93.7 ha. Being created in the 60s of last century, the park was practically free of any human care in the last 20 years. Now, arboreal community is represented by 61 tree and shrub species with the dominance of *Quercus robur* L., *Acer platanoides* L., *Robinia pseudoacacia* L., *Ulmus minor* Mill., *Fraxinus excelsior* L., *Fraxinus pennsylvanica* Marsh., *Tilia platyphyllos* Scop., *Populus alba* L., *Gleditzia triacanthos* L., *Larix sibirica* Ledeb., *Acer pseudoplatanus* L., *Phellodendron amurense* Rupr. Herein, *T. platyphyllos* trees grew in the second tier, hence were shaded. In addition, undergrowth (5 – 7 years old) was found around the adult large-leaved linden trees. One side of the park is adjacent to the highway with non-stop intercity heavy traffic of passenger and a truck transport. Four study sites were chosen at a distance of 20 m, 270 m, 660 m and 965 m from the highway (plot 1, plot 2, plot 3, and plot 4 respectively).

Plot 5. Botanical Garden of Dnipropetrovs’k National University (48°26'14.09"N, 35°02'35.11"E) was considered as conventional control because of low pollution level. Herein, *T. platyphyllos* trees were planted as a mono-species community, and exposed to sunshine abundance.

Plot 6. It was a land along the roadside of Gagarin Avenue (48°25'49.75"N, 35°02'27.19"E), where *T. platyphyllos* solitary trees were subjected to chronic influence of the passenger cars exhausts and sunshine.

Plot 7 located at the square "20th anniversary of Victory Day" (48°25'53.92"N, 35°01'00.22"E). It has an area of about 2.0 ha, surrounded on all sides by trucks highways. Woody plant community is represented by *A. platanoides*, *R. pseudoacacia*, *F. excelsior*, *U. minor*, *T. platyphyllos*, *Betula pendula* Roth, *A. negundo*, *G. triacanthos*. Here, large-leaved linden trees grew in the second tier in the shade, and were exposed to chronic strong pollutant action.

The plots 1, 2 and 7 are under two factors impact: a) shading; b)strong pollution. The local environmental conditions for the rest plots are following: plot 3- a)shading; b)moderate pollution; plot 4 – a)shading; b) slight pollution; plot 5 (control) – a)light; b) low pollution; plot 6– a)light; b) strong pollution.

Data collection

Data on the composition of the woody communities at the study plots were obtained during the period May-June 2016. The leaves of *T. platyphyllos* were collected into a clear dry weather in the mean of July 2016 from 5–7 same-age trees in each studied plot simultaneously. Leaves intended for morphometric measurements and counting of the stomata were placed in plastic bags, while the second part of the leaves was frozen immediately for biochemical analysis.

Data analysis

The measurement of stomata density was performed in accordance with Grant and Vatnick (2004) using a light microscope Carl Zeiss Jena, and the results were expressed as stomata number per mm^2 of leaf surface.

Chlorophyll content (Chl a, Chl b, and a total chlorophyll value) was determined according to Wintermans and De Mots method (1965) in the ethanol extracts of tree leaves. Results were expressed in mcg of chlorophyll per g fresh weight (mcg/g FW).

Glutathione-S-transferase activity was assessed with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate according to method of Habig et al. (1974). The assay mixture, contained 0.1 M Tris buffer, pH 8.0, 100 µl of GSH, and 200 µl of sample, was incubated during 10 min at 30° C. The change of the optical density was detected at 340 nm during four minutes after addition of 100 µl CDNB, and the enzyme activity was expressed in nanoM CDNB/ sec ·g FW (nanokatal/g WW).

The reduced glutathione (GSH) content determination was based on spectrophotometric registration of reaction with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent) in accordance with method of Anderson (1985) in modification. No-protein extracts were obtained by homogenization of 200 mg fresh leaves with 2.5 ml of 5% sulfosalicylic acid followed by centrifugation at 10,000 g for 10 min. Optical density of assay mixture (110 µl of 0.1 M K-phosphate buffer contained EDTA, pH 7.8, and 300 µl of sample) was detected at 412 nm 3 minutes after addition of 0.013 M Ellman's reagent. GSH content was calculated by using calibration graph, and expressed in nanoMol GSH/g FW. Total glutathione content was determined through the same procedure after reduction of oxidized glutathione in the non-protein extracts using zinc dust (20 mg/ml of sample) as described by Woodward and Fry (1932). Oxidized glutathione (GSSG) content was determined from the difference between total glutathione and GSH followed by halving, and result was expressed in nMol GSSG /g FW.

Catalase (CAT) activity evaluations according to Goth (1991) as well as guaiacol-peroxidase (GPOD) activity determination in accordance with Ranieri et al. (2001) were conducted as has been described before (Lykholat et al., 2016). All determinations of morphometric indexes and stomata number, as well as metabolites content and enzymes activity required three replicates. Data represent mean values and standard deviations (±SD). Significance of differences was estimated using Student's t-test (P<0.05).

RESULTS AND DISCUSSION

Variability of morphometric indexes, stomata density, and chlorophyll content was revealed in leaves of *T. platyphyllos* depending on the local environmental conditions, as shown in Table 1.

Table 1. Effect of local environmental conditions on growth and chlorophyll content of *T. platyphyllos* leaves (Mean ± SD)

Plot	Parameter, units				
	Leaf surface area, cm^2	Leaf FW/surface area, mg/cm^2	Stomata density, units per mm^2	Total chlorophyll, mg/g FW	Ratio Chl a/Chl b
1	40.9±11.0	1.16±0.09	452±18	3.25±0.14	4.00
2	41.5±10.8	0.84±0.21	442±9	3,87±0.14	3.10
3	44.3±12.9	0.83±0.09	393±12	3.82±0.12	3.80
4	55.7±14.5	0.68±0.12	337±14	3.47±0.14	4.20
5	100.9±21.1	0.92±0.23	302±14	3.87±0.13	3.65
6	72.1±13.7	0.96±0.31	440±9	3.85±0.15	3.68
7	69.5±12.1	0.81±0.11	395±11	3.45±0.13	4.21

Reaching its maximum at the conditional control, leaf surface area of *T. platyphyllos* was decreased both at most contaminated plot 1, plot 6 and plot 7 (60%, 29% and 31% below control, $P<0.05$), and away from pollution in shaded leaves (45% and 56%, at plot 3 and plot 4 respectively, $P<0.05$). Similar differences of leaf area were revealed by Gillner et al. (2015) in sun adapted and shaded leaves of different urban trees. In contrast, stomata density, being a minimal in control linden leaves, increased markedly (in range 12%–50%, $P<0.05$) in both lighted and shaded leaves under pollutant influence. Results agree with Carins et al. (2013) data that leaf size plasticity can provide an efficient acclimation of stomatal conductance to contrasting evaporative conditions of sun and shade. In addition, the predominant stomatal density in relatively more polluted and lighted leaves are in accordance with opinion of Fraser et al. (2008) that highest stomatal density will be at high combined stress. In our study, rather high correlation ($r= – 0.66$) between leaf surface area and stomata density pointed to coordinated physiological features change in linden leaves, depending on increase in light and pollution. This assumption is in one line with data about stomatal regulation as an efficient short term dynamic adaptation of *T. platyphyllos* to water stress (Breda et al., 2006). Control linden leaf thickness was exceeded due to strong pollution influence both in shaded and lighted leaves (25% and 11%, respectively at plot 1 and plot 6, $P<0.05$). Results resonate with Sperlich et al. (2015) data about severe drought-induced increasing leaf mass per area. They can also be attributed to lignification processes under stressful conditions (Ranieri et al., 2001). High total chlorophyll content was found not only in control leaves of *T. platyphyllos*, but in shaded and lighted leaves at contaminated plots. The lowest chlorophyll levels (16% and 11% below control, $P<0.05$) were found in shaded leaves on the most polluted plot 1 and plot 7, respectively. At the same time, Chl a/Chl b ratio exceeded the control in all linden leaves, excluding leaves at plot 2. The results obtained indicate intraspecific differences in the rate of photosynthesis in leaves of large-leaved linden, caused by local environmental conditions. The variability of the photosynthetic process allows its adaptation to water stress (Aranda et al., 2015), as well as seasonal acclimation to drought in sunlit and shaded leaves (Sperlich et al., 2015). Total glutathione content was higher in leaves of *T. platyphyllos* at the most polluted plot 1, plot 6, and plot 7 (respectively 7%, 15%, and 7% above control, $P<0.05$) as shown in Figure 2 (a). However, pool of reduced glutathione was the highest in control leaves, falling in all other cases (4-8%). Control GSH/GSSG ratio (equal to 2.4) was also reduced at all contaminated plots, reaching a minimum (1.3) at plot 6. Varying the glutathione redox status in mature linden leaves means adaptation to local environmental conditions and reflects the adaptive capacity in general. GST activity was declined in shaded leaves at the polluted plots (in a range 11 – 32% below the control level), but not in lighted polluted leaves (Fig.2, b).

Glutathione-S-transferases are large family of enzymes, which catalyze the reaction of GSH conjugation with various xenobiotics providing their detoxification (Edwards et al., 2000). Decrease in defense enzyme activity in *T. platiphylos* leaves could be related to its inhibition due to excessive pollutant influence, just in the shaded leaves. Multilevel protection system provides that plants are able also to use non-enzymatic pathway detoxification of pollutants, including through direct conjugation with reduced glutathione (Noctor et al., 2002).

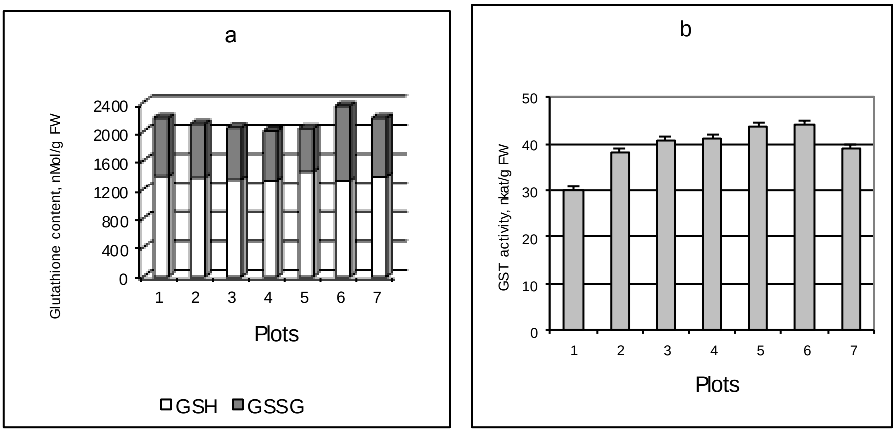

Figure 2: Effect of growth local conditions on T. platiphylos leaves glutathione (GSH and GSSG, nanoMol/g FW) content (a) and activity (nano Mol/sec ·g FW) of glutathione-S-transferase (b).

It is quite possible that enhanced glutathione accumulation could play a compensatory role in toxicants neutralization in case of inhibiting the enzymatic pathway in shaded polluted linden leaves.

At the same time, glutathione-dependent system functioning in sunlit polluted leaves of *T. platyphyllos* requires more detailed study. In our study, activities of catalase and guaiacol-peroxidase were the highest in the lighted linden leaves at polluted plot 6 (Table 2).

Table 2. Effect of local environmental conditions on catalase and guaiacol-peroxidase activity of *T. platyphyllos* leaves (Mean ± SD)

Plot	Factors	Enzyme, units	
		CAT, cMH_2O_2/ sec·g FW	GPOD, mcM/sec·g FW
1	Shading; strong pollution	9.55±0.9	40.21±2.6
2	Shading; strong pollution	7.94±0.7	33.07±1.4
3	Shading; moderate pollution	7.52±0.8	36.86±3.8
4	Shading; slight pollution	6.48±0.6	29.14±1.3
5	Light; low pollution	19.65±1.8	38.76±2.3
6	Light; strong pollution	20.78±1.7	53.33±2.8
7	Shading; strong pollution	4.85±0.5	10.64±1.1

Activity of CAT in shaded leaves of *T. platyphyllos* was significantly lowered relative to conditional control (in a range 51 – 67%) at all plots, especially at plot 7. The results obtained are consistent with previously reported (Lykholat et al., 2016) catalase activation in leaves of oak and ash for countering photorespiration due to temperature and light increasing. However, low level of catalase activity in the shaded linden leaves can not be unambiguously regarded as the evidence of insufficient active hydrogen peroxide eliminating. Difficulties in assessing the role of catalase appear because of complexity and redundancy of plant antioxidant system (Mhamdi et al., 2010). Anyway, intraspecific variation in catalase activity is undoubtedly an important element in *T. platyphyllos* adaptation capacity. Activity of guaiacol-peroxidase exceeded the control level in both shaded and sunlit linden leaves at polluted plot 1 (4% above control, unreliable difference) and plot 6 (38%, $P<0.05$), while declined at all other plots. Results reflect the multiplicity peroxidase role in physiological processes since has been shown (Ranieri et al., 2001; Lee et al., 2007) that stress-induced peroxidase activation may be more related to enzyme involving in processes of lignifications than in protecting against oxidative stress.

Taking into account the increase in linden leaf thickness founded at plot 1 and plot 6, we can assume that the increase in lignification is an adaptive response to pollution regardless of light level.

CONCLUSION

In the present study, large range of intraspecific variability of *T. platyphyllos* leaves morphological and metabolic traits induced by local environmental conditions was observed. Dissimilar effects of pollutant action have been identified in the sunlit and shaded linden leaves. Pollution-induced decrease in leaf area was more marked in the shaded leaves, whereas leaf thickness and stomata density were more declined in the lighted leaves at contaminated plots. Sunlit polluted leaves contain a greater total chlorophyll amount; whereas shaded leaves accumulated more Chl a. Lighted polluted leaves have the highest total glutathione pool together with lowest redox state of glutathione. Pollution-induced activation of glutathione-S-transferase, guaiacol-peroxidase and catalase was higher in the sunlit linden leaves. Comparative analysis of sunlit and shaded leaves features away from the pollution allowed predicting responses of *T. platyphyllos* to increase light intensity and temperature. Presumed adaptive changes may include increase in leaf area and leaf thickness. However decrease in stomata density, enhancing total chlorophyll content together with Chl b increasing; growth of glutathione redox state. Despite a slight increase in the total glutathione pool, moderate activation of peroxidase followed by increasing lignification; multiple increase in catalase activity. It can reduce the negative effect of photo respiration. Study results permit to conclude that the forecast for the survival of *T. platyphyllos* trees under climate change in steppe zone is more favorable than negative

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