

Ecological and biological characteristics of *Betula pendula* in the conditions of urban environment

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This study focused on the influence of polluted environment on various indicators of *Betula pendula* Roth and their applicability to the bioindication of the condition of urban environments in one of the largest industrial cities of Ukraine in the steppe zone, namely Kryvyi Rih. Our investigations have been carried out for three years (2016–2018) in eight *B. pendula* plantations exposed to different levels of aerotechnogenic loading: to a low pollution level – in Kryvyi Rih Botanical Garden of the National Academy of Sciences of Ukraine (control), Poliana Kazok Square and Heroiv ATO Park, to an average pollution level – along three highways with heavy traffic and those exposed to a high pollution level – near the enterprises of Private JSC ‘Northern Iron Ore Dressing Works’ (Northern GZK) and Public JSC ‘ArcelorMittal Kryvyi Rih’. Morphometric, anatomical and statistical methods were used to identify the most sensitive indicators of the impact of air pollutants. As a result of this research, changes associated with greater level of technogenic pollution were revealed in the morphological and anatomical parameters of the leaf blade, namely: reduction in the length of the petiole to 14.3%, in the length of the leaf blade to 8.4% and its width to 12.8%; a decrease in length, width and area of the stomata up to 29.4%, 25.9%, 48.1%, respectively; an increase in thickness of the leaf blade to 23.0% and its tissues, as well as the stomata density per 1 mm² of the leaf to 47.3%. We also registered a decrease in the length of male catkins to 7.4% and an increase in their width to 13.7% associated with greater environmental pollution. The maximum and minimum values of the indicators were recorded near the mining and processing works and close to metallurgical plants. In addition, in order to determine the most sensitive indicators of *B. pendula* response to urban pollution, we carried out a comparative analysis of all the studied parameters and those, the results of which were published earlier. As a result of this analysis, these indicators were ranked according to the level of differences in relation to the control values in decreasing order: differences in 1.5 and more times in comparison with the control – seed germination, curved leaf apex, fluctuating asymmetry, crown volume, pollen viability, the amount of abnormal pollen, vital state of trees, seed quality, crown area; 25–50% differences from control – stomata area, thickness of the lower epidermis, stomata density on leaf blade, seed productivity, pollen fertility, width of fruit (female) catkins, number of scales in fruit (female) catkins, thickness of palisade parenchyma, tree height, stomata length, pollen tube length, trunk diameter at the height of 1.3 m, thickness of the upper epidermis, stomata width; a 10–24% change in indicators – leaf thickness, length of fruit (female) catkin petiole, weight of 1000 seeds, leaf petiole length, male catkin width, leaf blade width, fruit (female) catkin length; the differences from control are less than 10% – the thickness of the spongy parenchyma, the polar axis of pollen, the leaf length, the equatorial diameter of pollen, the length of male catkins and the purity of the seeds.

Keywords: pollution; urban environments; leaf blade; male catkins.

Introduction

In various countries of Europe, North America, and China, representatives of the genus *Betula* L. are used as indicators of the state of urban environments (Kurteva & Stambolieva, 2007; Onete et al., 2010; Ha & Martines, 2018). In the course of investigations on effects of air pollutants, exhaust gases and heavy metals on the state of the environment, a variety of plant indicators are applicable, namely phenological phases of plants (Jochner et al., 2013; Jochner et al., 2015), vital state of plantations, the tree number in populations (Nock et al., 2013), the level of crown defoliation (Augustaitis et al., 2010), the reproductive ability of plants (Brown & Wilkins, 1986; Franiel & Blocka 2008; Franiel & Babczynska, 2011), the fertility and viability of their pollen and seeds (Callagher et al., 2011; Cui-nica et al., 2013), linear growth of annual shoots (Samecka-Cymerman et al., 2009; Franiel & Babczynska, 2011; Kolon et al., 2015), leaf damage (chlorosis-necrosis) (Fostad & Pedersen, 1997; Kowacic & Nolic, 2005), changes in their morphology (Franiel & Więski, 2005; Riikonen et al., 2005), in the anatomical structure (Dobrovolska et al., 2001; Agurajjuja

et al., 2015), stomata sizes, their conductance and density on the leaf blade (Paakkonen et al., 1998; Oksanen, 2003; Janjić et al., 2017), the content and ratio of photosynthetic pigments (Hoshika et al., 2013; Petrova et al., 2014; Czaja et al., 2015), the intensity of photosynthesis (Riikonen et al., 2005; Mäenpää et al., 2011), the level of activity of antioxidant enzymes, the accumulation of low molecular weight compounds that detoxify xenobiotics (Czaja et al., 2015; Šuškalo, 2018), the level of accumulation of heavy metals in the vegetative organs (Kitao et al., 1997, 1999; Kirkey et al., 2012), their effects on the biometric parameters of seedlings (Kirkey et al., 2012), as well as on cytogenetic ones (Vostrikova, 2007; Kalaev et al., 2010).

The influence of the urban environment on plants is illustrated by comparative studies of the effects of polluted air in Wuhan (China) and Union City (USA), located near New York on the density and number of leaf stomata in trees, including species of the genus *Betula* L. (Ha & Martínez, 2018). Another study has revealed a number of heavy metals accumulated in the leaves of *Betula pendula* Roth, growing along highways with heavy traffic in Bolzano (Italy). This species is recommended as a

good bioindicator for assessing urban pollution (Dadea et al., 2016). The increased content of heavy metals in different years, as well as of Cl^- , NO_3^- , $(\text{SO}_4)_2^-$ anions was recorded the leaves of *B. pendula*, exposed to emissions from industry and exhaust gases of the city of Nitra (Czech Republic) (Supuca et al., 2008). Other researchers investigated the content of these substances in timber of *B. pendula* in Southern Lithuania (Butkus & Baltrėnaitė, 2007), as well as in the roots and stems of birch seedlings grown on a contaminated substrate (Mleczek et al., 2017). The investigations of Likholat et al. (2018) on representatives of the genus *Tilia* L. showed that the adaptive potential of plants to changing environmental conditions can be determined by the composition of epicuticular waxes of tree leaves. These data also agree with other investigations, according to which epicuticular wax indices are indicative of abnormalities in the *B. pendula* assimilation apparatus due to excessive concentrations of SO_2 in the Ore Mountains region (Bednařova & Kučera, 2011).

B. pendula was used as an indicator species for assessing the environmental situation in five regions of Croatia (Kovacic & Nolic, 2005), in the capital of Bulgaria – Sofia (Kurteva & Stambolieva, 2007) and in zinc-lead dumps in near Katowice (Poland) (Franiel & Babszyska, 2011). Sensitivity of *B. pendula* pollen to atmospheric pollutants, such as carbon dioxide, ozone, and sulfur dioxide, was tested in laboratory conditions (Cuinica et al., 2013), as well as that of photosynthetic leaf pigments (Rey & Jarvis, 1998; Petrova, 2011; Petrova et al., 2017). Most of the investigations are devoted to the accumulation of heavy metals in leaves in different countries, namely Poland (Piotrowska & Panek, 2012; Zakrzewska & Klimek, 2017; Borgulat et al., 2018), Italy (Dadea et al., 2016), Sweden (Goransson, 1994), Czech Republic (Hrdlička & Kula, 1998, 2011), Serbia (Alagić et al., 2013; Serbula et al., 2014; Pavlovic et al., 2017), Russia (Popova, 2018). The investigations of accumulation of these substance in other organs, namely shoots, roots (Dmuchowski et al., 2014) and even in the seeds of *B. pendula* are less numerous (Kicińska & Gruszecka-Kosowska, 2016). There are also studies of the content of other chemical elements in the assimilation apparatus of *B. pendula*, such as S, N, P, etc. (Hrdlička & Kula, 2004, 2009; Sklyarenko & Bessonova, 2018). Research on antioxidant activity and secondary metabolite content in the plant leaves and fruits is also important (Khromykh et al., 2018). Suskalo et al. (2018) determined that protein concentration, isoenzyme peroxidase profiles, content and antioxidant activity of common phenols and antifungal activity change in the leaves of *B. pendula* from urban areas compared to forest habitats, which is an adaptive response to a specific habitat conditions. In Finland, the effects of CO_2 and O_3 on the accumulation of phenolic compounds in the leaves of *B. pendula* clones were investigated (Peltonen et al., 2005). The previous studies also proved that the leaf hydropathy index is a good criterion for determining the effects of polluted urban environments (Kardel et al., 2012; Krutul, 2014). It was revealed that the largest amount of airborne particulate matter with a diameter of less than $10 \mu\text{m}$ harmful to health accumulates on the leaves of *B. pendula*, compared to other species (Dzierzanowski & Gawroński, 2011). However, there have been no comprehensive studies using a variety of indicators and reflecting the response to the influence of environmental pollution so far.

Nine species of the genus *Betula* are found in the natural flora of Ukraine, of which *B. pendula* is the most common. This species grows in almost all the country's natural climatic zones, including steppe, occurring in the valleys of the Samara and Northern Donets rivers (Brygadyrenko, 2015). In the steppe regions of Ukraine *B. pendula* plantings date back to the early 19th century, later birch trees were widely introduced in urban greening in the second half of the 20th century, especially in the 1970–1980s. In the plantations of the cities of Donbass the share of this species is 1.8%, and it is considered relatively stable (Polyakov, 2009). In the stands of the town of Kryvyi Rih, *B. pendula* is ubiquitous and characterized by high winter, drought, dust, and gas resistance. It is used in greening of parks, squares, alley and roadside plantations, as well as in house adjoining territories in solitary and group plantings (Fedorovskiy & Mazur, 2007). However, a decline in the health condition of both individual birch trees and plantations was observed in recent years in this large industrial region (Korshykov & Petrushkevych, 2017). There is an urgent need for a comprehensive study

of the viability of *B. pendula* in the conditions of Kryvyi Rih, with one of the highest pollution levels in Ukraine due to emissions from large industrial enterprises and exhaust gases, and identification of the parameters indicative of tree acute response to the action of air pollutants.

Here we attempt to comprehensively assess the impact of polluted urban environments on *B. pendula* to identify the most sensitive indicators, reflecting plant response and useful for bioindication of the environment in a large industrial city in the steppe zone of Ukraine.

Material and methods

Research in *B. pendula* plantations was carried out in 2016–2018 in one of the largest industrial cities of Ukraine, namely Kryvyi Rih, an industrial center with diverse manifestations of negative technogenic impacts on the environment (Ekologichnyj pasport, 2017). The main pollution sources are metallurgical, mining and transport enterprises. According to the Main Department of Statistics in the Dnipropetrovsk Region, for the period of our investigations the volume of pollutant emissions into the environment of Kryvyi Rih was 266.5–342.9 thousand tons, 80% of them were emitted by the operating metallurgical plant of Public JSC “ArcelorMittal Kryvyi Rih”. The annual amount of harmful substances from exhaust gases is significantly smaller. But much greater car numbers in recent years, the duration of vehicle operation, the absence of neutralizers in the bulk of domestic and old foreign car brands, resulted in the growing contribution of mobile emission sources to the total air pollution background of the city (Ekologichnyj pasport, 2017; Rehionalna dopovid, 2018). Since Kryvyi Rih extends for 126 km, the pollution levels are different throughout city districts and places of tree growth. Therefore, the study areas were selected taking into account their location along the stretch of the city in three districts of Ternivskiy, Pokrovskiy and Metalurgiyniy and their levels of technogenic loading (Fig. 1, Table 1). Trees grown in the Kryvyi Rih Botanical Garden were taken as control.

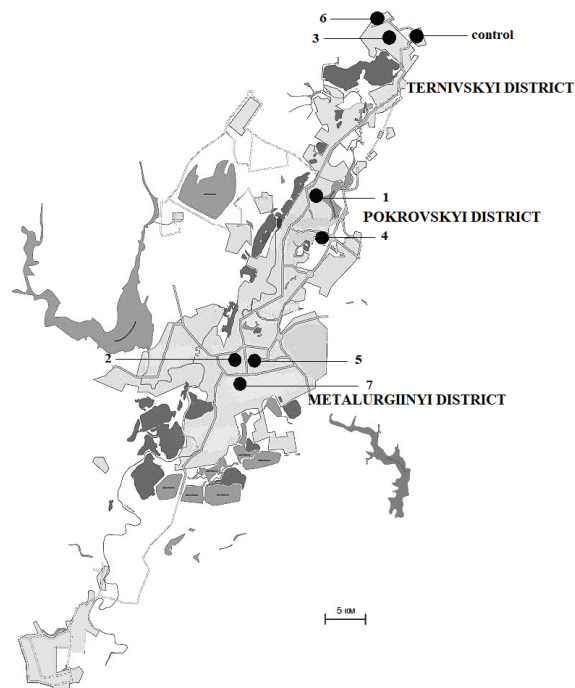


Fig. 1. Map of the study area: 1–7 – sample areas

In the course of our investigation, we sampled leaves from 30–40-year-old trees of *B. pendula*, their age being determined by literature data (Buxtiyarov, 2005; Fedorovskiy & Mazur, 2007).

Leaves were sampled to measure their morphometric parameters after the end of intensive growth, in late July – early August 2016. Each sample included 100 leaves. A total of 800 leaf blades from 80 trees was collected. Sampling followed the method recommended by Zakharov et al. (2000): trees were of about the same age, leaves were sampled from unshaded trees, from the bottom of a similar-sized crown, from the

maximum number of branches available, relatively evenly located around the tree, from shortened shoots; all leaves were packed in a plastic bag, labelled with collection place and stored in the refrigerator for up to two days. Dimensions of the leaf blades were measured using calipers.

Table 1
Sample areas for research

The level of aero technogenic loading	Administrative district	Sample areas	Habitats
Low	Temivskyi	control	Kryvyi Rih Botanical Garden of the NAS of Ukraine
	Pokrovskyi	1	Poliana Kazok Square
	Metalurgiyni	2	Heroiv ATO Park
Average	Temivskyi	3	Cherkasova Street
	Pokrovskyi	4	Elektrozavod's'ka Street
	Metalurgiyni	5	Metalurhiv Avenue
High	Temivskyi	6	near Northern GZK
	Metalurgiyni	7	near Public JSC 'ArcelorMittal Kryvyi Rih'

At the end of August 2017, 10 male catkins were sampled from 10 trees in each plantation, from the maximum available branches, relatively evenly located around the tree. Morphometric parameters were measured using calipers.

The investigations of the leaf anatomical structure and stomatal apparatus were carried out at the end of June 2018. Leaves were sampled from each plantation on the southern side of the crown from three trees that were not blocked by other objects or trees, three were samples taken. Cross sections were made from the middle part of the leaf blade in triplicate using MS-2 sliding microtome without material impregnation, after which they were transferred to microscope slides to obtain a temporary preparation (Barykina et al., 2000).

The stomatal apparatus was investigated by way of making cellulose replicas according to the Molotkovskiy method (1935). A thin layer of transparent perfume varnish was applied to the lower surface of the leaf blade, namely in three parts (upper, middle and lower) and allowed to dry. A replica was removed using a thin adhesive tape and transferred onto a preparation glass.

Temporary section preparations and stomatal replicas were examined using a Carl Zeiss Primo Star light microscope (Germany) at a magnification of 40×10 . The leaf sizes (thickness of the leaf blade, upper and lower epidermis, palisade and spongy parenchyma) and stomata, as well as their number per 1 mm^2 , were determined by photographs taken with a Canon PowerShot A620 digital camera using Axio Vision Rel. 4.8.2.0. (06-2010) program. The replication of measurements of the leaf anatomical structure was 30-fold; the morphometric parameters of stomata were measured in 100 units for each plantation. The stomata areas (SA) were calculated according to the formula:

$$SA = (SL \times SW \times \pi) / 4,$$

where SL is the stomata length, SW is the stomata width (Balasooriya et al., 2009).

To determine the number of stomata per 1 mm^2 , their quantity was first calculated in the field of view of the microscope at a magnification of 40×10 . The area of the microscope field was determined by multiplying distal displays of the horizontal and vertical rulers of the working area of the program. The density of stomata was calculated as follows:

$$N = \frac{n}{S},$$

where N is the number of stomata per 1 mm^2 , pcs; n is the number of stomata in the microscope field, pcs; S is the area of the microscope field, mm^2 .

The results were processed by standard methods with the calculation of \bar{x} – the mean value, SE – the standard error. To compare the samples of mean values, the Tukey criterion of honest significant difference of group means was used (McDonald, 2014). This criterion allows correct performance of multiple pairwise comparisons of mean values. Differences were found to be statistically significant at $P < 0.05$. To determine the most sensitive indices of *B. pendula* response to urban pollution, we used a comparative analysis of all the indicators investigated and those the results of which were published earlier.

Results

In Kryvyi Rih region, *B. pendula* is a rather common species, often found in parks and squares, near office buildings, in adjoining territories and roadside plantations, near large industrial enterprises, and in other parts of the city. A phenomenon has been reported of *B. pendula* successfully colonizing ore mining dumps of Kryvyi Rih by seeds brought in from the nearby tree stands. This species is found in many dumps; four such locations were investigated in detail and 11 loci, or clusters, revealed, numbering from dozens to hundreds of plants of different ages that no one has ever planted here. The area occupied by *B. pendula* in the studied loci of different dumps was from 0.08 to 2.73 ha, the number of individuals of different ages ranged from 91 to 1507, and their density was from 1.2 individual trees up to 51.9 per 100 m^2 , depending on the particular habitat. *B. pendula* settled in different dump parts: slopes, berms, more or less flattened surfaces, crevices between large stones, pits, etc. Trees grow on rocks that are extremely heterogeneous in physical and chemical composition, mechanical size. These loci are an element of the general population field of *B. pendula* on separate dumps. Settling of *B. pendula* in dumps where plants can successfully grow and develop began about 30 years ago. *B. pendula* belongs to the pioneer species which were one of the first to populate ore mining dumps and adapt to specific extreme growth conditions on these newly formed lands, which did not exist in the steppe landscapes before. Thus, *B. pendula* has actually naturalized in iron ore dumps. Due to its high viability and abundance in the Kryvyi Rih area, this species is quite applicable for use in bioindication of this contaminated environment. Reduced leaf sizes were observed in trees exposed to emissions from mining complexes and metallurgical plants or exhaust gases. In the trees growing in areas with low pollution rates the leaf petiole length was 23.1 mm (control), 22.5 and 22.3 mm (in the square and parkland – SA1, SA2), whereas in the rest of the studied plantations it varied from 22.2 to 19.8 mm ($F = 5.71$; $P < 0.001$), the minimum indicators being recorded in trees near Public JSC ‘ArcelorMittal Kryvyi Rih’ (SA7) (Table 2). Other parameters also decreased with an increase in the pollution rates: the length of the leaf blade varied from 56.2 to 51.5 mm ($F = 6.54$; $P < 0.001$), and the width ranged 49.3–43.0 mm ($F = 14.27$; $P < 0.001$). The most sensitive indicator among all characteristics of leaf morphology is its width, as its differences are significant after the Tukey test in all urban plantations (SA2–7), with the exception of the Poliana Kazok Square (SA1), compared to the control plantation.

Table 2
Leaf morphometric parameters of *B. pendula* ($\bar{x} \pm SE$, $n = 100$) in different plantations of Kryvyi Rih

The level of aerotechnogenic loading	Sample areas (SA)	Petiole length, mm	Leaf length, mm	Leaf width, mm
Low	control	23.1 ± 0.48^a	56.2 ± 0.75^a	49.3 ± 0.66^a
	1	22.5 ± 0.43^{ab}	55.7 ± 0.80^{ab}	47.6 ± 0.45^{ab}
	2	22.3 ± 0.34^{ac}	55.8 ± 0.58^{ac}	46.4 ± 0.76^c
Average	3	20.6 ± 0.57^d	53.2 ± 0.65^{ad}	43.1 ± 0.39^d
	4	22.2 ± 0.57^{ac}	53.4 ± 0.61^{ac}	45.1 ± 0.97^c
	5	21.3 ± 0.41^{af}	54.3 ± 0.95^{af}	44.1 ± 0.49^f
High	6	22.2 ± 0.46^{ab}	51.8 ± 0.59^e	43.5 ± 0.52^b
	7	19.8 ± 0.44^b	51.5 ± 0.65^b	43.0 ± 0.42^b

Note: * – from this table on: the identical Latin letters stand for statistically insignificant differences of mean values in a comparable pair in a series by Tukey criterion (HSD).

The impact of air pollutants and exhaust gases on *B. pendula* causes changes in the leaf anatomical structure, which are accompanied by an increase in thickness of the leaf blade. On the leaf cross-section, its structure is rectilinear, mesophile of the dorsoventral type, palisade tissue is represented by cylindrical cells located tightly between each other, spongy parenchyma consists of several cell layers, between which there are large numbers of intercellular spaces. The cells of the upper and lower epidermis are arranged in one layer. The leaf thickness significantly changed depending on the place of tree growth from 169.1 μm (in the Botanical Garden Arboretum taken as control) to 208 μm (near Public JSC ‘ArcelorMittal Kryvyi Rih’ – SA7) ($F = 423.3$, $P < 0.0001$, Table 3).

Table 3Anatomic structure of leaf blade in *B. pendula* ($\bar{x} \pm SE$, $n = 810$) from different plantations in the conditions of Kryvyi Rih

The level of aerotechnogenic loading	Sample areas (SA)	Leaf thickness, μm	Upper epidermis, μm	Parenchyma		Lower epidermis, μm
				palisade	spongy	
Low	control	169.1 \pm 0.57 ^a	20.3 \pm 0.20 ^a	67.1 \pm 0.33 ^a	65.0 \pm 0.37 ^a	16.7 \pm 0.17 ^a
	1	173.4 \pm 0.50 ^b	21.4 \pm 0.19 ^b	67.7 \pm 0.23 ^{ab}	65.4 \pm 0.37 ^{ab}	18.9 \pm 0.16 ^b
	2	172.2 \pm 0.62 ^c	20.7 \pm 0.20 ^{ac}	68.4 \pm 0.34 ^{ac}	66.1 \pm 0.28 ^{ac}	17.0 \pm 0.14 ^{ac}
Average	3	189.5 \pm 0.97 ^d	23.9 \pm 0.31 ^d	74.2 \pm 0.40 ^d	69.6 \pm 0.58 ^d	21.8 \pm 0.24 ^d
	4	187.3 \pm 0.57 ^e	25.4 \pm 0.17 ^e	71.8 \pm 0.34 ^e	68.5 \pm 0.23 ^e	21.6 \pm 0.18 ^e
	5	189.0 \pm 0.44 ^f	24.1 \pm 0.16 ^f	76.3 \pm 0.25 ^f	68.4 \pm 0.32 ^f	20.2 \pm 0.19 ^f
High	6	199.9 \pm 0.84 ^g	27.4 \pm 0.22 ^g	78.5 \pm 0.38 ^g	69.3 \pm 0.49 ^g	24.7 \pm 0.23 ^g
	7	208.0 \pm 0.69 ^h	26.5 \pm 0.19 ^h	88.3 \pm 0.46 ^h	70.8 \pm 0.34 ^h	22.4 \pm 0.16 ^h

Note: see Fig. 2.

According to the classification of Vasiliev (1988), the leaf blade of *B. pendula* in seven studied stands is considered thin, with the exception of trees near Public JSC “ArcelorMittal Kryvyi Rih” (SA7), where the leaves were of average thickness (more than 200 μm). An increase in leaf thickness occurs in plants of all city plantations due to thickening of its tissues: the upper and lower epidermis – up to 35% ($F = 137.13$; $P < 0.001$) and 47.9% ($F = 217.98$; $P < 0.001$), as well as palisade and spongy parenchyma – up to 31.6% ($F = 411.72$; $P < 0.001$) and 8.9% ($F = 22.24$; $P < 0.001$), respectively. The maximum leaf thickness was observed in trees located near mining complexes and metalworks: the upper and lower epidermis were as thick as 27.4 and 24.7 μm (SA6), and the thickness of palisade and spongy parenchyma was 88.3 and 70.8 μm (SA7), respectively. Intermediate values were recorded in trees growing in areas with an average level of technogenic pressure from air pollution (SA3–5), and were significantly higher than in plants of Kryvyi Rih Botanical Garden (control). So, the total leaf thickness ranged from 187.3 to 189.5 μm , which exceeded the control by an average of 11.5%, the upper and lower epidermis were thicker by 20.5% and 26.9%, palisade and spongy parenchyma – by 10.4% and 5.9%, respectively. Obviously, all these changes in the leaf anatomical structure of *B. pendula* in city plantations should be considered as adaptive to the effect of air pollutants.

In urban environments the changes in *B. pendula* occur not only in the leaf morphological and anatomical structure, but also in stomatal apparatus. Our investigations have shown that under higher technogenic pressure the stomata were significantly smaller, their density was greater, and F-ratio calculated for all parameters indicated statistically significant differences between the mean values of four indicators in different

plantations relative to the control (Table 4). In stands in relatively clean areas (control, SA1, SA2), the mean length, width and area of leaf stomata varied within 28–32, 18.4–19.7, 407.5–501.8 μm . In the stands exposed to exhaust gases we observed lower by 16.5%, 12.0% and 27.1% measurements than in the Botanical Garden, and near mining complexes and metalworks (SA6, SA7) they were smaller by 24.2%, 20.6%, 40.0% respectively. The smallest values were found in plantations near Public JSC “ArcelorMittal Kryvyi Rih” (SA7), where the average length, width and area of stomata were 22.6 μm ($F = 73.24$; $P < 0.001$), 14.6 μm ($F = 37.12$; $P < 0.001$) and 260.4 μm^2 ($F = 66.36$; $P < 0.001$).

As for the density of stomata, their number per 1 mm^2 of leaf varied in different plantation samples on average from 92.9 to 136.8 pcs/mm^2 ($F = 51.64$; $P < 0.001$), increasing under the influence of greater aerotechnogenic loading (Table 4). Even in plants growing in conditions of low pollution rates, namely in the park and square (SA1, SA2), this indicator was 10.6% higher compared to the Botanical Garden (control), it was higher by an average of 26.6% in roadside plantings (SA3–5), and it was by 42.5% higher in areas with a high level of anthropogenic pressure, that is near industrial plants (SA6, SA7). Thus, there is an obvious dependence in the leaf response of *B. pendula* to the level of pollution of the urban environments: the higher it is, the smaller the stomata sizes are, while their total number is increasing.

In many bioindicator plants, such as *B. pendula*, not only vegetative organs, but also generative ones are sensitive to aerotechnogenic pollution. For example, male inflorescences of *B. pendula*, the catkins containing pollen, differed in their sizes among trees under investigation (Table 5).

Table 4Morphometric parameters of stomata in *B. pendula* ($\bar{x} \pm SE$, $n = 100$) from different stands in Kryvyi Rih

The level of aerotechnogenic loading	Sample areas (SA)	Length of stomata, μm	Width of stomata, μm	Areas of stomata, μm^2	Number of stomata per μm^2
Low	control	32.0 \pm 0.46 ^a	19.7 \pm 0.33 ^a	501.8 \pm 13.85 ^a	92.9 \pm 2.00 ^a
	1	28.0 \pm 0.29 ^b	18.4 \pm 0.25 ^b	407.5 \pm 8.79 ^b	101.9 \pm 2.22 ^{ab}
	2	28.7 \pm 0.28 ^c	18.9 \pm 0.28 ^{ac}	426.0 \pm 8.19 ^c	103.6 \pm 1.76 ^c
Average	3	27.7 \pm 0.34 ^d	17.7 \pm 0.23 ^d	388.9 \pm 8.86 ^d	110.9 \pm 2.07 ^d
	4	26.3 \pm 0.26 ^e	17.2 \pm 0.28 ^e	356.7 \pm 7.83 ^e	112.4 \pm 2.72 ^e
	5	26.2 \pm 0.21 ^f	17.1 \pm 0.21 ^f	351.2 \pm 5.41 ^f	129.6 \pm 1.52 ^f
High	6	25.9 \pm 0.33 ^g	16.7 \pm 0.25 ^g	342.0 \pm 7.82 ^g	128.0 \pm 2.50 ^g
	7	22.6 \pm 0.27 ^h	14.6 \pm 0.22 ^h	260.4 \pm 5.90 ^h	136.8 \pm 2.13 ^h

Note: see Fig. 2.

The length of the catkins in plants with an increase in aerotechnogenic loading slightly decreased – only to 7.4%. The calculated F-ratio for the available number of degrees of freedom is 1.89, which corresponds to a significance level of $P = 0.068$, that is, the null hypothesis that there are no differences between group means cannot be rejected. Therefore, the differences between the average lengths of the catkins in different plantations are statistically insignificant. In investigated trees an increase in width of catkins was observed 3.9–13.7% ($F = 6.29$, $P < 0.001$). The results of the Tukey test indicated the presence of statistically significant differences between indicators of catkin width in the control and experiments (i.e. with a significance level of 0.05 and smaller) in the four investigated stands, which grow in areas with medium and high levels of aerotechnogenic loading, namely in the Cherkasova, Elektroavodsk’ka Streets, near

Northern GZK and Public JSC “ArcelorMittal Kryvyi Rih” (SA3, SA4, SA6, SA7). Thus, according to the data obtained, it was found that emissions from combines and exhaust gases from vehicles have a negative effect on the assimilation apparatus of *B. pendula*, more on its anatomical structure than on the morphometric parameters of leaves and slightly affects the size of male catkins. To determine the most sensitive indicators of this effect for bioindication purposes of polluted environments, their comparative analysis is needed.

Discussion

According to preliminary data from our studies, it was revealed that under the industrial conditions of Kryvyi Rih, the vital state of eight

plantations of *B. pendula* and the biometric parameters of trees differed significantly from each other and were smaller compared to the control (Kryvyi Rih Botanical Garden) The lowest indices were recorded in trees near mining complexes and metalworks: near Northern GZK the height of *B. pendula* trees was 12.6, the trunk diameter was 23.9 cm; and near Public JSC “ArcelorMittal Kryvyi Rih” it was 11.9 m and 20.9 cm, which is on average by 28.8% and 20.8% lower than control. With an increase in the air technogenic pressure, the viability of plantations also deteriorated: in parkland this indicator was no less than 90% (Heroiv ATO Park) and reached 96.7% (Botanical Garden, Arboretum), in roadside stands it ranged 63.3–80.0%, and near enterprises it did not exceed 40%. At the same time, emissions caused also significant decrease in tree crown volumes and projection areas. This fact is indicative of the negative visually manifested effects of emissions on the growth and development of *B. pendula* trees. It is a proven fact that under extreme climatic conditions of Kryvyi Rih a critical age and top drying of *B. pendula* begins at the age of 40, even in the Botanical Garden. The proportion of top-dry trees increases significantly in plantings exposed to emissions from large plants – Northern GZK and Public JSC “ArcelorMittal Kryvyi Rih” (Korshykov & Petrushkevych, 2017).

Table 5
Morphometric parameters of male catkins ($\bar{x} \pm SE$, $n = 100$) in various plantings of *B. pendula* with different levels of technogenic air pollution

The level of technogenic air pollution	Sample areas (SA)	Catkin length, mm	Catkin width, mm
	control	68.0 ± 1.09 ^a	5.1 ± 0.07 ^b
Low	1	67.6 ± 1.47 ^{ab}	5.3 ± 0.07 ^{ab}
	2	67.5 ± 1.03 ^{ac}	5.5 ± 0.08 ^{ac}
Average	3	64.1 ± 1.67 ^{ad}	5.6 ± 0.10 ^d
	4	66.8 ± 1.23 ^{ac}	5.8 ± 0.10 ^e
	5	65.0 ± 1.51 ^{af}	5.5 ± 0.08 ^{af}
High	6	63.0 ± 1.57 ^{ae}	5.7 ± 0.11 ^e
	7	64.3 ± 1.40 ^{ab}	5.7 ± 0.12 ^h

Note: see Fig. 2.

In the leaves of woody plants, considered among the most plastic and sensitive plant organs (Butnik & Timchenko, 1987) as they are in direct contact with the environment (Waugh et al., 2006), changes occur at the morphological, anatomical and physiological levels under the influence of technogenic air pollution (Shyam et al., 2008). Research reports of Avdashkova & Tyulkova (2017) devoted to *B. pendula* growing under polluted environments in Gomel (Belarus) showed a decrease in leaf length and width from 6.37 and 4.87 cm (in control) to 4.40 and 3.71 cm (in experimental areas) in correlation with an increase in the pollution rates. The same was noted by us in trees exposed to the emissions and exhaust gases. Short-term and long-term exposure to increased concentrations of ozone caused decrease in the leaf size of *B. pendula* seedlings in the first experiment, by 1.5–1.7 times, respectively (Oksanen & Saleem, 1999).

An important morphometric indicator of plant response to changes in environmental conditions is the level of fluctuating asymmetry (FA) of the leaves: the greater deviation is, the worse the conditions are (Zakharov et al., 2000). An increase in FA in trees near copper-nickel plants was reported in the work by Kozlov et al. (1996). In 2016 in the investigated plantations in Kryvyi Rih, the FA values for the trees from parkland did not exceed 0.021, while for the trees from roadsides they varied within the range 0.058–0.076 and in the areas with the highest pollution levels they increased to 0.082 (near Northern GZK) and 0.101 (close to Public JSC ‘ArcelorMittal Kryvyi Rih’) (Petrushkevych, 2018b), i.e. they increased 5-fold compared to control. A reliable indicator reflecting the level of technogenic air pollution is the curve of the leaf apex, considered by Franiel & Wieski (2005) as the most important when evaluating the interpolar variability of the leaf blade. Preliminary results of our research showed that the smallest number of leaves with a curved apex (5–7 pcs) per 100 individuals of the total sample was among trees from conditionally clean zones (Kryvyi Rih Botanical Garden, the square and parkland). In roadside stands this indicator increased significantly on average by 4.3 times (19–24 pcs), and near mining complexes and

metalworks plants it was higher by 7.3 times (38 and 35 pcs) (Petrushkevych, 2018b).

Many authors consider changes in the leaf anatomical structure to be a manifestation of the adaptive reaction of plant assimilation apparatus for their successful growth under extreme conditions (Egorova & Kulagin, 2008). In Belarus, in response to deterioration in the quality of environment a thickening of the *B. pendula* leaf blade 133.8–190.0 μm was observed mainly due to the thickening of the upper and lower epidermis, the maximum values were registered near the Krasnoselsky Construction Material Plant. At the same time, the parenchyma coefficient (the ratio of the height of the columnar to the spongy parenchyma) also changed 0.6–0.9 (Nikolaychuk, 2017). An increase in the leaf thickness of *B. pendula* was also noted by Woof et al. (1999), who examined the effects of ultraviolet radiation on its anatomical structure. They determined that the leaf thickness was 137.9 μm with a low level of UV radiation, 151.4 μm with a high level and it was 150.3 μm with a combined effect of low and high ozone concentrations. In Kryvyi Rih, a thickening of the leaf blade 169.1–208.0 μm was also revealed in the worse environmental situations: the leaf thickness was on average 11.5% greater on roadsides, and 20.6% greater near industrial enterprises compared with control.

Estimates of stomata density are usually used to characterize the leaf ability to regulate water exchange. These estimates largely depend on growing conditions, are characterized by a wide amplitude of variability, and can be considered as an indicator reflecting adaptive potential of the species (Belaeva & Butenkova, 2018). Paakkonen et al. (1993) recorded an increase in stomata density on *B. pendula* leaves under the influence of high ozone concentrations in seedlings of five birch clones, this indicator varied 3–25%. Yanich et al. (2017) consider changes in the number of stomata and the size of stomatal apparatus in *B. pendula* indicative of their resistance to air pollution in urban environments. Under the conditions of Kryvyi Rih, the density of stomata on the leaf blade significantly increased with an increase in the level of technogenic pollution: the average number of stomata in trees from Kryvyi Rih Botanical Garden was 92.9 pcs, it increased to 110.9–129.6 pcs/mm² in roadside stands, and reached 136.8 pcs/mm² in trees growing near enterprises.

Pollutants can very significantly affect male gametophyte (pollen), accompanied by a change in the morphological parameters of pollen grains, an increase in abnormal pollen fraction, a decrease in fertility and viability (Shevtsova et al., 2012; Cuinica et al., 2013), as well as sowing qualities of seeds and subsequent development of germs (Wardle, 1970). The negative impact of anthropogenic pollution on pollen quality was also reported in a number of foreign investigations. For example, Cuinica et al. (2013) studied the gas resistance of pollen, each sample of which was exposed to various atmospheric pollutants: CO, O₃ and SO₂ in two different concentrations: the first concentration corresponded to the current atmospheric temporal level acceptable for the protection of human health; the second level was two times higher than the limit concentration. Pollen viability of *B. pendula* was reduced when exposed to a high concentration of SO₂. An important result of these studies is that even with a low level of pollutants, which is below the standard level of safety for human health, pollen viability decreased to 25% at the maximum gas concentration, while in the control it was 39%. Studies in France showed that fresh pollen samples completely lost their biological characteristics and reproduction function in industrial and roadside areas (Cerceanu-Larrival et al., 1994).

In 2017, the smallest pollen grain sizes were found in *B. pendula* trees growing near mining complexes and metalworks in Kryvyi Rih. The length of the polar axis is shorter in these plants by 4–9%, and that of the equatorial diameter – by 5.5–7.8% compared with the pollen of trees from the Botanical Garden. The amount of abnormal pollen and the spectrum of abnormalities increased in trees exposed to air pollution. Five main types of anomalies were revealed and identified, of which 12 specific abnormalities were named: “dwarf”, “giant”, 2-, 4-, 5- and multi-aperture pollen, that with exine disorder, shriveled pollen, 5-aperture dwarf pollen grain, pollen with 4 asymmetric apertures, the latter two anomalies are regarded as complex (Petrushkevych & Korshykov, 2018). Only half of the above mentioned disorders was found in all plantations, namely a “dwarf”, “giant”, pollen, that with 4 apertures, an asymmetric and shri-

veled pollen grain, and also that with exine anomaly. The pollen with four apertures was most common – from 1.2% to 3.1%, and the rarest were complex anomalies: 5-aperture dwarf pollen grain was recorded only in plants near Northern GZK, the share of them was 0.1% of all anomalies in this plantation, pollen with 4 asymmetric apertures was found in trees near Public JSC “ArcelorMittal Kryvyi Rih” in the proportion of 0.4%. The total amount of abnormal pollen in plants of the Botanical Garden was 3.5%, in roadside plantations it varied within 6.2–7.2%, and near enterprises it reached 11.1% (Public JSC “ArcelorMittal Kryvyi Rih”).

In the trees of *B. pendula* exposed to exhaust gases and industrial emissions in Kryvyi Rih, the quality of pollen deteriorated significantly. The portion of pollen filled with starch, i.e. fertile, in the trees from the Botanical Garden was 91.4%, in roadside plantations this indicator decreased on average by 10.8%, and near mining complexes and metalworks it was reduced by 26.3%. Pollen germination was even worse: in the Kryvyi Rih Botanical Garden the percentage of viable pollen was 49.1%, and in plantations near Northern GZK and Public JSC “ArcelorMittal Kryvyi Rih” it was 27% and 14.3%, respectively. In addition, the environmental effects of pollution also affected the growth of pollen tubes during pollen germination under laboratory conditions, their length in the plants from the Botanical Garden, the square and parkland was 41.8–45.9 µm, in the roadside trees it was 37.2–39.1 µm, and near the industrial plants it was 33.7–35.2 µm (Korshykov & Petrushkevych, 2018). These findings show that the reproductive system of *B. pendula* is sensitive to the deterioration of the urban environment due to air pollution with exhaust gases and emissions from large industrial enterprises.

Measurements of the size of female catkins in *B. pendula* plantations growing in Kryvyi Rih in 2017 showed a maximum decrease in the petiole length, the length of the catkins and its width in the plantation near Public JSC “ArcelorMittal Kryvyi Rih” by 21.4%, 8.3%, 33.3% respectively in comparison with the trees from the Botanical Garden. Seed productivity also decreased: the number of seeds per catkin in plantation near Public JSC “ArcelorMittal Kryvyi Rih” was 21.1% smaller compared to the Botanical Garden, where it was on average 446.9 pcs, although the reproductive scales in the catkins of trees near Public JSC “ArcelorMittal Kryvyi Rih” were by 5.7% more numerous. A larger number of seed scales by 17.1–32.0% was also noted in plants of other plantations. At the same time, the average number of seeds per catkin varied within the range of 451.5–641.4 pcs in all plantations, that is 0.9–43.5% greater than in the trees from the Botanical Garden (Petrushkevych, 2018a). Increased seed productivity in *B. pendula* in a polluted environment was also noted in other industrial regions (Franiel & Babczyńska, 2011).

Under conditions of environmental pollution a decline in seed purity and weight was revealed in *B. pendula* trees that grow in Kryvyi Rih, from 99.6% and 0.158 g (control, Heroiv ATO Park) to 96.9% 0.126 (Public JSC “ArcelorMittal Kryvyi Rih”), that is by 2.7% and 20.3% lower compared to the Botanical Garden. These values were intermediate in roadside plantings and Northern GZK, and on average they were lower than in Kryvyi Rih Botanical Garden by 0.7% and 11.9%. The indicators of seed quality and germinating power were smaller as well in the trees from urban plantations. Thus, the maximum indicators were recorded in Kryvyi Rih Botanical Garden, where 65% of full-grained seeds and 17.7% of sprouted seeds were found; in roadside stands these values decreased on average by 29.6% and 45.2%, respectively, near mining complexes and metalworks it did not exceed 35.0%, and the germinating power ranged between 1% to 2%. The energy of seed germination in all plantations reached only 14% and also decreased alongside with increasing pollution to 1% (near Public JSC “ArcelorMittal Kryvyi Rih”) (Petrushkevych, 2018a). Franiel & Babsinskaya (2011), who studied germination capacity and energy of *B. pendula* seeds collected from a zinc-lead dump in the city of Katowice and background plantings, found that although the seed germination energy from the dump plants was slightly higher by 16.3%, their germination was significantly lower (by 39.9%) compared with the rural area. Therefore, it can be argued that high air technogenic pollution causes inhibition of the generative system of *B. pendula*, which is accompanied by deterioration in the quality of plant seeds and a decrease in their germination.

Conclusion

Thus, the comparative results of our investigations on reactions of *B. pendula* to an urban environment show that the most negative impact is caused by the mining complexes and metallurgical plants, to a smaller extent, by exhaust gases. Indicators of environmental pollution which are actually applicable to monitoring of urban environments, can be ranked according to their sensitivity: great differences compared with the control (1.5 times or more) – seed germination, the curve of leaf apex, fluctuating asymmetry, crown volume, pollen viability, the amount of abnormal pollen, vital state of trees, seed quality, crown area; differences from the control values by 25–50% – stomata area, the thickness of lower epidermis, the density of stomata on the leaf blade, seed productivity, pollen fertility, width of fruit (female) catkins, number of scales in fruit (female) catkins, thickness of palisade parenchyma, tree height, stomata length, pollen tube length, trunk diameter at the height of 1.3 m, thickness of the upper epidermis, stomata width; a 10–24% change in indicators – leaf thickness, petiole length of fruit (female) catkins, 1000 seed weight, leaf petiole length, male catkin width, leaf blade width, fruit (female) catkin length; the differences from control are less than 10% – the thickness of the spongy parenchyma, the polar axis of the pollen, the leaf length, the equatorial diameter of the pollen, the length of the male catkins, seed purity. Therefore, it is recommended to use *B. pendula* trees to determine the level of environmental pollution using leaf parameters with indicators (as shown by our investigations) that have 1.5-fold differences from control values. In order for *B. pendula* to retain its decorative qualities for a longer time, it is recommended to plant trees of this species in relatively clean areas: parks, squares, and also in non-urban areas.

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