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Physiological and Biochemical Characteristic Features of Small-Leaved Lime (*Tilia Cordata* Mill.) in Urban Environment.

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ABSTRACT

The article presents the physiological and biochemical features of small-leaved lime growing in urban plantings of different ecological categories: parklands, highways and plantations of sanitary protective zones of industrial enterprises. The content of photosynthetic pigments, secondary metabolites - tannin and ascorbic acid, peroxydase enzyme activity in leaves is significantly influenced by the degree of technogenic impact, vegetation period and the spatial orientation of the assimilatory organs of plants.
Keywords: small-leaved lime, woody plants, chlorophylls a and b, carotinoids, ascorbic acid, peroxydase activity, tannins.

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INTRODUCTION

The development of resistant species in plantations in sanitary protective zones of large industrial enterprises and along highways taking into account their physiological and biochemical characteristics is still relevant to this day. Such issues are particularly important as it is necessary to select tree species that can survive not only in the extreme conditions of the urban environment, but also have positive influence helping to optimize and improve it [1]. One of these species of plants is a small-leaved lime (*Tilia cordata* Mill.) which is widely used in landscaping of large industrial cities. There is a well-known ability of limes to intense absorption of sulfur dioxide and carbon dioxide, and it, in its turn, leads to a decrease in the rate of photosynthesis and early yellowing of leaf blade [1, 2].

A small-leaved lime is of great interest in the scientific aspect as an object for learning the adaptation mechanisms in the urban environment and, in practical terms, as a widely demanded species in landscaping of industrial centers. In this connection, the aim of our research was to study the features of adaptive reactions of this type on the biochemical level, to identify the role of secondary metabolites in the adaptation system of this type in the urban environment. The study was carried out in the plantations of a large industrial center of the Ural region of Russia in Naberezhnye Chelny.

Naberezhnye Chelny is a part of the Republic of Tatarstan which is located in the Middle Volga region. The average annual rainfall is 555 mm. The average annual temperature is approximately 2...3.1 °C. Naberezhnye Chelny is a major industrial center with the population of 530 thousand people. The major industries in the city are mechanical engineering, electric power, construction, food processing. Kamskii Automobile Plant is the main (township-forming) enterprise. On the basis of the "Report on the ecological state of the Republic of Tatarstan for 2012 we gave the characteristic of the level of air pollution in the areas where woody plants grow. The integrated air pollution index (API = 15.3) describes the state of air pollution in the city as very high. There was identified **t**he excess of permissible exposure level of benzo(a)pyrene, formaldehyde, phenol, carbon and nitrogen oxides [3].

METHOD

The small-leaved lime species, as the objects of study, were selected in plantations of different environmental categories: plantings along highways **(**major highways are Auto 1 and Mira Avenue); plantings in sanitary protective zones (SPZ) of industrial enterprises such as plc. "Kamaz": plants "Liteinii" and "Kuznechnii" are the main polluters of the city. Chelninskii forest district (forest and steppe zone of 9539 hectares, the forest and steppe region of the European part of the Russian Federation) and the landscape zone of the city park "Grenada" were chosen as conventional control zones (CCZ).

The sample plots were laid in a regular way (5 plots in each area; the size of each plot is not less than 0.25 hectares). To study the physiological and biological indices within the test plot (TP), the selection (10 plants of each species) and numbering of chosen woody plants were carried out and their state of life was assessed. The chosen species of plants were in a good living and middle-aged generative ontogenetic state (g2). During the active vegetation period of trees, notably in June, July and August in chosen species of plants we selected leaves of the middle formation on the annual vegetative growth (in one third part at the bottom of the crown of studied plants growing in the southern and northern exposures). Within the test plots we also took the soil samples (mixed sample composed of individual samples taken by the method of envelop).

In the laboratory we identified the content of chlorophyll *a* and *b*, carotinoids in the leaves of woody plants by spectrophotometer method (spectrophotometer ПЭ-5400 ВИ, Russia) in acetone extracts (the absorption is 662, 644 and 440.5 nm, respectively). The concentration of pigments was calculated by using Holm-Wettshtein equations. The quantitative content of ascorbic acid was identified in accordance to the GOST 24556-89 (titrimetry method). The content of condensed tannins in the leaves of woody plants was identified by using the permanganatometric method (Leventhal method modified by Kursanov); peroxides activity was measured by colorimetric method of Bojarkin A. M. [4, 5]. The biochemical analyses were performed in the Ecology and Plant Physiology laboratory of Biology Faculty of the branch of FSAEI HPO “Kazanskii (Volga region) Federal University” in Elabuga. The study was conducted during two vegetation periods (2011-2012).

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The statistical package «Statistica 5.5» was used for mathematical processing of the results. For interpreting the obtained materials we used the methods of descriptive statistics and multivariate analysis of variance (LSD-test was used for the subsequent assessment of differences by the method of multiple comparisons).

MAIN BODY

The most important indicator of the assimilation activity of plants is the content of chlorophylls *a* and *b*, carotinoids in leaves (Table 1). The multivariate analysis of variance of the results of the study revealed that the complex of growing conditions (the level of significance of $P<10^{-5}$), the period of vegetation ($P<10^{-5}$), the exposure of leaves arrangement ($P<10^{-5}$) and the interaction of these factors ($P=3.98 \cdot 10^{-5}$) influenced significantly the content of chlorophylls *a* and *b,* carotinoids in *Tilia cordata* leaves.

The content of chlorophyll *a* in the leaves of a small-leaved lime had similar dynamics during the period of active plant vegetation in all tested plantations: it increased in July in comparison with June, and then it decreased in August, but its indices were higher than in June. As for comparing the content of chlorophyll *a* in the leaves of plants in the plantations of sanitary protective zones and along highways, in June it was higher than in plantations of conventional control zone (CCZ), and in July and August it was significantly lower. The most significant difference was observed in the plantations along highways in August. The content of chlorophyll *a* in the leaves of northern exposure was by 0.76 and in the leaves of southern exposure by 0.49 mg/g of dry substance lower than that in the conventional control zone (if Smallest Statistical Difference SSD_{05}=0.01). One of the reasons for the observed reduction in chlorophyll content can be the ability of woody plants' leaves to absorb nitrogen-containing contaminants from the atmospheric air (nitrogen oxides, ammonia) and involve them in metabolism, that causes inhibition of synthesis of chlorophyll in the leaves and, as a consequence, the depression of photosynthesis [6, 7].

Table 1. Dynamics of physiological and biochemical indexes content in Tilia cordata leaves (*Tilia cordata* Mill.) growing in different categories of plantations in the city of Naberezhnye Chelny

Month of vegetation	Leave exposure	Indexes					
		Chlorophyll *a*, *mg*/g dry substance (SSD_{05}=0.01)	Chlorophyll *b*, *mg*/g dry substance (SSD_{05} = 0.01)	Carotinoids, *mg*/g dry substance (SSD_{05} = 0.02)	Ascorbic acid, mg/% (SSD_{05} = 2.1)	Peroxides activity, conventional unit (SSD_{05}= 0.02)	Tannins, % (SSD_{05}= 0.01)
	Conventional control zone						
June	northern	1.13	1.29	1.08	316.8	1.40	0.43
	southern	1.03	1.56	0.95	329.6	1.54	0.50
July	northern	2.85	2.42	2.50	176.5	4.12	0.63
	southern	2.63	2.86	2.27	186.3	4.21	0.67
August	northern	2.34	1.84	1.93	112.4	2.72	0.99
	southern	1.88	2.10	1.47	133.4	2.38	1.10
	Sanitary-protective zones of industrial enterprises						
June	northern	1.34	1.65	1.91	391.6	2.12	0.36
	southern	1.17	1.73	1.42	440.1	2.46	0.38
July	northern	2.57	2.71	1.93	156.3	2.96	0.74
	southern	2.45	2.91	1.25	175.0	3.22	0.78
August	northern	1.75	2.40	1.64	153.6	1.83	0.95
	southern	1.58	2.70	1.40	190.3	1.90	0.96
	Highway plantations						
June	northern	1.28	1.52	1.53	170.4	1.22	0.33
	southern	1.21	1.60	1.01	174.2	1.56	0.32
July	northern	2.53	2.71	1.89	122.8	2.99	0.72
	southern	2.39	2.89	1.23	106.1	3.31	0.73
August	northern	1.58	1.86	1.17	101.4	1.81	0.89
	southern	1.39	2.00	1.10	92.5	1.99	0.89

In all plantations the content of this pigment was higher in the leaves of northern exposure than in the leaves of southern exposure. It should be noted that the high productivity of woody plants is also provided by a large number of functionally different (light and shadow) leaves. They are distributed in the crown of the tree so that maximum output of light energy is used effectively. Therefore, the environmental strategy of woody plants is to provide optimum photosynthesis under different light status [8].

The dynamics of chlorophyll *b* content in the leaves of small-leaved lime in plantations of the city was similar to chlorophyll *a*, but the highest concentration of pigment was typical to leaves in southern exposure. Moreover, the comparison of the plants' indices in plantations of sanitary protective zones and along highways with plants in conventional control zone showed that chlorophyll *b* content in conditions of intensive technogenic impact was higher during all periods of observation. The obtained results allow us to state the highest sensitivity of chlorophyll *a* and the ability of chlorophyll *b* to function in conditions of intense anthropogenic impact. The obtained results are somewhat different from the research results of photosynthetic pigments content in leaves exposed to alkaline dust pollution caused by cement production [9], where chlorophyll *b* turned out to be more sensitive to the conditions of pollution.

The scientific literature provides data on the protective role of carotinoids in the conditions of stress [10, 11, 12]. During the study we observed the dynamics of the carotinoids content in the leaves which is similar to chlorophylls; the carotinoids content was higher in the leaves of northern exposure. In June the plants growing in the plantations along highways and in sanitary protective zones had significantly higher indices than those growing in conventional control zones (at 0.06-0.45 and 0.47-0.83 mg/g of dry substance respectively if SSD_{05}=0.02**)**. In July and August, on the contrary, the plants in these plantations had lower carotinoid content in comparison with the plantations in conventional control zone. It can be assumed that the carotinoids can be used as antioxidants so their concentration was decreased. Acidic gases reduce chlorophyll synthesis and its activity so it involves earlier leaf aging. However, the leaf's symplast acidification presumably activates the redox reactions in it where the ascorbic acid (AA) is actively involved in regulation, so the dynamics of its content in the leaves can be used to assess the resistance of individual plant species, as well as to indicate the quality of environment [7, 13, 14].

A multivariate variance analysis of results showed that the complex of growth conditions ($P<10^{-5}$), the vegetation period ($P<10^{-5}$) and the exposure of leaves arrangement ($P=1.9\cdot10^{-5}$) influenced significantly the ascorbic acid content in the leaves of small-leaved lime. The ascorbic acid content in leaves was decreased during the vegetation period in all types of plantations. The maximum content of this metabolite was observed in June in plants growing in sanitary protective zone of industrial enterprises, where the leaves in southern exposure had 440.1, and the leaves in northern exposure had 391.6 mg /% of ascorbate and the indices were significantly higher than those in conventional control zones. In July the ascorbate content was decreased sharply (up to 156.3-175.0 mg/%), and in August it was already 153.6-190.3 mg/% , the indices were higher than those in conventional control zones. The highest content of ascorbate was observed in leaves in southern exposure. In plantations growing along highways the ascorbic acid content in the leaves of small-leaved lime was significantly lower in comparison with zone of conventional control during the whole period of observation, which is most likely connected with the protective function of this metabolite and it can be explained by its spending to neutralize pollutants with oxidative activity.

The multivariate variance analysis of research results showed that the complex of growth conditions ($P<10^{-5}$), the vegetation period ($P<10^{-5}$), the exposure of leaves arrangement ($P=1.96\cdot10^{-5}$) and the interaction of these factors (P=0,03) had a significant influence on peroxydase activity in the leaves of small-leaved lime. At the beginning of vegetation period in June the maximum peroxydase activity was observed in plants growing in plantations of sanitary protective zones of industrial enterprises (in the leaves in southern exposure it was 2.46, in northern exposure it was 2.12, which respectively by 0.9 and 0.72 standard units more than in plants growing in conventional control zones (if SSD_{05} = 0.02). These indices are significantly higher than in plantations along highways. During the vegetation period peroxydase activity in the leaves of lime was decreased and it was noticed in all of the studied types of plantations. Moreover, the plants in plantations of sanitary protective zones and along highways had significantly lower levels of peroxydase activity in the leaves in comparison with those growing in conventional control zones.

The analysis of the results showed a significant effect of all studied factors and their interaction (P < 0.5) on the content of tannins in leaves. As for the dynamics of tannin content, it was increased during the

vegetation period in plants growing in all types of plantations. The maximum quantity of tannins was found in plants growing in conventional control zones at the end of the active vegetation period (in August), but in each period of observation the content of tannins in lime's leaves in plantations of sanitary protective zones and along highways was significantly lower in comparison with the plants growing in plots of control. In the urban environment the spending of this metabolite on adaptive reactions lasts for a long time in a small-leaved lime, so its content is reduced in comparison with parklands and suburban plantings. A similar trend has already been noted in our previous publications [15]. It should be noted that the leaves of lime in southern exposure during the vegetation period had more tannins in leaves compared with those in northern exposure.

CONCLUSIONS

The content of different photosynthetic pigments in the leaves of small-leaved lime has a similar dynamics during the period of active plant growth in all studied plantations: it increases in July compared to June, and then it decreases in August, but its indices are higher than in June. Leaves of lime in northern exposure have a higher content of chlorophyll *a* and carotinoids, and leaves in southern exposure have a higher content of chlorophyll *b*. In the conditions of intensive technogenic impact the content of chlorophyll *a* and carotinoids in the leaves at the beginning of the active period of plant vegetation are significantly higher in conventional control zones, and in July and August, on the contrary, the indices in these plantations are significantly lower in comparison with parkland and suburban plantings. The content of chlorophyll *b* in leaves of *Tilia cordata* in urban areas is higher than the indices in conventional control zones. Peroxydase activity in the leaves of plants growing in the plantations of sanitary protective zones in June has higher indices than in the control plantations, and then, on the contrary, it decreases. In the plantations along highways peroxydase activity in the leaves is significantly lower than in the control plantations during the whole period of active plant vegetation. During the vegetation period the ascorbic acid content in the leaves of small-leaved lime decreases in all types of plantations. In plantations of sanitary protective zones and along highways the content of ascorbate is substantially lower in comparison with those in parklands and suburban plantings. The urban environment conditions also lead to a decrease in tannin content in leaves. Although, in general, it is common for tannins that they are accumulated gradually and have the highest level of concentration in August.

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