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Influence of anthropogenic factors on anatomo-morphological and physiological indices of needles of common pine trees

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Abstract. The article presents the results of the influence of anthropogenic factors on the changes in anatomo-morphological and physiological features of the structure of pine needles (Pinus sylvestris L.) growing in different habitat conditions in Kstovo, Nizhny Novgorod region. Necrosis and desiccation of needles in places with the most intensive motor transport traffic exceeded the control variant by 30.0-72.0 %, respectively. The experimental variant showed changes in the anatomical structure of pine leaf: epidermis thickness increased (by 60%), the area of assimilation tissue decreased by 34.6%, the diameter of resin passages decreased (by 5.0%) and their number (by 60.0%), the distance between conductive bundles (by 26.7%) and their diameter (by 37.8%), compared to the control variant. Under conditions of anthropogenic load, changes in the pigment composition of pine needles were observed: the content of chlorophyll a decreased by 9.1%, chlorophyll b - increased by 29.1%, carotenoids did not change, compared to the control variant. Thus, the results obtained expand the understanding of morphology, anatomy and pigment composition of needles of common pine growing under urban conditions. Quantitative changes in these indicators depend on the growing area.

1 Introduction

The particularities of growth and development of woody plants under urban conditions have been studied by many scientists [1-2]. However, important characteristics reflecting the peculiarities of their adaptation to unfavorable environmental factors have not been studied in sufficient depth and require further study.

According to scientists [3-4], the response of plants to the action of unfavorable environmental factors reflects the dependence of plant assimilation apparatus on the quality and degree of atmospheric air pollution. Therefore, the assimilation apparatus of plants, in particular conifers, working year-round, develops adaptations to unfavorable environmental

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factors, which are manifested in changes in morphological, anatomical, and physiological parameters of leaf plate structure. In this connection, the study of the structure of photosynthetic apparatus of coniferous plants under conditions of anthropogenic pollution is relevant at present, since photosynthesis determines plant ontogenesis. Therefore, anatomo-morphological and photosynthetic changes in plants are the main indicators of the state of the plant organism under various degrees of influence of unfavorable environmental conditions.

Studies by a number of authors have established that coniferous plants growing in unfavorable conditions differ from plants from natural growing places in morphological-anatomical and physiological indicators of leaf structure. They are characterized by smaller and thickened leaf plates, contain a large number of smaller stomata, and also contain a lower amount of chlorophylls and increased carotenoid content in the leaf [5-6]. As a result, these indicators lead to the disruption of physiological processes, and as a consequence, there are more pronounced signs of xeromorphic plants in conditions with increased anthropogenic load [7-8].

The pigment complex of the leaf of woody plants is the most important physiological indicator that can reflect to a greater or lesser extent the influence of anthropogenic pollution. At the moment, there is no consensus among researchers dealing with this problem regarding the pigment content in leaf chloroplasts. Some researchers note an increase in the content of pigments in leaves, others, on the contrary, note in their works a decrease, or state the absence of changes [7-8].

In this regard, the purpose of our work is to study the anatomo-morphological and physiological features of the structure of the needle structure of the common pine (Pinus sylvestris L.) growing in different growing conditions of the city of Kstovo, Nizhny Novgorod region. To meet this goal it is necessary to solve the following tasks: 1) to identify and analyze changes in morphological parameters of pine needles (Pinus sylvestris L.) depending on anthropogenic environmental factors; 2) to identify and analyze changes in anatomical parameters of pine needles (Pinus sylvestris L.) depending on anthropogenic environmental factors; 3) to identify and analyze changes in the pigment complex of pine needles (Pinus sylvestris L.) depending on anthropogenic environmental factors.

2 Materials and мethods

The research was conducted on the territory of Kstovo, Nizhny Novgorod region. Today Kstovo is a developing industrial center and is characterized by a moderate level of environmental pollution. In addition to industrial enterprises, the state of the city's atmosphere is negatively affected by exhaust gases from motor vehicles. Motor transport accounts for more than 80% of the total amount of pollution.

The object of the study was the common pine (Pinus sylvestris L.), growing in different ecological categories of plantings: forest massif (control variant) and plantings along the highway (experimental variant).

Material for the study was collected in April-May 2023. For anatomical studies, plant material (needles) was collected from trees of the same age, 20 needles each. To study the anatomical structure, needles were fixed in 70% ethyl alcohol solution. Transverse sections were made from the middle part of the leaf lamina and placed in glycerol. The following parameters were determined: epidermis thickness, diameter of resinous passages, distance between resinous passages, diameter of conductive bundles and distance between them, width and thickness of the transverse section of needles.

Studies of the anatomical structure of needles were carried out using a Biolam microscope at a magnification of 20×8 with an ocular micrometer, object - micrometer, camera and software [9].

Morphological indices such as percentage of needle damage, degree of needle desiccation and needle length were determined [10].

When studying the content of photosynthetic pigments, the spectrophotometric method (PE-5400 VI) was used to determine the content of chlorophyll with extraction of pigments with 100% acetone at wavelengths: for chlorophyll a - 662 nm, for chlorophyll b - 644 nm, for carotenoids - 440.5 nm. The Holm-Wetstein formulas were used to calculate pigment concentrations.

$$C_a = 9.784 \cdot D_{662} - 0.990 \cdot D_{644} ;$$
$$C_b = 21.426 \cdot D_{644} - 4.650 \cdot D_{662};$$
$$C_{a+b} = 5.134 \cdot D_{662} + 20.436 \cdot D_{644};$$
$$C_{car} = 4.695 \cdot D_{440,5} - 0.268 \cdot C_{a+b},$$

After establishing the concentration of pigments in the extract, their quantitative content (X, mg/g) in pine needle pomace was calculated using the formula:

$$X = V \cdot C \cdot 100 / m \cdot 1000,$$

where V - volume of alcoholic extract, ml; C - concentration of pigment in alcoholic solution, mg/ml; m - suspended weight, g [11].

The obtained results were processed statistically [12].

3 Resuts

The impact of atmospheric pollution on plants is a complex biochemical phenomenon that primarily affects photosynthetic processes causing morphological changes in leaf plates (necroses, desiccation, needle length). The results of the morphological characterization studies are presented in Figure 1.

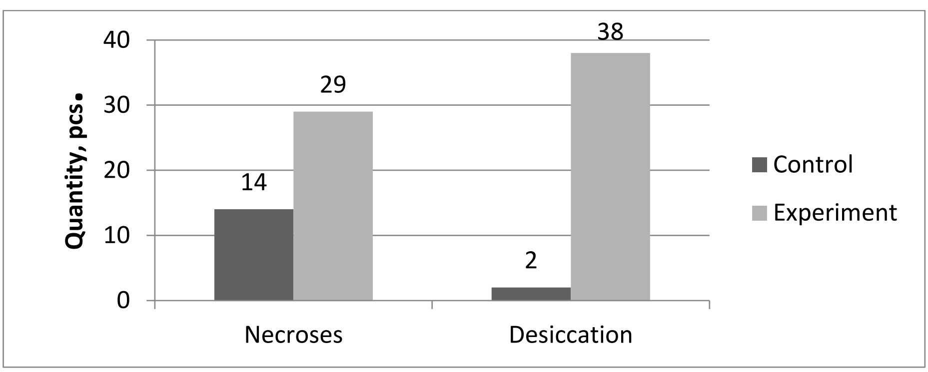

Fig. 1. Influence of anthropogenic factors on morphologic indices of needles of common pine (Pinus sylvestris L.).

As a result of the experiment it was found that the greatest damage to the needles, revealed in the form of necroses, occurred in the experimental variant. The excess of this indicator amounted to 30% compared to the control variant (forest massif).

The degree of needles desiccation in the experimental variant exceeded the control by 72.0%.

The length of needles in the experimental variant decreased by 10.0% compared to the control variant. Thus, under the influence of specific environmental conditions near pollution sources, the appearance and morphological features of pine plants can change, which is confirmed by our study.

During ontogenesis of coniferous plants under the influence of anthropogenic factors there were changes in the anatomical structure of leaf plates (Table 1).

Table 1. Influence of anthropogenic factors on the anatomical structure of the leaf plate of the common pine (Pinus sylvestris L.).

Variant	Epidermis thickness, mm	Diameter of resin passages, mm	Distance between conductive bundles, mm	Diameter of conductive bundles, mm	Distance between resin passages, mm	Width of needle cross section, mm	Thickness of needle cross section, mm
Control (inside the forest)	2.5	10.0	22.5	22.5	25.0	172.5	81.0
Experimental (plantings along highway)	4.0	9.5	16.5	14.0	40.0	132.5	69.0
HPC_{05}	*0.2*	*0.1*	*0.2*	*0.4*	*0.7*	*0.6*	*0.5*

In the experiment it was found that anthropogenic factors influenced the anatomical structure of the leaf plate of common pine. It was found that the epidermis thickness in the experimental variant exceeded the control variant by 60.0%. The diameter of conductive bundles and the distance between them in the control variant exceeded the experimental variant by 37.8 - 26.7 %, respectively. The decrease in the width of the transverse slice (by 23.2 %) and thickness of the transverse slice (by 14.9 %) in the experimental variant led to a decrease in the assimilatory tissue of the needles. Also, the diameter of resin passages decreased by 5.0 % and their number by 60.0 % compared to the control variant.

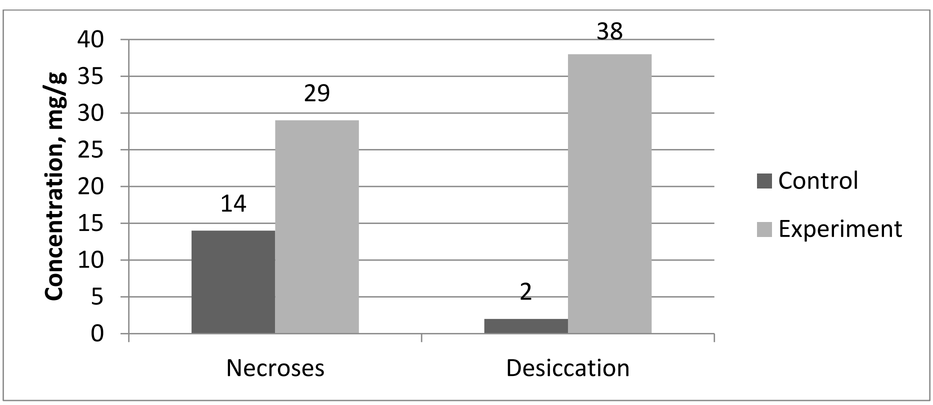

Fig. 2. Influence of anthropogenic factors on photosynthetic pigments of leaf plate of common pine (Pinus sylvestris L.).

The main assimilating organ of the common pine is leaves (needles), which are capable of photosynthesis all year round.

Due to this, the leaf apparatus adapts to different environmental conditions. An important physiological trait reflecting the influence of the growing environment is the content of pigments in the leaf laminae of common pine.

During the month of May, the content of chlorophylls a, b and carotenoids in leaf plates of common pine was studied. The results of the studies are presented in Figure 2.

The maximum content of chlorophyll a was recorded in the leaves of the control variant (by 9.1 %). The content of chlorophyll b in the experimental variant exceeded its content in the control by 29.1 %. The content of carotenoids in the experimental variant had an insignificant excess of 4.7%, within the experimental error.

4 Discussion

Analysis of the data presented in Figure 1 shows that the highest number of spots and necroses on needles was observed in the experimental variant (near the highway). The excess of this indicator amounted to 30% compared to the control variant (forest massif).

The number of needles with signs of desiccation in the experimental variant exceeded the control variant by 72%, which is a consequence of unfavorable environmental conditions.

A slight increase in the length of needles (by 10%) was observed in the control variant compared to the experimental variant.

Analyzing the results of measurements (Table 1), we see that anthropogenic factors had a significant impact on the anatomical structure of the leaf lamina.It was found that the epidermis thickness in the experimental variant exceeded the control variant by 60.0 %. This is probably due to the strengthening of barrier functions of the plant, which limit the entry of harmful substances into the mesophyll and conductive bundles from the environment. The diameter of conductive bundles in the control variant exceeded the experimental variant by 37.8 %. The control variant also showed an increase in the distance between the conductive bundles, which amounted to 26.7 % compared to the experimental variant. A significant increase in the diameter of conducting bundles and the distance between these bundles, which is filled with transfusion parenchyma, the cells of which partially perform the conducting function, contribute to the strengthening of the process of excretion of toxic substances. The decrease in the width of the transverse slice (by 23.2 %) and thickness of the transverse slice (by 14.9 %) in the experimental variant led to a decrease in the assimilatory tissue of the needles, which contributes to a decrease in photosynthetic processes. Also, the diameter of resin passages decreased by 5.0 % and their number by 60.0 % compared to the control variant.

Thus, in the anatomical structure of leaf plates of common pine with increasing degree of atmospheric air pollution there is a shift towards strengthening of xeromorphic properties. These properties are manifested in such indicators as thickening of epidermis, reduction of leaf surface of needles, reduction of diameter of resin passages and their number, reduction of diameter of conductive bundles and distance between conductive bundles, which allows plants to adapt to existing unfavorable environmental factors.

The main criterion of the condition of coniferous woody plants in urban space is the state of photosynthetic pigments in the leaf lamina. Since photosynthetic activity of plants determines growth and reproductive processes, therefore, the pigment complex is an indicator of plant growth and development.

The highest content of chlorophyll a was observed in the conifer of the control variant (Fig. 2). The excess amounted to 9.1 %. The content of chlorophyll b in the experimental variant exceeded its content in the control by 29.1 %.

Some authors report [13] that high levels of motor vehicle emissions reduce the content of all green pigments, with chlorophyll a decreasing in greater amounts and chlorophyll b slightly less. Other authors show in their studies that chlorophyll b accumulates to a greater extent than chlorophyll a under the influence of anthropogenic load. It is also known that more stable species accumulate more chlorophyll b, which, according to some scientists, prevents chlorophyll a from being destroyed [14].

Recently, scientists have been discussing the physiological basis for the intensity of the photosynthesis process when plants are affected by motor vehicle emissions, which is characterized by a certain ratio of chlorophylls (chlorophyll a/chlorophyll b) in the pigment system. In the norm, this ratio should be equal to: 2.1-3.1 [15]. Studies have shown that this indicator in the control is equal to the ratio of 3:1, in the experimental variant - 2:1. This is a sign of good intensity of photosynthesis processes on both variants.

Carotenoids are an obligatory component of photosynthetic apparatus of plants. They take part in the stabilization of chloroplast membranes and proteins of antenna complexes; absorb and dissipate excitation energy, working as photoprotectors of the photosynthetic apparatus; they are potential suppressors of dangerous triplet chlorophyll and singlet oxygen [16].

The content of carotenoids in the experimental variant had an insignificant excess of 4.7%, within the error of the experiment. According to some scientists, carotene may act as a certain protective function for chlorophylls, while others do not note the connection of yellow pigments with the problem of adaptation to the environment [3, 16].

5 Conclusion

In the conditions of growth with anthropogenic load, changes in morphological indices of pine needles were observed: the excess in needle damage is 30%, in the degree of desiccation - by 72%, in the length of needles - by 10%, compared to the control variant.

Under the conditions of anthropogenic load, changes in the anatomical structure of the pine leaf were observed: epidermis thickness increased (by 60.0%), the area of assimilation tissue decreased by 34.6%, the diameter of resin passages decreased (by 5.0%) and their number (by 60.0%), the distance between conductive bundles (by 26.7%) and their diameter (by 37.8%), compared to the control variant.

Under conditions of anthropogenic load, changes in the pigment composition of pine needles were observed: the content of chlorophyll a decreased by 9.1%, chlorophyll b - increased by 29.1%, carotenoids did not change, compared to the control variant.

Thus, the results obtained expand the understanding of morphology, anatomy and pigment composition of needles of common pine growing in different urban conditions. Quantitative changes in these indicators depend on the growing area.

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