POLLUTION

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THE USE OF INDICATORS OF PLANT PHOTOSYNTHETIC AND ENZYMATICE ACTIVITY IN BIOINDICATION STUDIES

Research article

Abstract

In the context of increasing anthropogenic impact on the environment, the study of the mechanisms of plant adaptation is an actual problem. For the diagnosis of ecological trouble, it is advisable to study the indicators characterizing metabolic processes in autotrophic plants. The change in the parameters of the photosynthetic apparatus and antioxidant system in silver birch (*Betula pendula*), European spruce (*Picea abies*) and dandelion (*Taraxacum officinale L.*) depending on the intensity of anthropogenic load was studied. The pigment composition of plants and the activity of antioxidant enzymes can be used as markers of the anthropogenic pollution level of a territory.

Keywords: environment, pollution, bioindication, polyphenol oxidase, chlorophyll, carotenoids.

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ИСПОЛЬЗОВАНИЕ ПОКАЗАТЕЛЕЙ ФОТОСИНТЕТИЧЕСКОЙ И ФЕРМЕНТАТИВНОЙ АКТИВНОСТИ РАСТЕНИЙ В БИОИНДИКАЦИОННЫХ ИССЛЕДОВАНИЯХ

Научная статья

Аннотация

В условиях усиления антропогенного воздействия на окружающую среду исследование механизмов адаптации растений является актуальной проблемой. Для диагностики экологического неблагополучия целесообразно изучение показателей, характеризующих протекание метаболических процессов у автотрофных растений. Изучено изменение показателей фотосинтетического аппарата и антиоксидантной системы березы повислой (*Betula pendula*), ели европейской (*Picea abies*) и одуванчика лекарственного (*Taraxacum officinale L.*) в зависимости от интенсивности антропогенной нагрузки. Пигментный состав растений и активность ферментов антиоксидантной защиты могут быть использованы как маркеры уровня антропогенной загрязненности территории.

Ключевые слова: окружающая среда, загрязнение, биоиндикация, полифенолоксидаза, хлорофилл, каротиноиды.

1. Introduction

Pollution is one of the most significant environmental factors in the modern world. There are various sources of pollution as internal combustion engines, chemical, metallurgical, and food industries. More than 500 thousand chemicals as heavy metals, oil products, pesticides, herbicides, etc. constantly enter the Earth's biosphere as products of technogenesis [1, P.59-66].

Plants are under the cumulative impact of emissions from transport and industrial enterprises. Some of these substances are accumulated and in some cases used by plant organisms. The adaptation of plants to rapidly changing concentrations of pollutants is slow. Physical factors also have a certain influence, such as vibration, noise, and electromagnetic fields.

As a rule, anthropogenic impact leads to a decrease in the productivity of plants, affects the metabolic processes, primarily the assimilation apparatus [2, P.50-55], [3, P.109-112].

The analysis of the ecological state of the environment is usually carried out on only a few parameters. A number of polluting factors, possible synergistic or cumulative effects are not taken into account. This does not provide a complete picture of the environmental stress experienced by living organisms [4, P.171-185].

According to the official sources [5], [6], the total amount of air pollutants from all emission sources in 2019 in the Tambov region was 150.59 thousand tons, including 74.71 thousand tons emissions from vehicles. Although the proportion of samples exceeding the maximum permissible dose was less than 1%, even at such pollution concentrations, changes in both metric and physiological and biochemical parameters of plant organisms are observed. Therefore, plants can be used as indicators of the ecological state in urban environment.

2. Methods

2.1. Determination of chlorophyll and carotenoids

A weighed portion of the plant material was ground in a porcelain mortar with the addition of sand, and the pigments were extracted with acetone. The extract was centrifuged at 4000 rpm for 10 min. The determination of pigments was carried out by spectrophotometric method (spectrophotometer СФ-2000) without preliminary separation of pigments in 100% acetone extract. Optical density was measured at following wavelengths: chlorophyll a - 662 nm, chlorophyll b - 644 nm, carotenoids - 440.5 nm.

The concentration of pigments was calculated by the formulas [7, P. 154-171]:

$C_a = 11{,}7 \cdot D_{662} - 2{,}09 \cdot D_{644}$,

$C_b = 21{,}19 \cdot D_{644} - 4{,}56 \cdot D_{662}$,

$C_{a+b} = 7{,}14 \cdot D_{644} + 19{,}1 \cdot D_{662}$,

$C_c = 4{,}695 \cdot D_{440,5} - 0{,}268 \cdot C_{a+b}$,

where C_a, C_b, C_{a+b} – concentration of chlorophyll a, b and their sum, respectively, mg/l;

C_c – total concentration of carotenoids, mg/l;

D_n is the optical density of the solution at a wavelength of n nm.

2.2. Determination of polyphenol oxidase activity

A sample of plant material was homogenized in the presence of polyamide and 1/15 M phosphate buffer solution (pH 7.4); then the extract was centrifuged. The activity of polyphenol oxidase was determined spectrophotometrically (spectrophotometer СФ-2000) using pyrocatechol as an oxidation substrate [8]. Optical density was measured at 420 nm in 2 s steps for 120 s. The program "Kinetics" was used.

3. Results and discussion

Silver birch (*Betula pendula*), European spruce (*Picea abies*) and dandelion (*Taraxacum officinale L.*) were used as the objects of the research as one of the most common species on the territory in the city of Tambov.

5 points of Tambov were selected for sampling plant materials (Table 1). The choice was based on the quantity and quality of anthropogenic load.

Table 1 – The plant material sampling points

1	Tambov-4 ("sleeping area")
2	Stop "Dynamo" (Sovetskaya st., 2; low traffic)
3	Stop "Pigment" (Montazhnikov st., 1; chemical plant)
4	Energetikov proezd, 7 (heat and power plant area)
5	Intersection of Sovetskaya and Moskovskaya streets (active traffic)

It was found that a maximum annual linear growth of spruce and birch shoots is observed at points with a minimum environmental load, located far from enterprises and active traffic flows (Fig. 1). This indicates favorable conditions for the growth of plants. In areas characterized by a high anthropogenic load (points 4 and 5), plant vegetation is suppressed (approximately 11 cm for silver birch (LSD_{05} = 6.52) and 10-12 cm for European spruce (LSD_{05} = 6.28)).

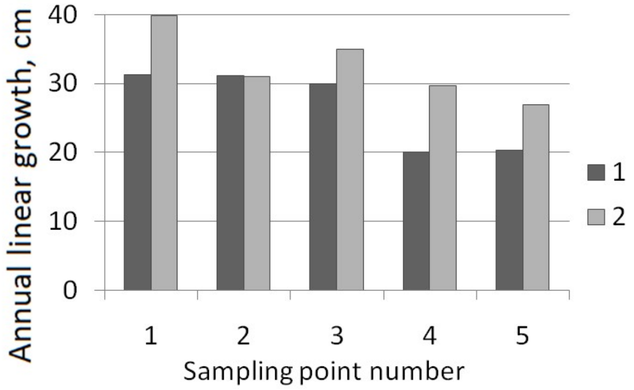

Figure 1 – Dependence of annual linear growth of silver birch *(Betula pendula)* (*1*) and European spruce *(Picea abies)* (*2*) from the point of growth

In this study, the amount of chlorophyll and carotenoids in Silver birch (*Betula pendula*), European spruce (*Picea abies*) and dandelion (*Taraxacum officinale L.*) was analyzed. Chlorophylls a and b and their accompanying carotenoids constitute a constant set of chloroplast pigments in the leaves of higher plants.

Changes in the amount and ratio of chlorophyll and carotenoids under anthropogenic stress have been noted by many researches. The main reason for the decrease in the content of chlorophylls in the presence of heavy metals is the suppression of its synthesis. Heavy metals can also affect the functioning of chloroplast membranes and proteins of photosystem [9], [10, pp. 229-236]. According to [11], [12], when the atmospheric air is polluted, the response of photosynthetic apparatus of plants is to decrease the ratio C_a:C_b and increase $(C_a+C_b)/C_c$.

Since the dependence of pigments content in plants on the level of environmental pollution was a subject of interest, it was proposed to take the amount of chlorophyll and carotenoids in plants growing at point 1 as etalon, and calculate the coefficients showing the change in the content of pigments:

$K_c = (C_a:C_b)_n/(C_a:C_b)_1$,

where $(C_a:C_b)_n$ is the ratio of the concentrations of chlorophylls a and b at plant material sampling point n; $(C_a:C_b)_1$ is the ratio of the concentrations of chlorophylls a and b at plant material sampling point 1;

$K_p = ((C_a+C_b)/C_c)_n/((C_a+C_b)/C_c)_1$,

where $((C_a+C_b)/C_c$ is the ratio of the sum of the concentrations of chlorophylls a and b to the concentration of carotenoids.

For all cases, K_c is less than one, i.e. a decrease in the ratio of the content of chlorophylls a and b is observed (Fig. 2). Different species have different sensitivity to contamination of the environment. There is a clear dependence of the pigment content on the intensity of anthropogenic load in leafs of birch.

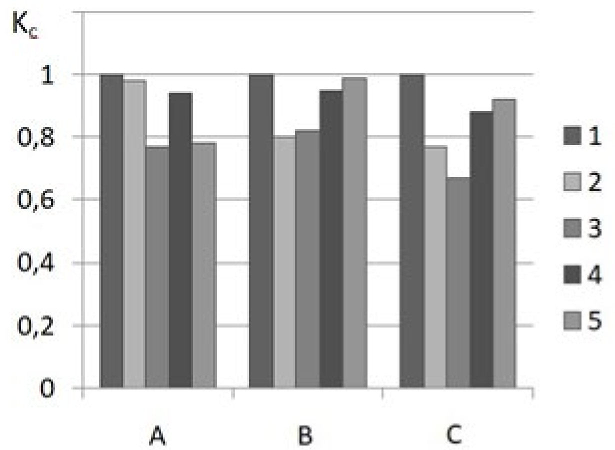

Figure 2 – Coefficient of change in C_a:C_b ratio (K_c) in silver birch *(Betula pendula)* (*A*), European spruce *(Picea abies)* (*B*), and dandelion *(Taraxacum officinale L.)* (*C*) depending on the point of growth (1-5, Tab.1)

The chemical nature of the pollution plays an important role. According to experimental data (Fig.3), the amount of carotenoids is sensitive mostly to emissions of the chemical plant. It leads to increase of $(C_a+C_b)/C_c$ ratios in all samples were analyzed.

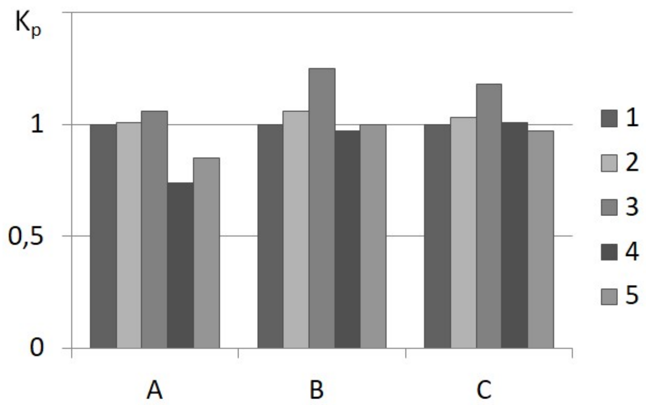

Figure 3 – Coefficient of change in $(C_a+C_b)/C_c$ ratio (K_p) in silver birch *(Betula pendula)* (*A*), European spruce *(Picea abies)* (*B*), and dandelion *(Taraxacum officinale L.)* (*C*) depending on the point of growth (1-5, Tab.1)

Almost any environmental factor leads to an excess of ROS (reactive oxygen species) in living cells. There are various mechanisms aimed at maintaining a certain optimal level of ROS. They involve enzymes and non-enzymatic components of antioxidant system (AOS). The response of AOS on the pollution was shown by [13], [14, P. 213–230], [15, P. 220-224].

Previously, the authors of the current study have shown a change in the activity of polyphenol oxidase (PPO) both in the model media containing salts of heavy metals, and at natural conditions [16, P. 255-257], [17, P. 347-348].

The significant change in PPO activity is observed in leafs of silver birch *(Betula pendula)* (Fig. 4). With an increase in anthropogenic load, the PPO activity in birch leaves rises. This might be the reaction of cells to an increase in the need for respiration, caused by a high content of pollutants [15, P. 220-224].

In pine needles, a similar phenomenon is also observed; however, at the points with maximum pollution by fuel combustion products, the PPO activity is extremely low. It is possible that the adaptive capabilities of the plant at a high level of pollution have been exhausted.

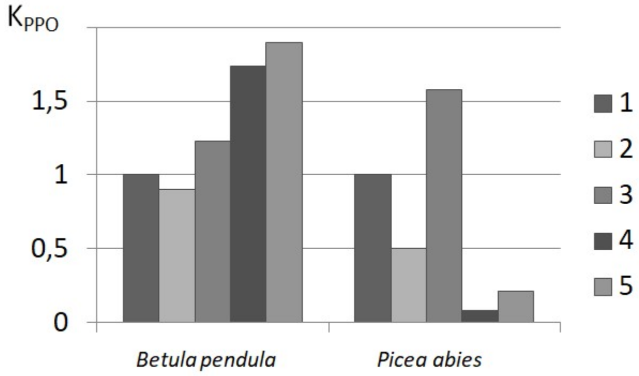

Figure 4 – Coefficient of change in polyphenol oxidase activity of silver birch *(Betula pendula)* and European spruce *(Picea abies)* depending on the point of growth (1-5, Tab.1)

Conclusion

Investigation of the state of the pigment system and antioxidant system of plants can be useful for the operational bioindication of contamination during environmental monitoring. Within the framework of the study, the most promising indicator of the level of anthropogenic pollution is the activity of polyphenol oxidase in birch leaves, which increases by 70-80% under unfavorable conditions.

Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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