INVESTIGAÇÃO DA COMPOSIÇÃO BIOQUÍMICA DO BORDO DA NORUEGA (*ACER PLATANOIDES L.*) NAS CONDIÇÕES DE ESTRESSE TECNOGÊNICO**AN INVESTIGATION OF THE BIOCHEMICAL COMPOSITION OF NORWAY MAPLE (*ACER PLATANOIDES L.*) IN THE CONDITIONS OF TECHNOGENIC STRESS****ИССЛЕДОВАНИЕ БИОХИМИЧЕСКОГО СОСТАВА НОРВЕЖСКОГО КЛЕНА (*ACER PLATANOIDES L.*) В УСЛОВИЯХ ТЕХНОГЕННОГО СТРЕССА**KUZMIN, Petr A.^{1*}; BUKHARINA, Irina L.²; KUZMINA, Ajgul M.³¹Kazan Federal University, Yelabuga Institute, Russia Federation.²Udmurt State University, Russian Federation..³Izhevsk State Agricultural Academy, Russian Federation.

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RESUMO

Um número bastante grande de publicações dedicadas ao estudo do papel de substâncias antioxidantes em plantas que crescem sob estresse antropogênico apareceu em revistas científicas. No entanto, esses problemas e questões não são estudados suficientemente. Este estudo teve como objetivo revelar as características da composição bioquímica do bordo norueguês (*Acer platanoides L.*) nas condições das plantações urbanas (pelo exemplo do grande centro industrial da cidade de Naberezhnye Chelny). O artigo apresenta os resultados de investigações sobre características de composição bioquímica do bordo norueguês nas condições de um grande centro industrial, a cidade de Naberezhnye Chelny (República do Tartaristão). Nas condições de carga tecnogênica intensiva, o bordo norueguês apresenta maior atividade da oxidase do ácido ascórbico nos estágios iniciais da vegetação ativa e menor atividade no final da vegetação, em comparação com as plantas nas zonas de controle convencionais (CCZ). Ao mesmo tempo, o teor de ácido ascórbico nas folhas também diminui durante a vegetação das plantas nas plantações da zona tampão de empresas industriais e plantações de tronco. Nas plantações da zona tampão de empresas industriais e plantações de tronco, o bordo norueguês foi caracterizado por um aumento na atividade da polifenol oxidase em comparação com as plantações de controle. O aumento da atividade da polifenol oxidase é acompanhado por uma diminuição no teor de taninos. O bordo norueguês em plantações de tronco apresenta menor atividade de peroxidase em suas folhas nas condições de carga mais intensa em julho do que nas plantações de CCZ e, em agosto, essa atividade é maior do que no grupo controle.

Palavras-chave: *Bordo da Noruega (Acer platanoides L.), atividade de polifenol oxidase, atividade de oxidase de ácido ascórbico, atividade de peroxidase, plantações urbanas.*

ABSTRACT

A reasonably large number of publications devoted to the study of the role of antioxidant substances in plants growing under anthropogenic stress have appeared in scientific journals. However, these problems and issues are not studied sufficiently. This study aimed to reveal the biochemical composition features of Norway maple (*Acer platanoides L.*) in the conditions of urban plantations (by the example of the large industrial center in the city of Naberezhnye Chelny). The paper presents the results of investigations of biochemical composition features of Norway maple in the conditions of a large industrial center, the city of Naberezhnye Chelny (the Republic of Tatarstan). In the circumstances of intensive technogenic load, Norway maple shows higher ascorbic acid oxidase activity at the initial stages of active vegetation and lower activity at the end of vegetation, in comparison with plants in the conventional control zones (CCZ). At the same time, the content of ascorbic acid in the leaves also decreases during the vegetation of plants in the buffer zone plantations of industrial enterprises and trunk plantations. In the buffer zone plantations of industrial enterprises and trunk plantations, Norway maple was characterized by an increase in polyphenol oxidase activity, in comparison with the control plantings. The rise in polyphenol oxidase activity is accompanied by a decrease in the content of tannins. Norway maple in trunk

plantations has lower peroxidase activity in its leaves in the conditions of the most intense load in July than in plantations of CCZ, and in August, this activity is higher than in the control group.

Keywords: Norway maple (*Acer platanoides* L.), polyphenol oxidase activity, ascorbic acid oxidase activity, peroxidase activity, urban plantations.

АННОТАЦИЯ

В научных журналах появилось достаточно большое количество публикаций, посвященных изучению роли антиоксидантных веществ в растениях, растущих в условиях антропогенного стресса. Однако эти проблемы и проблемы недостаточно изучены. Целью данного исследования было выявление особенностей биохимического состава клена обыкновенного (*Acer platanoides* L.) в условиях городских насаждений (на примере крупного промышленного центра в городе Набережные Челны). В статье представлены результаты исследований особенностей биохимического состава клена обыкновенного в условиях крупного промышленного центра, г. Набережные Челны (Республика Татарстан). В условиях интенсивной техногенной нагрузки клен норвежский проявляет более высокую активность оксидазы аскорбиновой кислоты на начальных этапах активной вегетации и более низкую активность в конце вегетации по сравнению с растениями в обычных контрольных зонах (ЗКК). В то же время содержание аскорбиновой кислоты в листьях также уменьшается при вегетации растений на плантациях буферной зоны промышленных предприятий и на стволовых плантациях. На плантациях буферной зоны промышленных предприятий и стволовых насаждениях клен обыкновенный характеризовался повышением активности полифенолоксидазы по сравнению с контрольными насаждениями. Увеличение активности полифенолоксидазы сопровождается снижением содержания дубильных веществ. Клен обыкновенный в стволовых насаждениях имеет более низкую активность пероксидазы в своих листьях в условиях наиболее интенсивной нагрузки в июле, чем на плантациях CCZ, а в августе эта активность выше, чем в контрольной группе.

Ключевые слова: Клен обыкновенный (*Acer platanoides* L.), активность полифенолоксидазы, активность аскорбинатооксидазы, активность пероксидазы, городские плантации.

1. INTRODUCTION:

In recent years, a relatively large number of publications devoted to the study of the role of antioxidant substances in plants growing under anthropogenic stress have appeared in scientific journals. However, these problems and issues are not studied sufficiently. The interaction features of enzymes and secondary metabolites involved in the formation of adaptive reactions of the plant organism are among these issues (Chupakhina *et al.*, 2012; Bukharina *et al.*, 2014; Galves-Valdivieso *et al.*, 2010; Maiti *et al.*, 2016).

Many investigations highlight the relationship between the adaptive capacity of the plant organism and the functioning of the non-enzymatic and enzymatic systems, individual elements of which are such substances as tannins and polyphenol oxidase, ascorbic acid, and ascorbic acid oxidases (Bukharina *et al.*, 2014; John *et al.*, 2008; Gill, Tuteja, 2010; Pachzkowska *et al.*, 2007; Podoprigora, and Korobov, 2017; Ivanova *et al.*, 2019; Rogatchev *et al.*, 2019). Polyphenol oxidase and peroxidase in combination with phenolic substrates, participate in the respiration process at the intermediate stages of hydrogen transfer. It was found that polyphenol oxidase activity increases in damaged

tissues of plants (Shagiakhmetov *et al.*, 2019). Ascorbic acid oxidase contributes to the elimination of reactive oxygen intermediates and participates in the protective reactions of plant organisms in the fight against oxidative stress (Sarika *et al.*, 2005; Ahmad *et al.*, 2010; Hattas, Julkunen-Tiitto, 2012; Kerio *et al.*, 2013;).

For example, during the study of antioxidant properties of the deciduous shrub *Cassia alata* L., scientists have found the features of the work of the enzymatic system and photosynthetic apparatus in different conditions of the abiotic environment. When abiotic stress occurs, catalase, ascorbate peroxidase, and glutathione, reductase activity increases (Ahmed, Anis, 2014). Other investigations have noted the role of antioxidant enzymes, proline, abscisic acid, phenols, and ascorbic acid in poplar plants and green tea. The specific reaction of these samples under stress was revealed, which is manifested in the increase in the activity of antioxidant enzymes and the reduction of the proline and abscisic acid levels (Garcia *et al.*, 2016; Kloseiko, 2016; Li *et al.*, 2014; Podoprigora and Korobov, 2017).

This study aimed to reveal the biochemical composition features of Norway maple (*Acer platanoides* L.) in the conditions of urban plantations (by the example of the large industrial

center in the city of Naberezhnye Chelny).

2. MATERIALS AND METHODS:

The object of investigation was Norway maple (*Acer platanoides* L.). The studied species grows in the city as a part of plantations of different ecological categories: trunk plantations (major highways Avto-1 and the Mira avenue) and the buffer zones (BZ) of industrial enterprises in OAO "KamAZ": foundry and blacksmith plants which are the primary pollutants of the city. The territories of Naberezhnye Chelny local forestry were chosen as conventional control zones (CCZ).

On the basis of sample plots description, (3 in each studied area, laid down by the regular method of different configurations, the size of at least 0.25 hectares, depending on the area of the studied category of plantations), it was made the selection and numbering of accounting plants (10 plants, from which the plants of good average generative ontogenetic condition were selected for biochemical studies) (Rodin *et al.*, 1968; Grishina, Samoylova, 1971). The schematic map of the sample plots location is shown in Figure 1.

The degree evaluation of air pollution in the places of woody plant growth was carried out on the basis of the materials of the "Report on the ecological state of the Republic of Tatarstan". The complex atmosphere impurity index (All=11.6) characterizes the state of air pollution in the city as very high (State..., 2016).

Within the boundaries of sample plots, selection and numbering of at least ten samples for the study of biochemical parameters was carried out. The calculation of the relative life state (RLS) of forest crop in plantations was carried out according to the method by V.A. Alekseev (1990)

$$RLS = \frac{100n_1 + 70n_2 + 40n_3 + 5n_4}{N},$$

where n_1, n_2, n_3, n_4 – the number of healthy, weak, very weak, and dying trees in the sample plot, respectively; 100, 70, 40, 5 – coefficients expressing (in percent) the life state of healthy, weak, very weak and dying trees;

N – the total number of trees in the sample plot (including dead trees). At the value of the relative life state from 100 to 80%, the forest crop is estimated as healthy, at 79-50% – weak, at 49-20% – very weak, at 19% and below – wholly destroyed.

In the period of active vegetation (in June, July, and August), the leaves of the middle

formation on the annual vegetative shoot (from the lower third of the south-facing crown) were taken from the accounting samples. The leaves of the intermediate creation are typical for the plant, developing in the middle zone of the shoot and performing the function of photosynthesis (Korovkin, 2007). In trunk plantations, the part of the south-facing crown was turned towards the roadway of the avenue directly. The selection of leaves was carried out in one day in all types of plantations.

In laboratory conditions, the content of condensed tannins in the leaves of woody plants was determined with the help of the permanganate metrical method (the Leventhal method modified by Kirsanov). The quantitative content of ascorbic acid was established following 24556-89 government standard (Chupakhina, 2000).

Ascorbic acid oxidase activity was determined with the method proposed by D.K. Asamov, S.T. Rakhimova (Ermakov *et al.*, 1987), which is based on the property of ascorbic acid to absorb light with a maximum wavelength of 265 nm. The activity of the enzyme was judged by the reduction of the optical density, taking into account that the degree of oxidation of ascorbic acid is proportional to the amount of the enzyme. Peroxidase activity was determined with the use of the calorimetric method (by A.M. Boyarkin) based on the determination of the benzidine oxidation reaction rate).

Polyphenol oxidase activity was determined by a spectrophotometric method based on the measurement of optical density of reaction products, which formed during the oxidation of pyrocatechol for a certain period of time (Ermakov *et al.*, 1987).

The analyses were carried out in triplicate for each accounting plant. The mathematical analysis of the measurement results was carried out with the use of the statistical package "Statistica 6.0". To interpret the obtained data, the method of variance multiple factor analysis was used (in the subsequent assessment of differences with the numerous comparison LSD test). In the process of comparison and analysis of the obtained results, significant differences between the signs were used (at $P < 0.05$).

3. RESULTS AND DISCUSSION:

In the territory of the study, the excess of the maximum permissible concentration of benz(a)pyrene, formaldehyde, phenols, carbon oxides, and nitrogen was determined. In the buffer

zones of industrial enterprises, the average annual MPC excess was observed for the following substances: carbon oxide – by two times; nitrogen oxides – by 3 times; sulfur dioxide – by 1.2 times; formaldehyde – by 5 times; phenol – by 1.7 times; benz(a)pyrene – by 1.9 times. In the trunk plantations zone, the average annual MPC excess was observed for the following substances: carbon oxide – by 3.4 times; formaldehyde – by 3.8 times; phenol – by 1.4 times; benz(a)pyrene – by 1.5 times.

The characteristics of plantations on the sample plots in the investigated areas are given in Table 1. The state assessment of plantations showed that in CCZ, the forest crop was estimated as healthy. But in buffer zones of industrial enterprises and in trunk plantations, the forest crop was weak. One of the substances with antioxidant properties is ascorbic acid, but the information in the literature is contradictory. In some sources, it is reported that under the influence of technogenic stress, the content of ascorbic acid decreases in others – vice versa.

Variance multiple factor analysis of the research results revealed the reliability of the influence of the complex conditions of the place of growth (significance level $p < 10^{-5}$), the vegetation period ($P < 10^{-5}$), as well as the interaction of these factors ($P < 10^{-5}$), on ascorbic acid oxidase activity in the leaves of Norway maple (Table 2).

The indicator of ascorbic acid oxidase activity in the leaves was compared among the plants growing in different types of plantations, i.e., in the conditions of the influence of anthropogenic load with varying intensity. In June, the activity of ascorbic acid oxidase in urban plantations was higher than among control samples in the park area. Further on, in July and August, Norway maple showed less enzyme activity among trees in the buffer areas of industrial zones and trunk plantations. At the same time, significant differences were found in August in the conditions of buffer zones of industrial enterprises and amounted to 3.17 activity units.

The analysis of the dynamics of ascorbic acid oxidase activity showed that in the zone of conditional control in the leaves of Norway maple, this index increased significantly from June to August.

In the leaves of Norway maple, the activity of this enzyme significantly decreased during the entire period of active vegetation, in the conditions of buffer plantations of industrial enterprises and trunk plantations. Thus, it can be concluded that the increase in ascorbic acid oxidase activity

during the growing season in the zone of conditional control and the decrease in the buffer zones of industrial enterprises and trunk plantations is typical for Norway maple.

Variance multiple factor analysis of the research results showed the reliable influence of the complex conditions of the place of growth ($P = 4.34 \cdot 10^{-5}$), vegetation period ($P < 10^{-5}$), as well as the interaction of these factors ($P < 10^{-5}$), on the content of ascorbic acid in the leaves of woody plants. The Norway maple growing in BZ plantations of the industrial enterprises, the content of ascorbic acid in leaves in comparison with control was higher: in June – by 76.1; in July – by 59.1, in August – by 22.3 mg/%.

The other tendency was observed in trunk plantations: the content of this metabolite, compared with CCZ, was significantly higher in June – by 111.7, in July, no significant differences were found, and in August it was slightly lower – by 9.6 mg% ($NSR_{05} = 6.5$ mg%). Thus, in Norway maple in the conditions of BZ of industrial enterprises and in trunk plantations, the content of this metabolite decreased during the growing season, which indicates the decrease in the activity of redox processes occurring in the leaves of plants. However, some samples in trunk plantations have differences in the dynamics of ascorbic acid content in the leaves. Thus, the increase of the degree of technogenic load leads to the increase in ascorbic acid content in the leaves of Norway maple.

The features of the content of condensed tannins in the leaves of Norway maple in connection with the conditions of the place of growth were investigated in this work as well. Variance multiple factor analysis of the research results revealed the reliability of the influence of the complex conditions of the place of growth ($P < 10^{-5}$), the vegetation period ($P < 10^{-5}$), as well as the interaction of these factors ($P < 10^{-5}$), on the content of condensed tannins in the leaves of Norway maple (Table 3).

Norway maple in all dates of observations (June, July, August) in the trunk plantations showed the significantly lower content of tannins in the leaves compared with the plantings of CCZ: in June – by 0.35; in July – by 1.06, in August – by 0.89 mg/g⁻¹gDW. Among the plantations of buffer zones of industrial enterprises, the picture was different: in June, the content of this substance was higher by 0.11 mg g⁻¹gDW, in July and August, on the contrary, lower, respectively – by 0.78 and 0.94 mg g⁻¹gDW, in comparison with plantations in the zone of conditional control.

The obtained results indicate the intensive use of this metabolite and its possible participation in the processes of adaptation of woody plants under technogenic stress. By the end of the period of the active vegetation of plants in the plantations of each category, the significant increase in the content of condensed tannins in the leaves of all the species of woody plants under investigation was observed ($P < 10^{-5}$).

Such results were observed in both years of research, which confirms the position that the condensed tannins are an active participant in the adaptation processes of Norway maple in the technogenic environment. Thus, the content of tannins in the leaves of Norway maple growing in the plantations of different ecological categories increased throughout the whole growing season.

To find out the cause of high or low content of tannins, intensive consumption of metabolite, or reduction of its synthesis under conditions of intense technogenic load, the activity of the enzyme involved in the synthesis of tannins was analyzed. The indicator of polyphenol oxidase activity in the leaves was compared among the plants growing in different types of plantations. Variance multiple factor analysis of the research results revealed the reliability of the influence of the complex conditions of the place of growth ($P < 10^{-5}$), the vegetation period ($P < 10^{-5}$), as well as the interaction of these factors ($P < 10^{-5}$), on polyphenol oxidase activity in the leaves of Norway maple (Table 2).

In Norway maple in plantations of BZ of the industrial enterprises in August, and in trunk plantations in July and August, the activity of this enzyme was authentically higher than among control samples in park plantations. The most significant differences were in August in the conditions of the most intensive technogenic load of the trunk plantations; they reached 3.07 activity units.

The dynamics of polyphenol oxidase activity in the leaves of Norway maple in plantations of different types have been studied as well. In park plantations, the significant increase in enzyme activity in July was observed and then the considerable decrease in August; the enzyme activity rates, despite a substantial reduction compared to July, were significantly higher than in June.

In buffer zones plantations and trunk plantations, enzyme activity increased in July and further expanded in August. Thus, the investigation of polyphenol oxidase activity in the leaves of Norway maple has allowed finding out

that in August, the highest values of this indicator in the buffer zones plantations, as well as in trunk plantations, are observed in comparison with park plantations.

Peroxidase activity in the leaves of Norway maple in plantations of different types has been studied as well. Variance multiple factor analysis of the research results revealed the reliability of the influence of the complex conditions of the place of growth ($P < 10^{-5}$), the vegetation period ($P < 10^{-5}$), as well as the interaction of these factors ($P < 10^{-5}$), on peroxidase activity in the leaves of Norway maple (Table 2).

Norway maple in plantations of BZ of the industrial enterprises and in trunk plantations in June showed peroxidase activity that was authentically higher than among control samples in park plantations (Figure 2). In July, the action of the enzyme of Norway maple was higher by 0.04 activity units in the plantations of BZ of industrial enterprises. In August, it was lower by 0.04 activity units than in CCZ.

Norway maple showed higher, in comparison with the control, indicators of enzyme activity only in June. In the following months, the enzyme activity of Norway maple in July was lower (by 0.09), and in August – higher (by 0.03 of activity units) than in the CCZ. The dynamics of peroxidase activity in the leaves of Norway maple in plantations of different types have been studied as well. In park plantations, the significant increase in enzyme activity in July was observed and then the decrease in August; the enzyme activity rates, despite a substantial reduction compared to July, were significantly higher than in June.

In the plantations of BZ of industrial enterprises and the trunk plantations, the dynamics of enzyme activity were similar, increased in July, and decreased by August.

4. CONCLUSIONS:

In the conditions of intensive technogenic load, Norway maple shows higher ascorbic acid oxidase activity at the initial stages of active vegetation and lower at the end of vegetation, in comparison with plants of CCZ. At the same time, the content of ascorbic acid in the leaves also decreases during vegetation of plants in the buffer zones plantations of industrial enterprises and trunk plantations. Thus, the increase in the degree of technogenic load leads to an increase in the ascorbic acid content in the leaves of Norway maple.

As for polyphenol oxidase activity and

tannins content, in the buffer zones plantations of industrial enterprises and trunk plantations, Norway maple was characterized by the increase in polyphenol oxidase activity, in comparison with the control plantings. The rise in polyphenol oxidase activity is accompanied by the decrease in the content of tannins, which indicates the active participation of tannins in the adaptive reactions of plants associated with the mechanisms of neutralization of pollutants.

In the plantations of BZ of industrial enterprises and in the trunk plantations in June, peroxidase activity in the leaves is higher than the same indicators of the control samples. Norway maple in trunk plantations has lower peroxidase activity in its leaves in the conditions of the most intense load in July than in farms of CCZ, and in August, this activity is higher than in the control group.

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Figure 1. The schematic map of sample plots location (the city of Naberezhnye Chelny): Buffer zones of industrial enterprises: 1, 2, 3 – blacksmith plant; 4, 5, 6 foundry plant; trunk plantations: 7, 8, 9 – Avto-1; 10, 11, 12 – Mira Avenue. The scale is 1:50,000.

Figure 2. Peroxidase activity in the leaves of Norway maple in plantations of different types, act.un

Table 1. The characteristics of plantations on the sample plots in the investigated areas

Indicators	Sample plot	Investigation area					
		Conventional control zone		Buffer zones		Trunk plantations	
		Naberezhnye Chelny forestry	Grenada park	Blacksmith plant	Foundry plant	Mira Avenue	Avto-1
Trees/shrubs density, pcs./ha	SP No. 1	416/32	336/40	412/20	440/36	464/12	460/12
	SP No. 2	480/28	352/64	440/32	336/52	420/16	420/44
	SP No. 3	388/24	392/56	384/44	392/56	440/28	440/20
Total number of trees/shrubs, pcs.	SP No. 1	104/8	84/10	103/5	110/9	116/3	115/3
	SP No. 2	120/7	88/16	110/8	84/13	105/4	105/11
	SP No. 3	97/6	98/14	96/11	98/14	110/7	110/5
Forest crop normality	SP No. 1	0.7	0.7	0.7	0.8	0.8	0.8
	SP No. 2	0.8	0.7	0.8	0.6	0.7	0.7
	SP No. 3	0.7	0.8	0.7	0.7	0.8	0.8
Relative life state of forest crop, according to the scale by V.A. Alekseev (1990)	SP No. 1	health	health	weak	weak	weak	weak
	SP No. 2	health	health	weak	weak	weak	weak
	SP No. 3	health	health	weak	weak	weak	weak
Plantations age, years	SP No. 1	45	45	35	40	35	40
	SP No. 2	65	40	55	40	45	40
	SP No. 3	60	40	40	35	45	45

Table 2. The content of ascorbic acid and ascorbic acid oxidase activity in the leaves of Norway maple in plantations of different types

Plantations types	Ascorbic acid oxidase activity, act.un.			Ascorbic acid, mg/%		
	June	July	August	June	July	August
Conventional control zone	3.42	4.25	5.62	246.1	199	158.8
Buffer zone	3.66	3.18	2.45	322.2	258.1	181.1
Trunk plantations	3.96	3.58	2.96	357.8	196.7	149.2
NSR ₀₅	0.03			6.5		

Table 3. *The content of condensed tannins and polyphenol oxidase activity in the leaves of Norway maple in different types of plantations*

Plantations types	Polyphenol oxidase activity, act.un.			Condensed tannins, mg/gDW		
	June	July	August	June	July	August
Conventional control zone	0.94	2.92	1.80	4.87	6.90	8.45
Buffer zone	0.96	2.95	3.51	4.98	6.12	7.51
Trunk plantations	0.96	3.41	4.87	4.52	5.84	7.56
NSR ₀₅	0.04			0.04		