Registered Amendments in the Structure of Betula Pendula Roth
Leaf Blades as Adaptation to Polluted Environment

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Abstract

Screening and evaluating tolerance of trees to soil and air pollution is a leading task, especially nowadays, when environmental pollution reached its limits. The diminishing of air pollution and climate alterations are the crucial duties for the humanity. Trees utilization for remediation and revitalization of contaminated locations as filtering system or as bio-accumulators are well known and recognized good and proper practice that alleviate the harmful impact of pollution and offer the best monitoring system. Trees are also bio-indicators toward different types of pollution. In the urban conditions trees enhance aesthetic view, reduce noise and dangerous pollutants, moderate temperature and wind, and protect homes. In that study assessment of Betula pendula Roth tolerance in relation to industrial pollution have been made, using for the conducted research the leaf blades alterations. In the result the conclusion is that silver birch showed plasticity and tolerance towards industrial pollution and can be successfully applied as a species for building green belts around polluted locations, or for afforestation of unfavourable environmental areas, and in a design of urban forest.

Keywords: Betula pendula, air pollution, leaf blades, urban forest.

Introduction

Air pollution as particulate matter (PM10, PM2.5), carbon monoxide, ammonia, heavy metals with toxic effect (Pb, Ni, Cd, As), polycyclic aromatic hydrocarbons (PAHs), sulphur dioxide, and nitric oxides possesses tendency of trans boundary distribution and are the main atmospheric pollution, which threats human health and welfare (Kaur et al., 2017; Kardel, 2018 and Gupta et al., 2019). The association between PM levels and people mortality along with other health risks have been confirmed (WHO, 2002 and 2003). Improving the urban living conditions by implementing trees as an urban forest is a well-known practice (Nowak et al., 2006; Gheorge and Ion, 2011 and Feder, 2019). In the urban environment trees provide economic, environmental, and social benefits for the human well-being through reducing noise, odor and air pollutants, cooling the cities during mid summers, offering shadow, and transpiring water into the air. Trees improve air quality, reduce the urban heat island effect, protect homes from heavy winds and storm water, making the towns and cities much healthier and more attractive place to live, and all that on the end boost the human mental health (Begum, 2019 and Feder, 2019).

The benefits of urban forests are undoubted (Sæbøa et al., 2012), the trees advance the health of city's people through improving physical activities, academic performance, social cohesion (Hanson and Frank, 2016; Begum, 2019; Feder, 2019 and Tirelli, 2019). The Italian organization at the European level, “Coldiretti”, published a list of top ranking tree species suitable to defeat air pollution (Tirelli, 2019). First in this special ranking is Norway maple (Acer platanoides L.), the best among anti-smog trees, followed by Silver birch (Betula pendula Roth.) (Tirelli, 2019). According to Begum (2019) in UK's the most popular street trees are Plane tree (Platamus x hispanica L), Sycamore (Acer pseudoplatanus L.), English oak (Quercus robur L.), Silver birch (Betula pendula Roth.), and Horse chestnut tree (Aesculus hippocastanum L) (Begum, 2019). According to Roloff et al., (2009) Betula pendula Roth is suitable for urban areas under climate change with concern to drought tolerance. Moreover Bolte et al., (2012) estimated silver birch to be a 'winner' in the face of climate change.

In this study the alterations of leaf blade structure of Betula pendula Roth growing in an industrially polluted environment have been estimated, in order to assess its tolerance towards anthropogenic contamination.

Materials and Methods

The study examines anatomical changes of leaf blades of Betula pendula exposure to industrial pollution. The plant materials were collected and conserved from two regions - polluted and control. The polluted area stayed in distance of two km. from the point source of main contamination, in the region of
metallurgical plant. The middle part of each leaf blade was cut and fixed in 90% ethanol-90 cm³, ice acetic acid-5 cm³, and formalin-5 cm³. On the cross-section of samples were measured the thickness of the cuticle, spongy and palisade parenchyma, and epidermis. Data were presented as mean ± standard deviation of \( n = 10 \). Means were compared by student’s t test and were considered to show significant differences for \( p \leq 0.05 \), \( p \leq 0.01 \) and \( p \leq 0.001 \), respectively.

**Results and discussion**

The silver birch (Betula pendula Roth) is a ubiquitous tree growing all over boreal and temperate Europe and Asia, figure 1 (EUFORGEN, 2009; Beck et al., 2016 and Vakulenko, 2018). *Betula pendula* Roth is a relatively small broadleafed, an aesthetics, fine-looking, but a short-lived tree up to 80 years, that is distributed particularly in northern regions and limited to mountainous areas in southern part of Europe (figure 1), since they do not tolerate prolonged summer drought (Beck et al., 2016). Silver birch is tolerant to a wide range of temperatures, wind and frost-resistant (Begum, 2019). Moreover, *Betula pendula* Roth is given as a tolerant species to urban environment, soil and air industrial contamination (Ivanova and Velikova, 1990; Kozlov et al., 1995; Hrdlička and Kula, 2004; Slodičák et al., 2008; Samecka et al., 2009; Hrdlička and Kula, 2011 and Begum, 2019).

![Figure 1. Native spatial range for Betula pendula (EUFORGEN, 2009).](image)

The study of air pollution in terms of trees may comprise parts of the tree, individual trees, or entire forests. However, the laminas reflect changes of environmental conditions for a relatively short time and are considered as a proper tool for investigation. In the field observations the leaves from the silver birch were the most affected among the tree species growing in the area of industrial pollution. On all leaves collected from the contaminated area a necrosis and chlorosis spots were presented. The necrosis was observed between the lateral veins and the periphery of the leaf blades and consists about 78% with the chlorosis. The chlorosis and necrosis spots are accepted as a good biomarkers of air pollution (Manning et al. 2002; Moraes et al., 2002). The development of visible lesions is generally followed with premature leaf-loss (Neighbour, 1988; Wright, 1988), which also was observed on the field. According to Wright (1988) such trees suffered of water-stress shedding their older leaves prematurely because of enhanced water-loss caused from incomplete stomatal closure and by increased cuticular transpiration.

For the test specimens of silver birch, the leaf surface average was very small approximately 9.37 cm²,
compared to the controls with the measured mean around 33.67 cm². Ordinary smaller leave surfaces are often detected under polluted conditions (Gratani et al., 2000; Kurteva and Dimitrova, 2014). The reduction of the leaf blade sizes in the polluted area are associated with slow initially obstructed development (Franiel and Więski, 2005), but it also can be accepted as an adaptive response toward air dust, because PM particles, aerosols and other air pollutants are directly absorbed on leaf surfaces and immediately influence plant function and structure (Agrawal, 2018), henceforth less surface area will be related to a smaller available amount of toxic absorption. Reducing the leaf area of silver birch are often observed reaction of trees growing under heavy air toxic load as high levels O₃ (Pääkkönen et al., 1997), sulphur dioxide and nitric oxides (Neighbour 1988 and Wright, 1988), as well as in an urban areas under heavy vehicle traffic (Dzierżanowski and Gawroński, 2011). Moreover in some studies a significant positive correlation between leaf areas and distance from the polluted source have been found (Areington et al., 2015).

In the study of morphological distinctive dimensions of vegetative development of birch growing in high polluted site, in Wrocław, the positive correlations were found between the concentration of Co, Cu, Fe, Ni and Pb in leaves and traffic intensity. Vegetative short shoots of Betula pendula Roth from polluted locations were significantly longer than that of the trees growing in a clean area, and furthermore that parameter were supposed by the authors to be used as a pollution bioindicator (Samecka et al., 2009). In this study were registered inhibitions of the annual twigs growth under industrial contaminated conditions, with a great significance (table 1).

The same results inhibition of the twigs linear growth of Betula pendula Roth under industrial pollution have been reported from Kulagin (1974), Ninova et al., (1986), Volkova and Belyaeva (1990). The reduction of a linear growth is a result from the stress reaction of plant to the pollution, and it is classified as an adaptive response of the tree species (Korshikov and Tarabrin, 1990). Reduction in the twigs growth in other tree species has been observed by Girs and Zubareva (1992), Kovács (1992), Gryshko (2002), Kurteva and Dimitrova (2014). The registered suppressed growth was remain during the all season and was concern not only the twigs, but also the leave blades, which were with less length and wide, p ≤ 0.001 (table 1), and with length/width leaf ratio 1.25 for contaminated and 1.38 for the control sites respectively. Nevertheless, the number of leave buds on the twigs remained the same for the both locations, polluted and control, approximately 5 up to 6. According to Kurteva and Dimitrova (2014) the number of leave buds is the most stable, hence less sensitive parameter of the vegetative growth connected to the different types and weight of contamination used for bio monitoring.

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### Table 1. Mean values of annual growth indexes for Betula pendula Roth (June).

| Growth indexes | Polluted area | Control | Differences in the mean |
|----------------|---------------|---------|-------------------------|
|                | ΔX ± SD       | ΔX ± SD | ΔX=x<sub>p</sub>-x<sub>c</sub> |
| Time of vegetation | June | October | June | October | June | October |
| Length of shoots | 6.01±1.17 | 9.27±2.12 | 12.33±4.2 | 12.39±3.67 | -6.37 | -3.12 |
| Length of blades | 3.45±0.43 | 3.28±0.43 | 5.39±2.09 | 5.75±0.74 | -1.94 | -2.47 |
| Wide of blades   | 2.75±0.27 | 2.81±0.34 | 3.89±0.57 | 4.61±0.71 | -1.14 | -1.82 |

* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.

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### Table 2. Cross section measurements of Betula pendula Roth leaf blades during the month of May.

| Indexes           | Mean ± SD | Differences in the mean |
|-------------------|-----------|-------------------------|
|                   | polluted, x<sub>p</sub> | control, x<sub>c</sub> | ΔX=x<sub>p</sub>-x<sub>c</sub> |
| Upper cuticle(μm) | 6 ± 2.4   | 6.33 ± 2.2   | -0.33 |
| Upper epidermis(μm) | 35.33 ± 8.5 | 30.33 ± 6.9 | 5** |
| Palisade thickness(μm) | 77.83 ± 14.5 | 100.17 ± 28.9 | -22.33*** |
| Spongy thickness(μm) | 166.67 ± 42.9 | 179.5 ± 32.5 | -12.83 |
| Lower epidermis(μm) | 21.67 ± 3.5 | 15.67 ± 2.17 | 6*** |
| Lower cuticle(μm) | 5 ± 0     | 5 ± 0       | 0    |

* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.
Consequently silver birch is a promising tree for human exposure to anthropogenic pollutants. The adaptations cause by industrial pollution of silver birch leave blades have been investigated on the lamina cross sections, and the results are shown in table 2.

The cuticle is the external layer of leaves and plays an important role in controlling the pollutant absorption and in preventing the fungi pathogens to enter in the lamina. Generally, under high polluted pressure the cuticle layer became thicker (Bednářová and Kučera, 2011), in this case there were no significant differences between the layers of upper and lower cuticles from both sites during May (table 2), but in June the lower cuticle became thicker (table 3).

Silver birch possess thicker upper wax layer 9.8 ± 1.5 μm and 10 ± 0 μm, for polluted and control site respectively, compared to the other tree species from the same area, which have been investigated previously (Dineva et al., 2018). The order of reducing the thickness of upper wax layer from the previous study Dineva et al., (2018) and this one is: Betula pendula Roth (9.8±1.5 vs 10±0 μm) ≥ Fraxinus americana L (5.21±0.99 μm vs 2.50±0.66 μm) Acer campestre L (4.75±0.5 vs 4.45±0.6 μm) ≥ Morus alba L (4.61±0.57 vs 4.09±1.02 μm) ≥ Platanus acerifolia Willd (4.15±1 vs 3.63±0.79 μm) ≥ Acer tataricum L (3.75±0.39 vs 2.95±0.75 μm) ≥ Acer negundo L (3.39±0.5 vs 2.05±0.62 μm) ≥ Acer saccharum L (2.83±0.49 vs 2.54±0.12 μm). According to Sæbøa et al., (2012) Betula pendula Roth is rich in waxes and captured PM mostly in the waxes, with largest accumulation 82.6% of the PM10 fraction (4.2–8.0 μg cm−2). Birch demonstrated three to over eight fold higher accumulation rate of smaller and wax-related PM, which related to over six fold more waxes on birch leaves than on other tested species (Dzierżanowski and Gawroński, 2011; Sæbøa et al., 2012). One of the best tree for capturing PM10, PM2.5, and PM1.0 is noticed to be Betula pendula Roth (Dzierżanowski and Gawroński, 2011 and Sæbøa et al., 2012). The efficient plant species for PM air pollution removal are applying in urban areas, for decreasing the human exposure to anthropogenic pollutants. Consequently silver birch is a promising tree for mitigating and improvement of air quality in polluted and urban places according to the cross sections and the obtained results.

Under industrial pollution, the lamina of birch became thinner 312.5±10.28 μmvs 337±10.41 μm in the control region, in May (table 2); and 314.52±44.44 μm vs 344.84±32.79μm in June (table 3). The changes of lamina structure expressed adaptation toward industrial contamination: thicker palisade layer; thinner parenchyma; smaller epidermis cells; thicker wax layer on the adaxial side of the lamina (table 3).

Jochner et al., (2015), did not find any significant correlation of the leaf morphological characteristics (mass, specific leaf areas and thickness), in birch, in short-/long-term pollution. The reason for that unclear view of air pollution effect on the plant leaves are perhaps due to the different type’s impacts, some of them with antagonistic influence, other with synergistic and in some cases just additive effects (Jochner et al., 2015). Numerous authors related the amendments in the leaf morphology and structure of trees to the type of pollution (Kurteva and Dimitrova, 2014). Under hard motor traffic the leaves of Castanea sativa developed largest surfaces, while under industrial pollution had the minimum size (Kurteva and Dimitrova, 2014). The influence of air pollutants on plants depended on their phytotoxicity, in the following order of decreasing phytotoxicity, are given by Garrec Jean-Pierre (2019): hydrofluoric acid (HF) > ozone (O3), sulfur dioxide (SO2) > nitrogen dioxide (NO2). Usually in the urban air pollution predominated sulphur dioxide (SO2) as well as in industrial, and fine particulates PM10, PM2.5, from coal smoke and motor vehicle emissions, which also enhance the concentrations of NO2, NO, and O3 pollutants directly or indirectly (Bell et al., 2011). Plants can act as bio-indicators or bio-accumulators depending on their sensitivity and tolerance to the air pollution, the tolerant to pollution plants easily grow in polluted areas serving as best filtering systems (Kaur et al., 2017). In the study of magnetic biomonitoring, Betula pendula Roth were recommended as a valuable

Table 3. Cross section measurements of Betula pendula Roth leaf blades during the month of June.

| Indexes | Mean ± SD | Differences in the mean |
|---------|-----------|-------------------------|
| polluted, $x_p$ | control, $x_c$ | $\Delta x = x_p - x_c$ |
| Lamina thickness (μm) | 314.52±44.44 | 344.84±32.79 | -30.32*** |
| Mesophyll thickness (μm) | 262.58±17.52 | 271.13±14.03 | -8.55*** |
| Upper cuticle(μm) | 9.8 ± 1.5 | 10 ± 0 | -0.003 |
| Upper epidermis(μm) | 21.12 ± 3.5 | 40 ± 2.5 | -18.87*** |
| Palisade thickness(μm) | 114.03 ± 16.23 | 101.93 ± 8.09 | 12.09*** |
| Spongy thickness(μm) | 148.55 ± 18.8 | 169.19 ± 19.96 | -20.64*** |
| Lower epidermis(μm) | 14.76 ± 2.23 | 18.71 ± 2.19 | -3.95*** |
| Lower cuticle(μm) | 6.2± 2.09 | 5 ± 0 | 1.21** |

*p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.
biodestructor of airborne particle matter (PM) pollution and the associated elements Al, Cr, Cu, Fe, Ni, Pb and Zn (Mira et al., 2019). Betula pendula Roth is proved to be very useful as a biomonitor of trace element contamination because of its resistance to industrial type of pollution (Ivanova and Velikova, 1990 and Kozlov et al., 1995).

Betula pendula Roth have been used for reforestation of sites heavily disturbed by man, for example natural recovery of industrial dumps (Franiel and Więcki, 2005), and on clear-cut areas, because of its ecological stability and creation of conditions for the revitalization of soils (Slojdícák et al., 2008). Birch plays an important role in the reclamation of toxic heaps, being a pioneer species for recovering vegetation (Franiel, 2001). Betula pendula Roth was found in urban locations to be a better bioaccumulator for the elements Cr, Cd, and especially for Zn compared to Acer platanoides L. and Aesculus hippocastanum L. (Petrova et al., 2014). Silver birch has been noted as a suitable species for long-term monitoring of contamination due to its resistance and tolerance to air pollution (Hrdlička and Kula, 2004). The tolerance to pollution makes the silver birch a common sight in urban landscapes such as industrial areas and roadsides, as well as parks and gardens (Begum, 2019).

The investigations of leaf blades are mainly used in assessing the destruction of forests, where air pollution is considered a major disturbing factor. The ambient air pollution initiated complicated leaf lamina modifications that are species-dependent and correlated to protective or adaptive mechanism of plants (Wuytack et al., 2011 and Noor et al., 2014). The study of vegetation cover using Landsat-8 revealed that there are conversely correlation between increasing the normalized difference vegetation index (NDVI) values and the levels of particulate matter in the atmosphere. Therefore enhancing of NDVI diminished the adverse effect of air pollutants (Gautam and Brema, 2019). According to Rolim et al., (2019), NDVI is responsive to changes in both the chlorophyll content and the intracellular spaces in the spongy mesophyll of plant leaves. Consequently, further investigations and gathering data for leaf blade structure alterations under different conditions are promising for extrapolation of remote sensing data.

Conclusions

In the present study, the valuation of Betula pendula Roth plasticity and tolerance towards industrial pollution have been made, using the leaf blade alterations. The reduction of leaf surfaces and the length of twigs have been registered in the polluted location, accompanied with chlorosis and necrosis spots and premature leaf-loss. Anyway, the number of annual buds remained in the same quantity. The amendments in the leaf structure have been noticed on the transverse sections related to the adaptation towards air pollution. The derived conclusion is that silver birch showed plasticity and tolerance towards industrial effluence and can be successfully applied as a species for building green belts around point sources of pollution, for remediation and afforestation of disturbed regions, or for establishment of urban forest.

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Snejana Boycheva Dineva

Snejana Boycheva Dineva

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