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BIO-INDICATION PERSPECTIVITY OF *BETULA PENDULA* ROTH. UNDER CONDITIONS OF ENVIRONMENTAL OIL POLLUTION

Abstract. The biological and ecological features of *Betula pendula* Roth. and its bioindicative perspectivity under the conditions of environmental oil pollution are analyzed. A wide areas of *Betula pendula* distribution are found both in the conditions of urbanized and natural territories of Ukraine, and in the natural zones of the Northern Hemisphere. The morphological, physiological and biochemical vitality indicators of the species under the oil pollution conditions are studied. The stability of the species linear foliar parameters and leaf area in the experimental area conditions are established, with the exception of the leaf asymmetry parameter, which doubles comparing to the background value. Peculiarities of *Betula pendula* assimilation organs necrotization under the influence of oil pollution are studied. It is found that the spotted type of necrosis is the dominant in the conditions of the background area, while "fish skeleton" and interveinal necrosis types are dominant in the oil deposit conditions. In the conditions of the background area, the necrotic lesions affect the smaller half of *Betula pendula* leaves, and at the same time, the largest number of leaves is affected by the second degree of necrosis. Under conditions of oil pollution 65% of the species leaf plates are affected by the fifth necrosis degree. This indicates that pollutants reach the plants above-ground organs through the root system. Under the influence of oil pollution there is a decrease in the buffer resistance of the species leaves' cells internal environment relatively to the background values. *Betula pendula* reacts sensitively to an additional stress factor – acid rain, and restores buffer resistance by only 38.2%, while in the conditions of the background area this value is 55.8%.

The most sensitive morphological bioindicative markers of *Betula pendula* vitality in the conditions of the environmental oil pollution are the fluctuating asymmetry coefficient and the leaves necrotic lesion degree. The parameter of buffer stability is characterized by the highest informativeness among other physiological and biochemical indicators.

According to a complex of morphological, physiological and biochemical indicators, *Betula pendula* shows an average sensitivity to environmental oil pollution and can be used as a biological indicator of the ecological state of man-made territories.

Key words: *Betula pendula*, oil pollution, environment, bioindication, morphological indicators, physiological and biochemical indicators.

INTRODUCTION

Environmental oil pollution is one of the global ecological problems of the last decades [14]. Nowadays various technical and biological methods of cleaning soil, water and air from oil components are used, as well as environmental monitoring of oil production areas ecological condition. Along with the analytical methods of controlling the level of environmental oil pollution, phytoindicative methods have also become widespread, which involve the use of plants sensitive to oil pollution, whose reactions analyze the current ecological situation in a certain area [1, 3, 4, 13].

Among the phytoindicative parameters, morphological indicators that reflect the growth and development of the plant, as well as physiological and biochemical one that reflect internal metabolic processes in the body, are the most informative. The classic reactions of plants in response to the influence of oil pollution are the suppression of growth processes, the appearance of premature aging signs and even the death of plants. A leaf, as a functionally active organ, reacts faster than others to environmental factors, therefore the level of man-made loading in a given area can be judged by the reaction of plant leaves [1, 15].

Betula pendula is one of the most common woody plants in the natural zones of the northern hemisphere and the main forest-forming species of Ukrainian mixed forests. *Betula pendula* is a valuable medicinal, essential oil and decorative plant used in the landscaping of Ukrainian settlements [2]. The species is characterized by its resistance to abiotic environmental factors – it tolerates frost, drought, and winds, grows on different soil types [1, 5]. In the case of *Betula pendula* vitality decrease, damage to the leaf plates by the fungus *Melampsorium betulinum* is observed, which causes rust disease of the species leaves, the appearance of local necrosis and premature yellowing of the leaves. *Betula pendula* leaves are very sensitive to spotting pathogens, which are caused by anamorphic fungi from the genera *Septoria*, *Phoma*, *Phomitopsis*, *Ascochyta*, *Gloeosporium*, *Marssonina* etc.

Many scientific publications are devoted to the impact of anthropogenic pollution of urbanized areas on *Betula pendula* life condition [7, 8, 10, 11, 16, 17], but there is not enough information about the oil pollution impact on this species and the prospects of using the species as an indicators of environmental oil pollution [6, 9].

MATERIALS AND METHODS

The research is conducted in Bytkiv-Babchensky oil and gas condensate field conditions, located in the Nadvirnyan district of Ivano-Frankivsk region.

Sampling of plant material is made from the lower part of the crown along its perimeter in tiers with one order of branching during the period of completion of the assimilation system full development (August-September). At the same time 8 *Betula pendula* individuals are analyzed, which grow in the zone of the deposit direct influence at a distance of up to 500 m. As controls plants from a conditionally ecologically clean territory – the "Gorgany" reserve – are chosen.

The necrotic lesions of leaf plates presence are determined visually. The necrosis damage degree is assessed using a 5-point rating scale according to S.S. Rudenko's method [12]: 0 – no damage; 1 – minor point necrosis is present; 2 – spotted necrosis up to 10 mm present; 3 – local necrotic spots that exceed 10 mm present; 4 – less than half of the leaf plate is dead; 5 – the greater half of the leaf blade is dead.

The leaves area is determined by the weight method. The coefficient of asymmetry is calculated according to the formula according to the generally accepted methodology [12].

The leaf asymmetry coefficient is determined by the formula:

FA ($FA = \sum |L-R| / (L+R) / 2$), where:

|L-R| – the difference between the left and right parts of the leaf;

L+R – the sum of the width of the left and right parts of the leaf;

FA is the leaf asymmetry coefficient.

Each morphometric parameter is studied 100 times.

The leaves acidity protoplast acidity is determined according to the generally accepted method. For this an express test called "acid rain" is used. After grinding fresh plant material weighing 2 g in 10 ml of distilled water and determining the pH value, 10 ml of 0.1N hydrochloric acid is added. After a day the pH value is determined, and the stability of plant cells buffer system is evaluated based on the difference between the initial and final acidity values of the leaf cells protoplast. The repeatability of experimental studies is fivefold.

Mathematical results processing is carried out using the variational statistical method. The reliability of the difference between the obtained experimental data and the background data is evaluated using the Student's t-test. The null hypothesis is rejected at $P \leq 0.05$. If the level of significance is in the range of $0.10 > P > 0.05$, then it is considered that there is a tendency for the manifestation of one or another process. All calculations are performed using the MS Excel 2007 editor and the Statistica 6.0 software package.

DISCUSSIONS

Betula pendula assimilation organs growth processes are characterized by relative stability in oil pollution environmental conditions. No significant difference in the length and width of the specie leaf is recorded in the plants of the experimental and control areas (Table 1).

Table 1. *Betula pendula* morphometric parameters in the background and oil-polluted territory

Morphometric parameters	Oil-polluted territory	Background territory
Leaf length, sm	4,93±0,19	4,81±0,14
Leaf width, sm	4,12±0,12	3,90±0,09
The left side of the leaf, sm	2,06±0,16	1,94±0,18
The right side of the leaf, sm	2,06±0,11	1,95±0,13
Leaf area, sm ²	7,11±2,20	6,51±1,77
Coefficient of asymmetry	0,04	0,02

A slight intensification of the species leaves growth under the influence of the deposit is noted, which is a rather rare phenomenon in stressful growth conditions. However, this species reaction indicates a sufficiently large potential of *Betula pendula* to adapt to extreme environmental conditions.

An increase in plant mass under stressful growth conditions can be considered as an adaptive and protective mechanism aimed at increasing the number of leaf cells and, accordingly, increasing plant survival.

There is no significant difference between the leaves area of experimental and control plants, which is a reason to consider *Betula pendula* as an ecologically plastic species. This tree species supports growth processes at a high level, which also indicates an effective phytoremediation ability.

Betula pendula leaves asymmetry coefficient doubles in the oil-polluted area compared to the background, which is a direct indication of unfavorable growth conditions for this species.

Necrotic damage occurs as a result of deep irreversible changes in the leaf plate due to the concentration of pollutants absorbed from the atmospheric air or soil in its separate areas. The topography of phytotoxicants distribution within the assimilation organs of woody plants depends on the industrial emissions composition, the concentrations of its individual ingredients,

the speed of their entry into the leaf and movement through the vessels [2, 3, 4]. Necrotized tissues of the leaf surface cannot perform photosynthesis, and therefore cease to perform the environment-creating function. This leads to the loss of plants vitality and its subsequent death.

Necrotic lesions of *Betula pendula* leaf plates have a different nature and degree of manifestation in plants of the background area and plants growing under the influence of the oil deposit (Table 2).

Table 2. *Betula pendula* leaf plates necrosis degrees in the background and oil-polluted territory

Necrosis degrees, % of the total number of leaves	Oil-polluted territory	Background territory
I	7	12
II	11	14
III	3	8
IV	16	3
V	65	4

In the conditions of the background territory less than the half of all examined *Betula pendula* leaf plates are characterized by the presence of necrosis on their surface. Predominant point and spot necrosis, which can occur in natural ecologically clean conditions due to certain climatic influences.

In environmental oil pollution conditions there is an increase in *Betula pendula* number of necrotized leaves, which is a signal of the plants protective mechanisms exhaustion. It is established that under the oil deposit influence 65% of *Betula pendula* leaves are characterized by the presence of spots larger than half of the leaf area.

In the conditions of the background area the point necrosis is the main necrosis type, which is quite natural given the fact that in a relatively clean territory there are practically no toxicants capable of causing serious damage to the assimilation organs (Table 3).

Table 3. *Betula pendula* leaf plates necrosis types in the background and oil-polluted territory

Necrosis types, %	Oil-polluted territory	Background territory
Edge	17	11
Spotted	21	21
Interveinal	30	4
“Fish skeleton”	32	5

However, it should be noted that *Betula pendula* is quite sensitive to the smallest concentrations of pollutants in the environment, which is manifested by the appearance of marginal and “fish-skeleton” necrosis in 11 and 5% of leaf plates, respectively.

Necrotic damages of plants assimilation organs in most cases are specific reactions to various stressors. Point necrosis occurs as a result of drops of sulfuric or nitric acids falling on a leaf during smog, fog or precipitation in the form of acid rain. Marginal necrosis is an evidence of heavy metal salts accumulation on the leaf blade edges.

Interveinal necrosis occurs in the process of nitrogen or sulfur oxides drops entering the leaf through the pores, that are transformed into nitric or sulfuric acid in the cytoplasm, which

are strong hygroscopic substances and rather quickly take moisture from carbohydrates that are formed in the process of photosynthesis.

The formation of free carbon burns part of the leaf, the free liquid evaporates, the coal is washed away by precipitation, resulting in the formation of dry blackish-brown tissue. “Fish skeleton” type necrosis is mostly caused by the entry of toxic substances through the root system.

In the conditions of environmental oil pollution the dominance of the “fish skeleton” and interveinal necrosis type is noted on *Betula pendula* leaves surface, which is a sign of toxic compounds entry both through the plants root system and through above-ground parts, in particular, leaves.

To counteract the chemical pressure of exogenous factors, cells have buffer systems that stabilize the pH of their environment. Displacement from the optimal pH value has a negative effect on metabolic processes, the formation and functioning of the protein-enzyme complex in the leaves. Therefore, by the indicator value of the leaves protoplast buffer capacity, it is possible to judge the resistance of the plant to man-made conditions [5].

The conducted studies of the woody plants leaves cells buffer system condition testify to the weakening of protective mechanisms in *Betula pendula* in response to unfavorable growth conditions (Table 4).

Table 4. *Betula pendula* buffer characteristics in the background and oil-polluted territory

Physiological and biochemical parameters	Oil-polluted territory	Background territory
the leaves protoplast pH	5,60±0,02*	5,28±0,02
the pH shift (Δ pH) of the leaf's protoplast	3,46±0,02*	2,33±0,04
pH pH, %	38,2	55,8

Note. * – differences with the control are significant at $P \leq 0.05$.

The increase of the pH value of the species leaf plates intracellular environment in the oil pollution conditions is due to the ingress of toxicants through stomata or the root system. In particular, heavy metals compounds help to reduce the natural acidity of plant protoplasts.

Betula pendula reacts sensitively to an additional stress factor – acid rain, and restores buffer resistance by only 38.2%, while in the conditions of the background area this value is 55.8%.

RESULTS

Under environmental oil pollution conditions, no reliable changes in *Betula pendula* leaf plates morphometric parameters compared to the background territory are recorded. This is a sign of the species ecological plasticity and the ability to adapt to adverse growth conditions. An exception is the coefficient of fluctuating asymmetry, which doubles under the influence of the deposit compared to the background area. *Betula pendula* reacts to the environmental oil pollution with the appearance of necrotic damage on the leaves. On the surface of the species assimilation organs all types and degrees of necrosis are represented both in the conditions

of the background and the experimental territory. “Fish skeleton” and interveinal necrosis are dominant in stressful growth conditions, which indicates the entry of contaminants both through the species leaf stomata and roots. Under the influence of the deposit the stability of *Betula pendula* leaf plates buffer system also decreases, compared to the background area. In general, *Betula pendula* shows an average sensitivity to the environmental oil pollution, which allows the species to be introduced into the greening of oil-polluted areas, where the plant will perform the dendro-indicative function as effectively as possible.

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АНОТАЦІЯ

БІОІНДИКАЦІЙНА ПЕРСПЕКТИВНІСТЬ БЕРЕЗИ ПОВИСЛОЇ В УМОВАХ НАФТОВОГО ЗАБРУДНЕННЯ ДОВКІЛЛЯ

Проаналізовано біологічні та екологічні особливості берези повислої та її біоіндикаційну перспективність в умовах нафтового забруднення довкілля. З'ясовано широкий ареал поширення берези повислої як в умовах урбанізованих та природних територій України, так і в природних зонах північної півкулі. Досліджено морфологічні та фізіолого-біохімічні показники життєвості виду під впливом нафтового рододовища. Встановлено стабільність лінійних фоліарних показників та площі листків в умовах дослідної території, за винятком параметра асиметрії листків, що зростає удвічі щодо фонового значення. Досліджено особливості некротизації асиміляційних органів берези повислої під впливом нафтового забруднення. З'ясовано, що домінуючим типом некрозу в умовах фонові території є плямистий, тоді як в умовах нафтового забруднення – некроз типу «риб'ячий скелет» та міжжилковий. В умовах фонові території некротичне ураження характерне для меншої половини листків берези повислої і при цьому найбільша кількість листків уражена другим ступенем некрозу. В умовах нафтового забруднення 65% листкових пластинок виду уражено п'ятим ступенем некрозу. Це свідчить про потрапляння забруднювачів через кореневу

систему до надземних органів рослини. Під впливом нафтового забруднення відзначається зниження буферної стійкості внутрішнього середовища клітин листків берези повислої щодо фонових значень. Береза повисла чутливо реагує на додатковий стресовий фактор – кислотний дощ, та відновлює буферну стійкість лише на 38,2%, тоді як в умовах фонові території це значення становить 55,8%.

Найчутливішими морфологічними біоіндикаційними маркерами життєвості виду в умовах нафтового забруднення довкілля є коефіцієнт флюктууючої асиметрії та ступінь некротичного ураження листків. Показник буферної стійкості відзначається найвищою інформативністю серед інших фізіолого-біохімічних показників.

За комплексом морфологічних та фізіолого-біохімічних показників береза повисла проявляє середню чутливість до нафтового забруднення довкілля та може використовуватися у якості біологічного індикатора екологічного стану техногенно-змінених територій.

Ключові слова: береза повисла, нафтове забруднення, навколишнє середовище, біоіндикація, морфологічні показники, фізіолого-біохімічні показники.