# BIOCHEMICAL ANALYSIS OF LEAVES OF TILIA CORDATA IN CONDITIONS OF TECHNOGENIC POLLUTION (ON THE EXAMPLE OF THE NABEREZHNYE CHELNY CITY)

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The article describes the physiological and biochemical characteristics of Tilia cordata species growing in the conditions of different contamination levels. Not only the contamination level has a significant impact on the content of the studied photosynthetic pigments and metabolites, but also the spatial orientation of the assimilatory organs of plants.

**Keywords:** Ttilia cordata (Mill.), technogenic environment, leaf photosynthetic pigments, ascorbic acid, peroxidase activity, tannins.

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Nowadays, large industrial centers are extremely open artificial systems created and entirely dependent on man as only a man is responsible for maintaining the ecological balance and improving the sanitary conditions. Such questions are of particular importance as it is necessary to select species of living organisms that can not only survive in the extreme conditions of urban environment but also have a positive influence on it helping to optimize and improve it (Bukharina and others, 2007). There has always been works to study the potential of plants as a factor in improving the quality of urban and technogeneous habitats. One of these species of plants is a small-leaved lime which is widely used in landscaping of large industrial cities. For example, it is known, that during vegetation period 1 kg of Tilia cordata leavescan accumulate up to 10 gr of sulphur dioxide and up to 10 kg of carbone dioxide and it, in its turn, leads to decreasing of photosynthetic rate and early yellowing of leaf blade. (Sergeichik, 1984;Kulagin, 1974;Bukharina,Povarnitsina, Vedernikov, 2007).

Naberezhnye Chelny is a city in the Republic of Tatarstan which is located in the Middle Volga region. The average annual rainfall in the region is 555 mm. The average annual atmospheric temperature is 2…3,1°C.

Naberezhnye Chelny is a major industrial centre with the population of 530 thousand people. Mechanical engineering, electric power, construction industry, food and processing industries are the main industries in the city. Kamskii Automobile Plant is the main enterprise forming a company town. On the basis of the "Report on the ecological state of the Republic of Tatarstan" we gave the characteristic of the air pollution level in the areas where woody plants grow. A comprehensive air pollution index (API=15,3) shows a very high level of air pollution in the city. There was found the excess of maximum permissible concentration of benzo(a) pyrene, formaldehyde, phenols and andoxides of carbon and nitrogen.

The object of study is a small-leaved lime (*Tilia cordata* Mill.). The studied species grows in the city in various ecological categories of plantations: along highways (the major highways are Auto 1, and Mira Avenue) and the sanitary-protective zones (SPZ) of industrial enterprises such as plc. "Kamaz": plants "Liteinii" and "Kuznechnii" are the main polluters of the city. The area of Chelninskii forestry (forest and steppe zone of 9539 hectares, forest and steppe region in the European part of Russian Federation) was chosen as conventional control zone (CCZ). The area of the city park "Grenada" was chosen for introduced species. The sample plots were laid in a regular way (5 plots in each area the size of which is not less than 0.25 hectares). To study the content of physiological and biological indexes in the plant leaves within the test plot (TP) the selection (10 plants of each species) and numbering of chosen woody plants were carried out and their living condition was assessed. The chosen species of plants were in a good living and middle-aged generative ontogenetic state ($g_2$).During the active vegetation period of trees, notably in June, July and Augustwe selected leaves of the middle formation on the annual growth (in one third part at the bottom of the crown of studied plants growing in southern exposure). Within the test plot we took the soil samples and carried out analysis of them (composite sample contained individual samples after the manner of envelop). The exposure was identified by compass and corresponded to the structure of the part of crown regarding to the north and south. In the plantations along the highways the southern exposure stretched to the avenue.

In the laboratory we identified the content of chlorophyll *a, b*and carotinoids in the leaves of woody plants by spectrophotometer method in acetone extracts (the absorption is 662, 644 and 440,5 correspondingly). The concentration of pigments was calculated by using Holm-Wettshteinequation. The quantitative content of ascorbic acid was identified in accordance to the State Standard 24556-89 (titration analysis). The content of condensed tannins in the leaves of woody plants was identified by permanganatometric method (Leventhal method modified by Kursanov), and peroxides activity – by colorimetric method of Bojarkin A. M. (Mokronosov, 1992; Workshop (Laboratory session)… 1991; Nikolaevskii, 1999). The analyses of vegetable samples were carried out in the Ecology and Plant Physiology laboratory of Biology Faculty of the Branch of Kazanskii (Privolzhskii) Federal University in Elabuga. The study lasted during two vegetation periods (2011-2012).

The statistical package «Statistica 5.5» was used for mathematical processing of the materials.

**Table 1**

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

| Month of vegetation | Leave exposure | Indexes | | | | | |
|---|---|---|---|---|---|---|---|
| | | Chlorophyll *a*, *mg*/g dry substance ($HCP_{05}$ = 0,01) | Chlorophyll *b*, *mg*/g dry substance ($HCP_{05}$ = 0,01) | Carotinoids, *mg*/g dry substance ($HCP_{05}$= 0,02) | Ascorbic acid, mg/% ($HCP_{05}$ = 2,1) | Peroxides activity, conventional unit ($HCP_{05}$= 0,02) | Tannins, % ($HCP_{05}$ = 0,01) |
| | Conventional control zone | | | | | | |
| June | northern | 1,13 | 1,29 | 8,08 | 316,8 | 1,40 | 0,43 |
| | southern | 1,03 | 1,56 | 7,95 | 329,6 | 1,54 | 0,50 |
| July | northern | 2,85 | 2,42 | 11,50 | 176,5 | 4,12 | 0,63 |
| | southern | 2,63 | 2,86 | 11,27 | 186,3 | 4,21 | 0,67 |
| August | northern | 2,34 | 1,84 | 10,93 | 112,4 | 2,72 | 0,99 |
| | southern | 1,88 | 2,10 | 9,47 | 133,4 | 2,38 | 1,10 |
| | Sanitary-protective zones of industrial enterprises | | | | | | |
| June | northern | 1,34 | 1,65 | 9,91 | 391,6 | 2,12 | 0,36 |
| | southern | 1,17 | 1,73 | 9,42 | 440,1 | 2,46 | 0,38 |
| July | northern | 2,57 | 2,71 | 10,93 | 156,3 | 2,96 | 0,74 |
| | southern | 2,45 | 2,91 | 10,25 | 175,0 | 3,22 | 0,78 |
| August | northern | 1,75 | 2,40 | 8,14 | 153,6 | 1,83 | 0,95 |
| | southern | 1,58 | 2,70 | 6,80 | 190,3 | 1,90 | 0,96 |
| | Highway plantations | | | | | | |
| June | northern | 1,28 | 1,52 | 9,53 | 170,4 | 1,22 | 0,33 |
| | southern | 1,21 | 1,60 | 9,01 | 174,2 | 1,56 | 0,32 |
| July | northern | 2,53 | 2,71 | 10,89 | 122,8 | 2,99 | 0,72 |
| | southern | 2,39 | 2,89 | 10,23 | 106,1 | 3,31 | 0,73 |
| August | northern | 1,58 | 1,86 | 7,57 | 101,4 | 1,81 | 0,89 |
| | southern | 1,39 | 2,00 | 6,10 | 92,5 | 1,99 | 0,89 |

For interpretation of obtained materials we used methods of descriptive statistics and multivariate analysis of variance (by cross-hierarchical scheme with the subsequent assessment of differences by multiple comparisons LSD-test).

The content of chlorophylls *a, b,* carotinoids, ascorbic acid, tannins and peroxides activity in leaves is the main indexes of physiological and biochemical state (Table 1). Multivariate analysis of variance of the results of the study revealed that complex of growing conditions (the level of significance of $P<10^{-5}$), the period of vegetation ($P<10^{-5}$), the exposure of leaves($P<10^{-5}$) and the interaction of these factors ($P=3,98\cdot10^{-5}$) influenced the content ofchlorophylls *a, b,* carotinoids in Tilia cordata leaves.The highest amount ofchlorophyll*a* andcarotinoids was observed in conventional control zone in July in the leaves of northern exposure 2,85 and 11,50 respectively, while the leaves of the southern exposure contained thehighest amount ofchlorophyll *b* 2,86 mg/g in dry substance.

The study has shown that in technogenic conditions in the early period of active vegetation the amount of photosynthetic pigments in leaves in comparison with given indexesin conventional control zones significantly rises:chlorophyll *a*by 0,15-0,21 and 0,14- 0,18 ($HCP_{05}$= 0,01); chlorophyll*b* by 0,23-0,36 and 0,04-0,17 ($HCP_{05}$= 0,01); carotinoids by 1,45 – 1,83 and 1,06 – 1,47 mg/g in dry substance ($HCP_{05}$= 0,02) respectively in the leaves in northern and southern exposures.Moreover, in the leaves in southern exposure the concentration ofchlorophyll*b*was higher during the whole period of active vegetation of plants.During the period of observation, on the contrarily, it was found a decrease in chlorophyll *a* andcarotinoids in leaf blade in comparison with the conventional control zones. Their significantly higher content was observed in the leaves in northern exposure in all categories of plantations.

Multivariate analysis of variance of the results of the study revealed that complex of growing conditions ($P<10^{-5}$), the period of vegetation ($P<10^{-5}$), the exposure of leaves($P=1,9\cdot10^{-5}$) influenced the content of ascorbic acid (AA)in Tilia cordata leaves.The highest amount of this metabolite was observedin Junein the plants growing in sanitary protective zones of industrial enterprises where the leaves in southern exposure had 440,1 and the leaves in northern exposure had 391,6 mg/%, and it is significantly higher than the indexes of conventional control zones.In July the content of ascorbic acid (AA) decreases sharply (to the level of 156,3-175,0), and in August this level was 153,6 – 190,3 mg/% with the higher content of ascorbate in the leaves

in southern exposure.In the plantations growing along highways the small-leaved lime species had some differential characteristics which resulted in considerable reduction of this metabolite in the leaves of plants in comparison with the control plantings during the whole period of observation.

Multivariate analysis of variance of the results of the study revealed that the complex of growing conditions ($P<10^{-5}$), the period of vegetation ($P<10^{-5}$), the exposure of leaves($P=1,96\cdot10^{-5}$) and the interaction of these factors ($P=0,03$) influenced significantly the peroxides activityin Tilia cordata leaves.In the early period of vegetation in June the highest level of peroxides activity was recorded at the plants growing in the sanitary protective zones of industrial enterprises (in the leaves in southern exposure 2,46, in northern exposure - 2,12; which, correspondingly, is higher by 0,9-0,92 and 0,72-0,90 conventional units than in plants growing in conventional control zones and in plantings growing along highways, if $HCP_{05}$= 0,02).Afterwards, peroxides activity in Tilia cordata leavesgrowing in the conditions of technogenic impact decreases but this activity in the leaves in southern exposure keeps going in July and August.

Multivariate analysis of variance of the results of the study revealed that the complex of growing conditions ($P<10^{-5}$), the period of vegetation ($P<10^{-5}$), the exposure of leaves($P=1,61\cdot10^{-5}$) and the interaction of these factors ($P=2,57\cdot10^{-5}$) influenced significantly the content of tannins inTilia cordata leaves.The highest level of tannins was found in plants growing in conventional control zones at the end of the period of active vegetation in August. In the conditions of urban environment the small-leaved lime, presumably, spend this metabolite on the adaptive reactions so its amount decreases. A similar trend has been noted in our earlier publications (Bukharina I.L., Kuzmin P.A, 2012).During the period of vegetation Tilia cordata leaves in southren exposure accumulate more tannins growing in sanitary protective zones of industrial enterprises by 0,01 – 0,04; in the plantations along highways – by 0,01 %, in comparison with this indexes in the leaves in northern exposure.

Thus, the small-leaved lime has a specific reaction to anthropogenic impact by changing its physiological and biochemical indexes. Not only has the level of technogenic impact a significant influence on the content of studied metabolites but also the orientation in space of the assimilatory organs of plants.

**References:**

1. Sergeichik S.A. Woody plantsand industrial environment optimization. – Minsk., 1984. – 167 p.

2. State report «On the state of natural resources andenvironmental protectionof the Republicof Tatarstanin 2011» (06.29.2012)., Access mode: http://www.eco.tatarstan.ru / rus / info.php? id =424234 (last access: 15.07.2012).

3. KulaginY.Z. Woody plantsandindustrial environment. – Moscow., Nauka [Science]., 1974., p. 125.

4. Mokronosov A.T. Photosynthesis: Physiological andecological andbiochemical aspects. - Moscow., Moscow State University Press., 1992., p. 319.

5. Workshop on Plant Physiology/ DPVictors. - Voronezh., Voronezh State University, 1991., p. 160.

6. Bukharina I.L., Povarnitsina T.M., Vedernikov K.E. Ecological andbiological characteristicsof treesinan urban environment., I.L. Bukharin, T.M. Povarnitsina, K.E. Vedernikov. – Izhevsk., Izhevsk State Agricultural FSEI, 2007. -216 p.

7. Nicholas V. Environmental assessment of pollution and ecosystem phyto indication methods. – Moscow., Moscow State Forest University, 1999. - 193p.

8. Bukharina I.L., Kuzmin P.A. Dynamics of tannin contentin the leaves of woody plantsin different plantationcategories (on the example ofthe town of Naberezhnye Chelny)., Research Bulletin SWorld. Modern scientific research and their practical application. Vol. J21201., 2012.

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