

ORIGINAL ARTICLE

Morphological and physiological parameters of woody plants under conditions of environmental oil pollution

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The morphological and physiological parameters of woody plants vitality under conditions of the environmental oil pollution are analyzed. The inhibition of investigated plants' leaves growth, the appearance of necrotic lesions and a decrease in the buffer stability of the assimilation organs' internal environment of trees under the influence of oil contamination are revealed. It has been established that *Acer platanoides* L. has the least buffering potential of leaves among all tree species, which are widely represented in natural and anthropogenic-modified ecosystems and grow under the conditions of the Bytkiv-Babchens'ke oil deposit. After artificial acidification of the homogeneous leaves of *Acer platanoides*, taken from the zone of the oil deposit influence and the background territory, the displacement of the acidity index was 3.32 and 2.96, respectively. All tree species under oil contamination conditions are marked by an increase of the leaves' internal environment acidity comparing with the background area.

Under oil deposit influence *Acer platanoides* is marked by the presence of the greatest lesions number of the leaf blades-the degree of necrosis is 4.19. The degree of leaf necrosis under the influence of oil pollution of the environment increases in the following row of investigated plants: *Salix caprea* L. → *Betula pendula* Roth. → *Populus tremula* L. → *Fagus sylvatica* L. → *Populus pyramidalis* L. → *Acer platanoides* L.

The destroying of the trees leaf tissue in stressful growth conditions indicates the weakening of the protective properties of the species, reveals their sensitivity to oil pollution. The appearance of necrosis of the "fish skeleton" type in *Fagus sylvatica* and *Acer platanoides* is established, which affects 11 and 23% of leaves of these species. The decrease in the leaves' area of *Populus tremula*, *Fagus sylvatica* and *Populus pyramidalis* is fixed at 55, 40, 20%, respectively.

The greatest value of the fluctuating leaf asymmetry coefficient is characteristic for *Fagus sylvatica* and *Acer platanoides*-0,09. *Salix caprea* and *Betula pendula* are identified as the most resistant species to the oil pollution and should be implemented in greening of the technogenically-transformed areas. These species are characterized by high phytoremediation ability and are effective phytomeliorants of the environment.

Fagus sylvatica and *Acer platanoides* are sensitive and informative phytoindicators of the ecological state of the oil-contaminated environment. These species are recommended for use in fitomonitoring studies to evaluate the quality of the environment.

Populus tremula and *Populus pyramidalis* are characterized by the average vitality among the studied tree species.

Keywords: Woody plants; bioindicators; bioremediators; stability; oil pollution; environment; morphological and physiological parameters

Introduction

Environmental oil pollution is one of the major global problems in the context of rapid industrialization and urbanization (Rai, 2016). Heavy metals as oil compounds are of particular danger because they have the ability to block enzymatic reactions in the body. Polycyclic aromatic hydrocarbons, which account for 75% of the oil components, stimulate the appearance of cell mutations. Today, there are known studies of the oil influence on growth, reproductive function, the content of photosynthetic pigments, enzyme activity, carbohydrate and protein content in grassy tissues (Nardelia, 2016; Panchenko et al., 2017; Tran et al., 2018). The trees in this aspect were studied to a much lesser extent, and the study of the promising nature of trees as phytoindicators and phytodesigns of the environment was carried out mainly in urboecosystems contaminated with heavy metals (Ojekunle et al., 2014; Hu et al., 2014; Franiel, Babczynska, 2011; Erofeeva, 2015 Steindor et al., 2016; Birke et al., 2018).

Fragmentary studies of the woody species vitality under oil pollution environmental conditions are represented in

publications by foreign authors (Mukherjee et al., 2015; Pedroso et al., 2016; Fasani et al., 2016). Perennial green plantations are known for their environment-specific function, the ability to react to the complex influence of environmental factors by a number of adaptive and destructive processes, the nature and extent of which can be judged on the level of technogenic environmental transformation (Glibovytska, 2017).

Reactions of different plants types to changed environmental conditions strictly correlate with their structural and functional characteristics. Among the most important indicators of the plants' vitality in specific living conditions are the leaf parameters (Gostin, 2016). The leaf blade as a functionally active body is more likely to respond to environmental changes than others and is most used in biomonitoring studies (Franiel, Babczynska, 2011; Ojekunle et al., 2014; Rai, 2016; Ord et al., 2016; Pedroso et al., 2016; Fasani et al., 2016; Alves-Silva et al., 2018).

The index of fluctuating asymmetry of leaves is considered an indicative sign of the organisms' development instability (Shadrina, Vol'pert, 2018). This indicator is used in ecological studies as an index of stress resistance of an organism in a polluted environment. According to the literature (Kozlov, Zvereva, 2015; Gostin, 2016), the rate of fluctuating asymmetry increases in response to stress.

Despite the fact that the pH of the intracellular plant's environment is an important parameter of cellular metabolism, studies using the acid rain simulation method are used very rarely. Foreign studies of the internal cells environment are carried out on four plants, dating from 1986 (Pfan, Heber, 1986). However, the problem of the occurrence of acid rain is global, one of the consequences of which is the decline in crop yields, damage and loss of greenery. Acid rain occurs as a result of the so-called acid oxides-sulfur and nitrogen-entering the environment and binding them with atmospheric water. The source of the ecosystem entering of these elements is the oil refining industry and motor transport. Therefore, this problem is inextricably linked with the theme of this work.

The ability of plants to resist acidification outside is determined by the size of the buffer capacity of the protoplast of plants, which indicates the level of plant's resistance and its adaptation to environmental conditions (Kuzminsky et al., 2016). Under oil environmental pollution the reaction of plant organisms to the additional stress factor-acid rain-will allow to identify the most adapted tree species, which can be used as remedyants of antropogenically-transformed environment.

The purpose of our work is to study the impact of oil pollution on the morphological and physiological indices of tree plants, the most common in landscaping cities and dominant in nature. Based on the results, we expect to find tree plants suitable for use as indicators or remedyants of the oil polluted environment.

Materials and methods of research

The research was conducted in the conditions of the Bytkiv-Babchens'ke oil deposit, located in the Nadvirna district of Ivano-Frankivsk region and is one of the oldest in Europe. The objects of the study were woody species most widespread in green plantations of Europe – *Salix caprea* L., *Betula pendula* Roth., *Populus tremula* L., *Fagus sylvatica* L., *Populus pyramidalis* L., *Acer platanoides* L.

The selection of plant material samples was carried out from branches of the same order of the lower crown part in the period of complete formation of the assimilation system (August-September) (Marghilik, 1961). An analysis of 8 trees of each species, located within a radius of 500 m from the oil deposit, was conducted. Morphological and physiological parameters were analyzed according to Rudenko's technique. The analysis was performed on 100 leaf blades of each species according to the following parameters: area, linear leaf parameters, coefficient of fluctuating asymmetry, type and degree of necrotic damage. Classification of the detected lesions was carried out according to Schubert's methodology (Rudenko, 2008). We distinguished the following types of necrosis:

- punctual-the presence of insignificant areas of leaves' damage in the form of points scattered over the surface;
- interweaving-the presence of necrotic patches between veins of leaves;
- edging-the presence of dead tissue edges of the leaf blades;
- "fish skeleton"-a combination of edging and interweaving necrosis.

There were 5 stages of necrosis:

- the presence of insignificant point necrosis of the leaf;
- presence of necrotic spots occupying area less than 1 cm;
- presence of necrotic spots occupying area of more than 1 cm;
- presence of necrotic spots, which occupy less than 50% of the leaf surface;
- presence of necrotic spots, which occupy more than 50% of the surface of the leaf;

The area of the leaves was determined by the product of length, leaf width and conversion coefficient. The coefficient of fluctuating asymmetry was determined by the difference between the right and the left part of the leaf.

To determine the acidity of the protoplast, fresh leaves weighing 1 g were triturated to a homogeneous mass in a porcelain mortar and washed in a glass of 10 ml of distilled water. After a day the pH of the homogenate was determined at pH-meter and 5 ml of 0.1 N hydrochloric acid was added. Repeated measurements were carried out in a day. The difference between the two meanings was found called the index of acid displacement (ΔpH). Repeatability of measurements was four times.

Research results

The stability of the leaves' buffer system of the investigated trees decreases under the influence of the oil deposit in comparison with the background area (Table 1).

Table 1. Acidity of the homogenate and the state of the buffer system of the leaves of wood species in the conditions of the Bytkiv-Babchens'ke oil deposit.

No	Type of plant	Background area		Oil deposit		
		pH	Δ pH	pH	Δ pH	
1	<i>Salix caprea</i> L.	4.30 0.07	\pm 1.56 0.09	\pm 5.34 0.06	\pm 2.92 0.12	\pm
2	<i>Betula pendula</i> Roth.	5.03 0.06	\pm 2.16 0.12	\pm 5.20 0.09	\pm 2.71 0.07	\pm
3	<i>Populus tremula</i> L.	5.49 0.10	\pm 2.41 0.08	\pm 5.38 0.13	\pm 3.14 0.07	\pm
4	<i>Fagus sylvatica</i> L.	4.02 0.04	\pm 2.79 0.05	\pm 4.73 0.04	\pm 3.23 0.11	\pm
5	<i>Acer platanoides</i> L.	4.35 0.08	\pm 2.96 0.06	\pm 4.95 0.11	\pm 3.32 0.14	\pm
6	<i>Populus pyramidalis</i> L.	5.29 0.11	\pm 2.71 0.08	\pm 5.41 0.13	\pm 3.12 0.05	\pm

Acer platanoides is characterized by the maximum instability of the buffer system in the stressful growth conditions. *Salix caprea* and *Betula pendula* are the most resistant woody species to oil pollution. Overall buffer system stability of the trees' assimilation organs to the impact of oil deposits is reduced in the next several species: *Betula pendula* → *Salix alba* → *Populus tremula* → *Populus pyramidalis* → *Acer platanoides*.

The acidity of the internal environment of the leaf blades of trees growing in the oil deposit conditions is higher comparing with the background area parameter.

There are changes in morphological parameters of trees that grow near the deposit, compared to individuals from relatively clean area (Tables 2 and 3).

Table 2. Morphological indexes of tree leaves in the conditions of the Bytkiv-Babchens'ke oil deposit.

No	Type of plant	Leaf length	Leaf width	Leaf square	Coefficient of fluctuating asymmetry
1	<i>Salix caprea</i> L.	7.95 \pm 0.14	2.57 \pm 0.23	13.48 \pm 1.21	0.03
2	<i>Betula pendula</i> Roth.	4.81 \pm 0.18	3.89 \pm 0.15	12.35 \pm 1.42	0.02
3	<i>Populus tremula</i> L.	4.35 \pm 0.09	4.44 \pm 0.19	12.75 \pm 1.63	0.07
4	<i>Fagus sylvatica</i> L.	6.72 \pm 0.12	5.48 \pm 0.21	24.30 \pm 2.34	0.09
5	<i>Acer platanoides</i> L.	7.78 \pm 0.11	8.46 \pm 0.12	43.44 \pm 2.54	0.09
6	<i>Populus pyramidalis</i> L.	7.89 \pm 0.04	8.68 \pm 0.09	45.20 \pm 2.13	0.05

Table 3. Morphological indexes of tree leaves in the conditions of background territory.

No	Type of plant	Leaf length	Leaf width	Leaf square
1	<i>Salix caprea</i> L.	8.04 \pm 0.17	3.02 \pm 0.05	16.03 \pm 1.28
2	<i>Betula pendula</i> Roth.	5.03 \pm 0.11	3.95 \pm 0.17	13.11 \pm 2.31
3	<i>Populus tremula</i> L.	7.11 \pm 0.13	6.07 \pm 0.13	28.48 \pm 1.42
4	<i>Fagus sylvatica</i> L.	8.49 \pm 0.27	7.19 \pm 0.22	40.29 \pm 3.12
5	<i>Acer platanoides</i> L.	8.67 \pm 0.23	8.93 \pm 0.11	51.09 \pm 3.42
6	<i>Populus pyramidalis</i> L.	3.21 \pm 0.08	9.37 \pm 0.14	56.96 \pm 2.76

All experimental tree species react to oil pollution of the environment, but to varying degrees. The linear leaf parameters and leaf area of *Populus tremula*, *Fagus sylvatica* and *Populus pyramidalis* are significantly reduced. The leaf area of these species under oil contamination impact decreases by 55, 40 and 20% relative to these parameter in control plants, respectively.

Betula pendula reveals the greatest resistance to contamination and the stability of growth processes, the area of the leaf blade of which decreases by 6% relative to the background value. In stressful growth conditions fluctuating asymmetry ratio is the highest in *Fagus sylvatica* and *Acer platanoides*, indicating a high sensitivity of species to environmental contamination by oil. The lowest value of the coefficient of asymmetry is recorded in *Salix caprea* and *Betula pendula*.

Experimental plants are marked by the appearance of necrotic lesions of leaves in conditions of influence of the deposit (Table 4).

Table 4. Types and degrees of tree leaves' necrosis in the conditions of the Bytkiv-Babchens'ke oil deposit.

No	Type of plant	Necrosis type, % of damaged leaves				Necrosis degree
		edging	punctual	interweaving	«fish skeleton»	
1	<i>Salix caprea</i> L.	19	27	25	-	1.72

2	<i>Betula pendula</i> Roth.	12	29	19	-	1.68
3	<i>Populus tremula</i> L.	19	32	27	-	2.03
4	<i>Fagus sylvatica</i> L.	22	37	30	11	3.05
5	<i>Acer platanoides</i> L.	55	15	3	23	4.19
6	<i>Populus pyramidalis</i> L.	26	38	23	-	3.21

The highest degree of necrotic damage of leaves is observed in *Acer platanoides*-4.19, the lowest-in *Betula pendula*-1.68. Edge type of necrosis and necrosis of the "fish skeleton" prevails in *Acer platanoides* individuals, point necrosis type dominates in *Fagus sylvatica* individuals, interweaving-*Populus tremula* individuals.

Discussion

The results of morpho-physiological studies of tree species under the influence of the oil deposit indicate the inhibition of plant growth processes, the appearance of necrosis and the weakening of the body's protective properties. According to the complex of analyzed tree parameters, the resistance to oil pollution of the environment increases in the following series of species: *Acer platanoides* → *Fagus sylvatica* → *Populus pyramidalis* → *Populus tremula* → *Salix caprea* → *Betula pendula*.

Reducing the leaf area is a classical adaptive response of the body to stress, aimed at reducing the area of contact with the environment and reorganization of metabolic processes. The appearance of necrosis of the "fish skeleton" type in the *Fagus sylvatica* and the *Acer platanoides* leaves is evidence of these species' exhaustion in these growing conditions, which is explained by the accumulation of contaminants by leaves and root system. The appearance of the edging necrosis type of plants leaves occurs due to the accumulation of heavy metals at the edges of the leaves and the subsequent death of the tissue. Heavy metals are known to block active centers of enzymes, which makes it impossible to carry out a number of necessary biochemical reactions in cells. This leads to premature aging and cell's death (Ojekunle et al., 2014).

Acer platanoides is characterized by the highest degree of leaves necrosis-4.19, with the percentage of damaged leaves in oil contamination conditions of 96%. This indicates the high sensitivity of the species to heavy metals as components of oil and its bioindicative perspective.

Foreign scientists also appreciate the ability of *Acer platanoides* to reflect the ecological environmental state and suggest that it can be used in fitomonitoring studies (Ord et al., 2016; Steindor et al., 2016).

Punctual and interweaving necrosis are reactions of plants to acid rain, which is caused by burns of fuel. These types of necrosis are inherent to the assimilation organs of *Populus pyramidalis* and *Fagus sylvatica* to the greatest extent compared with other species of trees. *Fagus sylvatica* is marked by a defeat of 100% leaf blades with necrosis, which is consistent with the literature data (Mauer, Palatova, 2011). According to the authors, *Fagus sylvatica* is unstable to high concentrations of pollutants in the environment and it is not recommended to introduce into natural and anthropogenically altered areas landscaping.

In our studies, *Populus pyramidalis* and *Populus tremula* occupy an intermediate position as species with medium resistance to oil pollution of the environment. According to foreign scientific results (Hu et al., 2014), *Populus tremula* is suitable for regeneration of soil poorly contaminated by heavy metals. *Populus tremula* can also be used for rhizoremediation of a wide range of organic pollutants in a complex with associated microbial populations (Mukherjee et al., 2015).

The prospects of phytoremediation are described in several works by foreign authors (Yavari et al., 2015; Ikeura et al., 2016; Lim et al., 2016; Kaur et al., 2017). The main attention is focused on an alternative method of purifying the environment from petroleum products-biological, which is environmentally safe and relatively inexpensive. We propose the use of sustainable tree species as perennial detoxifiers of harmful components of oil. According to foreign studies (Varga et al., 2009), *Salix caprea* is a dominant among the common species in Europe due to its high resistance contamination. Great contribution to the species tolerance to contamination of the environment make fungi symbionts, which reduce the flow of heavy metals inside the plant due to chelating organic acids available in the cells of fungi.

Our research has established that *Betula pendula* has maximum pollution resistance and phytoremediation perspectives. The species is relatively more studied in terms of its metal-bearing ability compared to other species (Samecka et al., 2009; Franiel, Babczynska, 2011; Pavlovic et al., 2017). *Betula pendula* is marked by low sensitivity to heavy metals and the ability to detoxify aliphatic chlorinated derivatives (Lewis et al., 2015). Being under the influence of environmentally contaminated petroleum products, the species reacts with the preservation of resources for the formation of seeds, maintaining the stability of growth processes and phenophases (Erofeeva, 2015). This testifies the high adaptive capacity of the birch veneer, its wide ecological plasticity and the ability to use it as a phytomelirant of oil-contaminated ecosystems.

Conclusions

In the conditions of oil pollution of the environment there is a slowdown of the processes of growth and development of woody plants, which is manifested in the reduction of area, linear foliar parameters, the appearance of necrosis and a decrease in the stability of the buffer leaf system. The tree species are characterized by uneven resistance to stressful conditions of growth, which is explained by the individual ecological potential of plants and the ability to adapt to pollution to varying degrees.

Acer platanoides and *Fagus sylvatica* are sensitive to the influence of oil contamination and can be used as bioindicators of the

environmental condition. *Betula pendula* and *Salix caprea* are resistant to oil pollution and can be used with the aim of technogenically-transformed ecosystems' restoration.

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