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Responses of functional traits of leaves and reproductive efforts in silver birch under an urban air pollution gradient

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Highlights
- Air pollution is a serious threat to human health in urban ecosystem
- Silver birch leaf and reproductive traits were tested as potential indicators of air pollution
- Changes in plant traits values were compared in 3 zones along the air pollution gradient
- Chl *b* content, *SLA*, *RWC*, number and length of fructification axes are good predictors of air pollution

Abstract: Urban vegetation is a fundamental element and the keystone of urban ecological systems. Significant air pollution can affect plant functional traits. In this work we study the response of selected leaf functional traits (leaf pigments concentration, leaf area, specific leaf area, relative water content) and reproductive effort (no of staminate inflorescences, no of fructifications, seeds no, no and length of fructification axes) of pioneer tree, *Betula pendula* Roth to air pollution stress in the urban ecosystem. 21 trees in 3 zones, growing under different long-term air $PM_{2.5}$ and PM_{10} (particulate matter ø ≤ 2.5 µm and ø ≤ 10 µm, respectively) concentration, land use and two seasons were studied. We confirmed air pollution stress has a profound effect on selected plant traits. The leaf chlorophyll *b* (Chl *b*) content, specific leaf area, and relative water content are highest in most polluted city centre and decrease to the peripheries, while chlorophyll *a* remains constant over zones. Moreover, the reproductive effort measured by the number and length of fructification axes were lowest in city centre. Overall the consistent patterns of variation of Chl *b* and reproductive effort in birch across gradient studied underscore their usability as easy to measure and low cost indicators of air quality in urban environments.

Keywords: adaptation to stress, *Betula pendula*, environmental stress, pollution zones, vegetative and generative traits

INTRODUCTION

The urban ecosystem is a complex phenomenon comprising a mosaic of different habitats that differ in origin, duration and frequency of disturbances, physicochemical parameters and diversity of life forms (Rebele, 1994). It includes spontaneous communities and landscaped greenery made of deliberately planted native and alien species. Cultivated vegetation can be isolated or spread over large areas such as gardens, orchards, parks, avenue trees, and wastelands both within and on the perimeter of towns and cities.

Urban vegetation is a fundamental element and the keystone of urban ecological systems. It provides various ecosystem services such as CO_2 and air pollution absorption and accumulation, water retention, heat island reduction and climate change mitigation (Hara *et al.*, 2021). However, it is subjected to environmental stresses such as elevated temperature, prolonged periods of drought, salinity, restricted space and various soil degradation forms (e.g. soil contamination and compaction, nutrient imbalance, impropriate soil chemistry, heavy metals) (Kazak, Błasik and Świąder, 2022). Environmental stress is any factor that can induce harmful chemical and physical changes in a plant. In natural conditions, stress factors do not act individually but in complex, interactive, cumulative, hierarchical, and sequential ways (Nawaz *et al.*, 2023). The complexity of the organism's response to stress makes it sometimes difficult to separate the action of the stress from its effect (Franiel, 2012). Functional leaf traits include morpho-physiological-phenological traits that directly influence an individual's growth, reproduction and survival by shaping its interaction with the environment. In contrast, mechanistic leaf traits are those that have clearly defined physiological roles, providing a more detailed understanding of processes such as resource uptake and use (Westoby, 1998).

Among the main urban environmental stressors, air pollutants, e.g. carbon monoxide (CO), SO_2, nitrogen dioxide (NO_x), ozone (O_3), volatile organic compounds (VOC's) and particulate matter (PM), have one of the most negative impacts on plants. However, knowledge of the effects of urban stress on plant functional traits remains limited, as some studies have measured traits under laboratory rather than field conditions (Abriha-Molnár *et al.*, 2024). In urbanised areas, they usually come from different sources: domestic (especially coal) heating, traffic, industry, building construction and renovation and agriculture. Pollutants strongly affect plant metabolic functions such as respiration, growth, and other morphological and biochemical characteristics of plants (Abriha-Molnár *et al.*, 2024).

Photosynthesis is a key process to plants' growth and development and the most sensitive to environmental changes; therefore, the structural and functional adaptations of photosynthetic apparatus in response to environmental stresses are of prime interest in monitoring air pollution (Rai, 2016; Jaszczuk and Bąba, 2024). Photosynthesising organisms contain pigments such as chlorophylls, carotenoids and phycobilins. Plant pigment composition depends on genetic and environmental conditions and show the inter and intra-specific variability.

The amount of solar radiation absorbed by a leaf largely depends on the adjustment of the photosynthetic pigment content of the leaf. The state of the inhibition of the pigments responsible for photosynthesis directly affects the production of energy required for the growth and development of individuals, and in the presence of stress, a low chlorophyll content can limit the photosynthetic potential of plants. Therefore, chlorophyll concentration are a good indicator of photosynthetic capacity, leaf nitrogen status, biomass production and overall plant vitality (Pompelli *et al.*, 2012).

Significant air pollution can affect other plant functional traits. Reduced leaf area, premature leaf shedding, reduced transpiration, fewer inflorescences, shorter length of fructification axes or dwarfing are all examples of the plant response to them. Analysis of functional traits makes it possible to predict how environmental changes affect the plant and its ability to adapt. The importance of these traits and their interrelationships can help to understand the mechanisms that shape plant diversity and function in ecosystems. Knowing and determining the value of individual functional traits complements knowledge of the resource energy balance of species inhabiting degraded areas. Leaf functional traits and some tree characteristics are important parameters that enable assessing pollution response and tree tolerance in natural and anthropogenic habitats. For example, specific leaf area (*SLA*), expressing the ratio of the light intercepting area of a leaf to a unit of dry matter, is a key functional trait. Species with low *SLA* invest more dry matter per leaf, long leaf persistence and often have low growth and photosynthetic rates (Shipley *et al.*, 2005). In contrast, species with high *SLA* invest less dry matter per leaf by growing rapidly and losing leaves quickly. Evergreen perennial species tend to have low *SLA*, especially in dry climates where water scarcity and low soil nutrients are common. Relative water content (*RWC*) is a useful indicator of a plant's water balance status because it expresses the absolute amount of water plants need to reach artificial full saturation (González and González-Vilar, 2001).

The air pollution e.g. PM in urban areas can reduce reproductive efficiency, flowering, fecundity and offspring viability, threatening population survival. In urban environments, stress conditions often lead to an increase in the number of inflorescences and fructifications, as plants redirect energy toward reproduction as a survival response, but this relationship is unclear. This species-specific allocation depends on resource availability and the population's life strategy, balancing energy between reproduction, development and survival under specific habitat conditions. The proportion of empty and full seeds is influenced by the number of inflorescences shaped by environmental pressures. Since species undergo constant evolutionary change, studying an entire species is impossible; research focuses on specific populations, with certain traits passed to future generations. Under mineral scarcity, stress-tolerant individuals emerge, making them valuable for reclaiming brownfield sites and thriving in urban environments. Birch, in particular, is well-suited for colonising such areas (Franiel, 2012). Studies on abiotic influences typically examine water, light, and soil properties, which are key factors in plant growth and development (Franiel, 2012; Chiam *et al.*, 2019; Tan, Lu and Wu, 2022).

We study responses of *Betula pendula* Roth population to complex abiotic stresses related to air pollution in urban ecosystem. We hypothesise differences in: (1) magnitude of the environmental stress between distinguished air pollution zones (2) reactions at the vegetative and generative functional traits level between the zones and growing seasons (spring and autumn).

MATERIALS AND METHODS

STUDY AREA

The city of Rybnik (50°05'N, 18°31'E) covers an area of 148.36 km^2 and lies in the southwestern part of the Silesian Voivodeship on the Rybnik Plateau (S. Poland). It is located in the Upper Silesian Coal Basin, covering the whole of the Rybnik Coal District and the Upper Silesian Industrial District. The average monthly temperature varies from −4°C (January) to 24°C (July). The highest rainfall occurs from May to September, with a peak in July. The average annual rainfall is 779 mm. The average wind speed is 14.7 $km \cdot h^{-1}$ (Weather Spark, 2024).

Rybnik was chosen as the model city to study the birch reaction to air pollution since it has been at the top of the rankings of cities in Poland polluted by particulates of anthropogenic origin especially of dust and gaseous pollutants (Airly, 2024). It came mainly from fuel combustion (39.0% of the total dust emission in the voivodeship).

The highest concentrations of PM_{10} dust recorded in Rybnik reached nearly 900 $\mu g \cdot m^{-3}$, and far exceed the daily average permissible PM_{10} dust concentration in Poland of 40 $\mu g \cdot m^{-3}$. The number of days with 24-hour average PM_{10} exceedances amounted to 18.4% of the analysed days (Jasiński, 2023).

The city area has been divided into three zones (Fig. S1). Zone 1 comprises the city centre area with the most densely built-up area and the highest traffic. The zone 2 shows the areas with less compact development and medium traffic. Zone 3 marks areas including commercial premises, allotment gardens, minor development and low traffic. Zone 3 is the least polluted, zone 2 is the moderately polluted zone, and zone 1 is the most contaminated zone (Tab. 1).

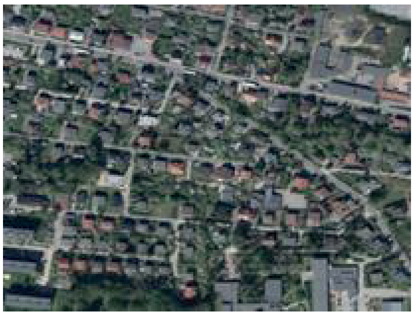
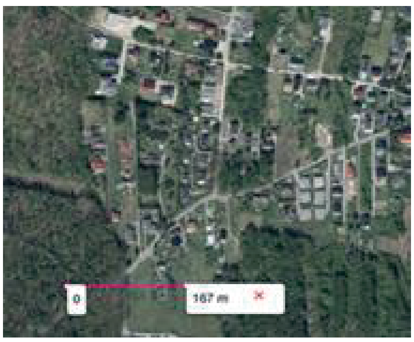
THE STUDY SPECIES

The birch tree (*Betula pendula* Roth) is a pioneer, native tree with broad ecological range, resistant to industrial pollution, that quickly adapts to harsh habitat conditions. The species commonly occur in natural forests and abandoned arable lands or as a result of secondary succession and afforestation (Jonczak *et al.*, 2020) and as well as on post-mining dumps or urbanised areas (Franiel, 2012; Possen *et al.*, 2014; Oksanen, 2021). It is characterised by rapid growth in youth, early fertility and seed dispersal by wind (Špulák *et al.*, 2010), but low shade tolerance (Hynynen *et al.*, 2010). Due to high resistance to chemical pollutants, birches are suitable for reclamation in degraded areas (Franiel, 2012).

PLANT FUNCTIONAL TRAITS

To estimate leaf functional traits, 21 birch trees were chosen, across the selected pollution zones. Seven trees were selected in zone one, nine trees in zone two and five trees in zone three. The

Table 1. The characteristic of landforms for each zone in Rybnik city

Zone	Description	Map	$PM_{2.5}$	PM_{10}
			$\mu g \cdot m^{-3}$	
1	city centre, with high-density areas and car exhausts because of heavy traffic		96.30	54.64
2	low-rise single-family houses with medium traffic and particulates from heating in houses from burning wood and coal		87.72	50.90
3	low-density areas, allotment gardens with low-traffic		26.87	23.00

Note: permissible values in Poland according to the Polish Standard: $PM_{2.5}$ = 20 $\mu g \cdot m^{-3}$, PM_{10} = 40 $\mu g \cdot m^{-3}$.
Explanations: $PM_{2.5}$ = particulate matter ø ≤ 2.5 µm, PM_{10} = particulate matter ø ≤ 10 µm.
Source: own elaboration based on data from: aqicn.org/station@68317/; aqicn.org/station/@194011/; aqicn.org/station/poland/slaski/rybnik-ul.-borki-37a/; maps from: https://streetmap.pl/rybnik/# .

surveys were conducted in two seasons spring until the end of May and autumn until the end of September. A total of 12 functional traits were analysed. Trees were selected free of any visible disease symptoms, fully growing and having a similar height (mean 12 m; Suunto PM 5/1520, Finland) and DBH (diameter at breast height, mean 38 cm; Calliper, Codimex-S, Poland) (Mahmud *et al.*, 2025).

DETERMINATION OF LEAF PIGMENTS

In spring and autumn from each of 21 trees, 15 leaves growing on short shoots from branches at a height of 1.3–2.0 m were collected, from the four sides of the tree crown to average the results. Birch leaves were collected the day before and kept in a refrigerator at 4°C. For each tree, plant material was cut into fragments, avoiding leaf nerves, and then 200 mg of leaves were weighed in duplicate and placed in 40 cm^3 screw-capped glass vessels, which were poured with 10 cm^3 of DMSO (dimethyl sulfoxide). The samples were incubated in a heated chamber for 72 h at 65°C. The leaf chlorophyll content was determined spectrophotometrically using the Hach Lange DR5000 spectrophotometer. For Chl *a*, *b* and carotenoids, extract absorbance was measured at the wavelength of 665, 649 and 470 nm, respectively (Pompelli *et al.*, 2012). The content of chloroplast pigments was calculated using the following Equations (1)–(3):

$$\text{Chl } a = 12.9\text{A}_{665} - 3.45\text{A}_{649} \quad (1)$$

$$\text{Chl } b = 21.99\text{A}_{649} - 5.32\text{A}_{665} \quad (2)$$

$$\text{carotenoids} = (1000\text{A}_{470} - 1.9\text{Chll } a - 63.14\text{Chl } b) \cdot 0.0047 \quad (3)$$

where: Chl *a* = chlorophyll *a*, Chl *b* = chlorophyll *b*; A = absorbance value for waves with the length in nm; the number of the particular pigments is quoted in $\mu g \cdot g^{-1}$ of fresh matter, while the weight of carotenoids is given in $mg \cdot g^{-1}$ of fresh weight.

A total of four functional traits in two repetitions were analysed in two seasons for 21 trees (336 samples).

LEAF FUNCTIONAL TRAITS OF *BETULA PENDULA*

10 leaves from the short shoots birch trees was collected from each of 21 trees in spring and autumn (420 leaves samples). The leaves were stored in water for one night at 4°C for tissues saturation. Then turgid fresh weight and leaf area were measured the next day. The leaves were scanned using Epson Perfection 700V photo scanner. The leaf area of each leaf (*LA*) was measured using ImageJ 4.3 software (Schneider, Rasband and Eliceiri, 2012). After leaf area measurement, the leaves were put in the heated chamber for several days at 70°C and were weighed to determine dry mass. Next, we calculated the following specific leaf area (*SLA*) as leaf area (mm^2) divided by dry mass (mg) (Pérez-Harguindeguy *et al.*, 2013).

For a sample of leaves from each tree, the water content of the plant tissues was determined and expressed as a percentage of the water content at full turgor (Eq. (4)):

$$RWC = \frac{TW - DW}{FW - DW} \cdot 100 \quad (4)$$

where: *RWC* = relative water content, *FW* = fresh weight of a leaf tissue, *DW* = leaf dry mass, *TW* = weight of the plant tissue at full turgor after 24 h of hydration in distilled water (Arndt, Irawan and Sanders, 2015).

REPRODUCTIVE EFFORT AND FECUNDITY OF BIRCH TREES

During the spring season, the number of staminate inflorescences on long shoots branches up to 2 m high on the four sides of each of the 21 trees was determined. Similarly, in the autumn, the fructifications were collected and counted. From the 5 fructifications collected from each tree (105 samples), the length of axes was measured using callipers, then seeds separately from each tree were mixed and 3 samples of 100 seeds were randomly prepared. The nuts were checked under a binocular. The numbers of empty and full seeds were determined according to the methodology in the works (Franiel, 2012; Franiel and Kompała-Bąba, 2021).

SOLID CONTAMINANTS WASHED OFF THE LEAF BLADES

This study assessed short-term surface deposition on 90 randomly selected leaves from trees of each of the three zones. The long-term accumulation of PM in the waxy layer of leaves was not within the scope of our analyses. Therefore, only the water-insoluble PM accumulated on leaf surfaces was assessed in this study. In the laboratory, leaf samples collected during the autumn season were immersed in distilled water in a 1 dm^3 beaker for 2 h. A porcelain Büchner funnel was used to siphon the precipitate, with the hole-punched bottom of the funnel being fitted with a filter. Whatman type 598 sieves with a diameter of 90 mm were used. These sieves capture particles of 8.0–10.0 μm. The funnel was set in a suction flask, which was connected to a water pump to create a vacuum. The filtrates were dried in a heated chamber at 60°C and then weighed on an analytical balance. The methodology for determining leaf area has been described previously. The particulate matter retention capacity (marked as PM) of the species was defined as the amount of PM accumulated per unit leaf area ($g \cdot m^{-2}$).

STATISTICAL METHODS

Principal component analysis (PCA) is performed for visualisation of the overall relationships between the functional traits in birch in the air pollution zones CANOCO 5.0 software (Šmilauer and Lepš, 2014). To test of the significance of the differences between the average values of the traits among air pollution zones, the non-parametric Kruskal-Wallis test was used. Dunn's multiple comparison test were used to show differences between zones. Moreover, the Mann–Whitney U test was used to determine the relationship between tree functional traits and pollution zones during spring and autumn. The statistical significance of differences were tested at $p < 0.05$ (Zar, 1999). The analyses were performed in Statistica 13 (TIBCO Software Inc., 2017).

RESULTS

THE COMPARISON OF CHOSEN MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS OF BIRCH GROWING IN DISTINGUISHED POLLUTION ZONES

The principal component analysis (PCA) shows the overall distribution of the morphological and physiological traits of the silver birch individuals growing in zones with different levels of air pollution in the ordination space of the 1st and 2nd PCA axes, which together explained 42.28% variation in the data (Fig. 1). The first PCA axis explains 27.28% of the variability in the dataset. Along this axis, physiological traits related to leaf photosynthetic pigment contents (especially Chl *b* in the zone 2 and 3 and Chl *a:b* ratio in zone 1) and traits related to leaf economic spectrum, such as specific leaf area, are grouped. On the other hand, the 2 PCA axes, which explains 15% of variation in the data, is mainly related to generative traits of birch (full versus empty nuts, number of fructification, number of staminate inflorescences, length of fructification axes).

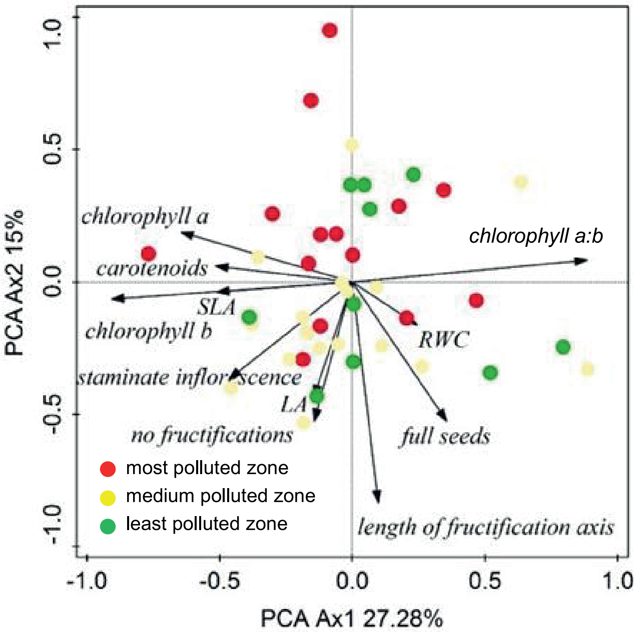


Fig. 1. The PCA analysis of silver birch functional traits in ordination space of 1 and 2 PCA axes; no = number, *LA* = leaf area, *SLA* = specific leaf area, *RWC* = relative water content; source: own study

PHYSIOLOGICAL TRAITS OF BIRCH TREES

Figure 2 shows the chlorophyll *a* (Chl *a*) levels in birch leaves in pollution zones from which the samples were taken. The average level of Chl *a*, between the zones was 1.563 $\mu g \cdot g^{-1}$ of (the median = 1.588 $\mu g \cdot g^{-1}$). The minimum Chl *a* value was 1.432 $\mu g \cdot g^{-1}$, and the highest was 1.577 $\mu g \cdot g^{-1}$. Significant differences were found between zones 1 and 3 and no such differences existed between zone 2 and others (Kruskal-Wallis (KW) test H = 7.590, $p < 0.023$). The level of Chl *b* differs between the zones (Fig. 2). The maximum Chl *b* value in the first zone reached 1.070 and the minimum – 0.805 $\mu g \cdot g^{-1}$. The median Chl *b* recorded was 0.420 $\mu g \cdot g^{-1}$ and the average Chl *b* between the zones was 0.666 $\mu g \cdot g^{-1}$ (KW test H = 29.165, $p < 0.0001$). The mean Chl *a:b* ratio value recorded was 2.407 (min. = 1.428, max. = 3.601). We found significant differences in Chl *a:b* ratio in zones 1 and 3 and between zones 2 and 3 (KW test H = 25.103, $p < 0.0001$) (Fig. 2).

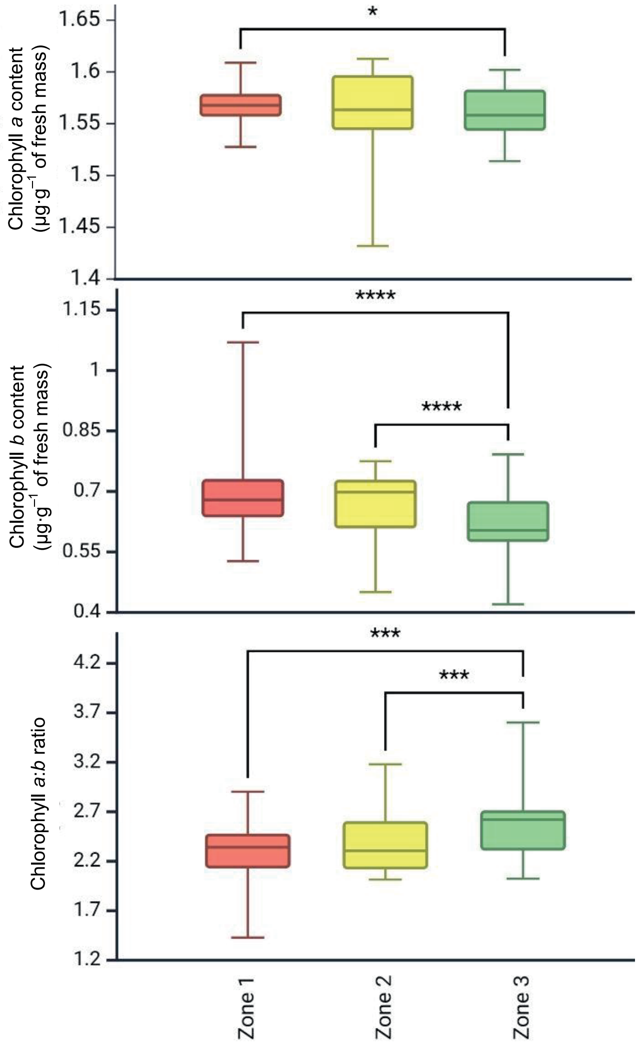


Fig. 2. The median of chlorophyll *a*, chlorophyll *b* content and *a:b* ratio in leaves of birch trees growing in polluted zones in the area of Rybnik; the lower and upper hinges which correspond to the first and third quartiles; the significance level represents Dunn's multiple comparison test results; * = $p < 0.05$, *** = $p < 0.001$, **** = $p < 0.0001$; source: own study

MORPHOLOGICAL TRAITS OF BIRCH TREES

The leaf area (*LA*) showed significant differences between pollution zones 1 and 2 as well as 2 and 3. However, no such differences were shown between the most polluted zone and the cleanest zone (Fig. S2). The average *LA* was 13.723 cm^2 (median = 13.236 cm^2). The minimum *LA* was 5.734 cm^2 in zone 1 and the highest 31.00 cm^2 in zone 2 (KW test H = 29.875, $p < 0.0001$).

The mean specific leaf area (*SLA*) value was 13.627 $mm^2 \cdot mg^{-1}$ and the median was 12.258 $mm^2 \cdot mg^{-1}$ (Fig. 3). The lowest *SLA* value was 6.789 $mm^2 \cdot mg^{-1}$ and the highest was 28.622 $mm^2 \cdot mg^{-1}$. Significant differences were found in the mean *SLA* value between zones 1 and 3 (KW test H = 19.739; $p < 0.001$). In contrast, no significant differences were found between zones 1 and 2.

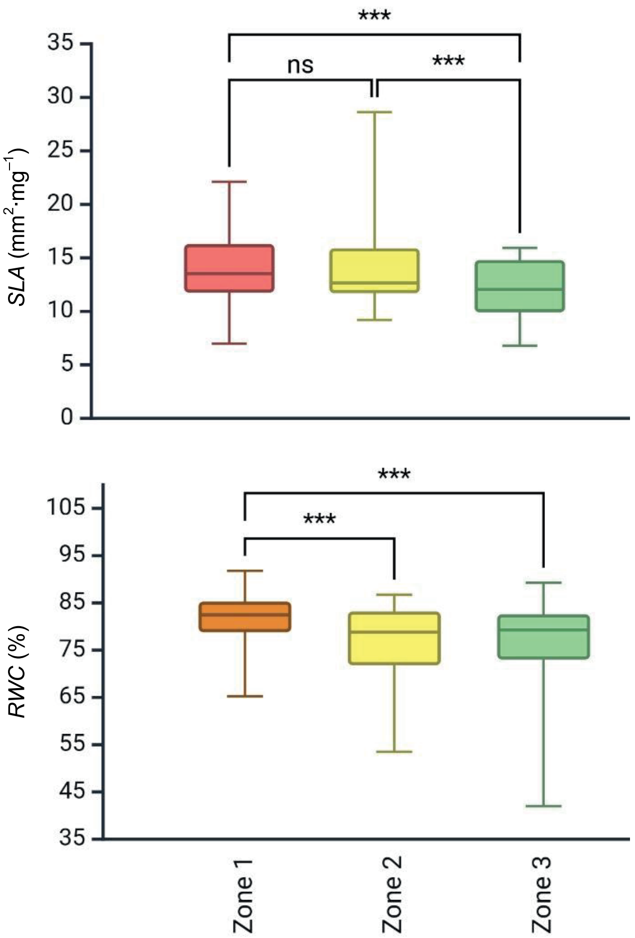


Fig. 3. The specific leaf area (*SLA*) and relative water content (*RWC*) of birch trees growing in polluted zones in the area of Rybnik; the significance level represents Dunn's multiple comparison test results; *** = $p < 0.001$; ns = non significant; source: own study

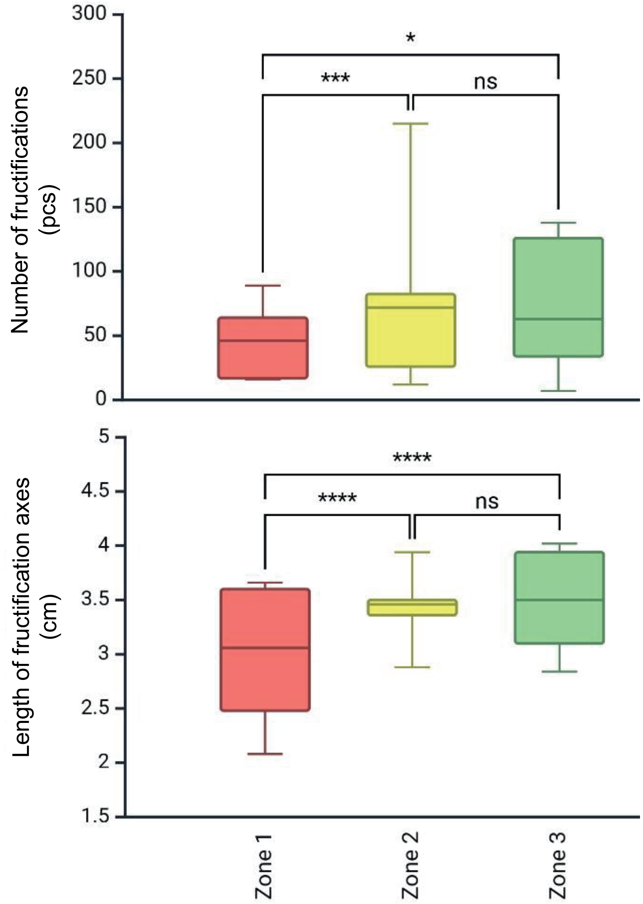


Fig. 4. The number of fructifications and length of fructification axes in birch trees growing in polluted zones in the area of Rybnik; the significance level represents Dunn's multiple comparison test results; **** = $p < 0.0001$; *** = $p < 0.001$; * = $p < 0.05$; ns – non significant; source: own study

The minimum *RWC* value was 41.99%, and the highest was 91.81%. The average *RWC* value was 77.92%. The median value was 82.50% (Fig. 3). There were significant differences between zone 1 and the other zones (2 and 3) in terms of leaf water content (KW test H = 22.83; $p < 0.0001$).

The average value of staminate inflorescences is 72.71. The minimum value of staminate inflorescences was 30 in zone 1, while the maximum was 169 in zone 2. There were no significant differences between zones in terms of staminate inflorescences (Fig. S3).

Significant differences were found in the number of fructifications between zone 1 and the other two zones (2 and 3). On the other hand, no significant differences were shown between zones 2 and 3. The highest number of fructifications was in zone 2, and they also had the highest value relative to a single study tree (215 pieces). The smallest value (7 pieces) for the number of fructifications occurred in zone 3. On average, there were 50.1 fructifications per tree. The Kruskal–Wallis H test = 18.97, $p < 0.0001$ (Fig. 4).

The comparison of the number of full and empty seeds in the distinguished pollution zones showed no significant differences between zones. For zones 1, 2 and 3, the smallest number of full seeds was 11, 17 and 16, respectively, and the largest number of empty seeds for the same zones were 88, 82 and 83 (Fig. S4).

The average length of the fructification axes was different between the zones (Fig. 4). The longest fructification axes were 4.02 cm in the third zone, and the shortest was 2.08 cm in the first zone. The average length was 3.32 cm. The median value was 3.44 cm. The H value of the Kruskal-Wallis test was 52.583, with a probability of $p < 0.0001$.

THE COMPARISON OF CHOSEN MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS OF SILVER BIRCH TREES BETWEEN SPRING AND AUTUMN

The detailed studies on morphological and physiological traits of silver birch conducted in two periods (spring and autumn) enabled us to detect some differences. The following variables proved to be significant ($p < 0.0001$): Chl *a* and Chl *b* concentration, Chl *a*:*b* ratio, carotenoids, *RWC*, and *SLA*. In contrast, no significant differences in *LA* between seasons existed. Table 2 shows the results of the Mann-Whitney U test.

POLLUTION WASHED OFF THE SURFACE OF LEAVES

The lowest value of particulate matter (PM) was recorded in the autumn in zone three and was 790.60 $\mu g \cdot cm^{-2}$. The highest value of PM was recorded in zone one – 888.15 $\mu g \cdot cm^{-2}$ (Tab. S1).

Table 2. The comparison of chosen functional traits of *Betula pendula* trees between seasons

Variable	Spring		Autumn		*Z*-test	$Z_{corr.}$	*p*
	median	min–max	median	min–max			
LA (cm^2)	13.236	6.003–25.009	13.245	5.734–31.000	−0.989	−0.989	0.323
Ch *a* ($\mu g \cdot g^{-1}$ FW)	1.554	1.432–1.577	1.588	1.514–1.613	−11.376	−11.379	0.001^{**}
Chl *b* ($\mu g \cdot g^{-1}$ FW)	0.715	0.450–0.805	0.656	0.420–1.070	3.810	3.811	0.001^{**}
Chl *a*:*b* ratio	2.171	1.935–3.179	2.433	1.428–3.601	−4.140	−4.141	0.001^{**}
Carotenoids ($mg \cdot g^{-1}$ FW)	18.048	13.808–20.812	25.759	24.038–26.756	−17.727	−17.732	0.001^{**}
RWC (%)	82.505	41.991–89.287	79.147	53.478–91.811	3.899	3.900	0.001^{**}
SLA ($mm^2 \cdot mg^{-1}$)	12.258	6.991–22.107	14.458	6.789–28.622	−3.979	−3.980	0.001^{**}

Explanations: *LA* = leaf area, Ch *a* = chlorophyll *a*, Ch *b* = chlorophyll *b*, FW = fresh weight, *RWC* = relative water content, *SLA* = specific leaf area, $Z_{corr.}$ = corrected *Z*-test, ** = $p < 0.001$.
Source: own study.

DISCUSSION

THE RELATIONSHIP BETWEEN STRESS FACTORS AND PIGMENTS CONTENT IN *BETULA PENDULA* LEAVES

Air environmental pollution significantly influence all plant life processes, but can result in the development of unique set of adaptive traits (Rai, 2016; Brestic and Allakhverdiev, 2022). One way to increase the chances of survival under stress is to maintain a favourable energy balance. It often results in stunted individual growth and a lack of protective functions of cellular structures necessary for survival (Franiel, 2012). Photosynthesis is one of the most sensitive physiological processes determining plant growth and development (Smith, 1992). Therefore structural changes in the photosynthetic apparatus are a good indicator of the environmental stresses affecting plants (Kompała-Bąba *et al.*, 2021; Bąba *et al.*, 2024).

The leaf chlorophyll *a* (Chl *a*) content is used as an indicator of plant reactions to environmental stresses as it is the least persistent plant pigment. While leaf Chl *a* and Chl *b* content in silver birch in Białowieża primeval forest is 5.54 and 1.36 $mg \cdot kg^{-1}$ DW (Przybylski, Ciepał and Palowski, 1994), the studies in urban areas of Plovdiv and Sofia, showed that the content of Chl *a* varied between 1.32 and 1.89 $mg \cdot g^{-1}$, while the content of Chl *b* – from 0.54 to 1.52 $mg \cdot g^{-1}$ (Petrova, 2011; Ivanova and Velikova, 1990). In cities, leaf chlorophyll concentration in plants may be lowered by air pollution. However, reverse pattern was found by Borowski (2008), where the chlorophyll content in birch trees growing in the city was higher than that of those in natural conditions (42.2 vs. 36.9 relative units). This is because micropollutants can interact with the activity of enzymes involved in chlorophyll biosynthesis (Prusty, Mishra and Azeezb, 2005). Although some studies by Borowski (2008) and Kuki *et al.* (2008) have shown that some species, on the contrary, respond by increasing the amount of chlorophyll under stress conditions, most species decrease the amount of leaf pigments.

In the present study, the Chl *a* content between the zones was at a similar level. The highest variability in Chl *a* values were reached in zone 2 (1.613–1.432 $\mu g \cdot g^{-1}$). The average Chl *a* concentration was 1.563 $\mu g \cdot g^{-1}$ and this value was lower than recorded by Petrova (2011). Similarly to Borowski (2008), the Chl *a* content of the leaves was lower in trees growing in near natural conditions than in trees growing in the street. In spring, Chl *a* content increases to intensify photosynthesis. In contrast, in autumn, it decreases to go into dormancy and the plants withdraw Chl *a* and can be replaced by carotenoids (Tab. 2). Another reason is the greater exposure of birch individuals to environmental stresses that can lead to chlorophyll degradation. The amount of Chl *a* should decrease during the autumn season. In the study, the Chl *a* content did not decrease in the autumn and even appeared to be higher than in the spring. Chl *b* reached its highest concentration in birch leaves in zone one (maximum 1.070 $\mu g \cdot g^{-1}$). The lowest value recorded was 0.420 $\mu g \cdot g^{-1}$ and was recorded in zone 3. The least Chl *b* was in the leaves of the tree in zone three, which is, by definition, the cleanest. The recorded levels of this pigment are lower than in studies by (Ivanova and Velikova, 1990). It may mean trees in the last zone are less stressed and do not need to withdraw Chl *a* and replace them with Chl *b*. The study also showed a difference between spring and autumn in the chlorophyll content of birch leaves. Maximum and minimum values in chlorophyll content were recorded in autumn. The median was also lower for the autumn season. The ratio of Chl *a*:*b* varies greatly between zones. The optimal ratio should be 3:1. The zone in the city centre (zone 1) has the lowest Chl *a*:*b* ratio and increase to the its peripheries. These differences are related mainly to high Chl *b* value, as the Chl *a* level is at a comparable between zones. Zone two is a transition zone, where the Chl *a*:*b* ratios are clearly higher than in zone one, but lower than in zone three. The lowest value recorded is 1.428. The highest is 3.601 (this is as much as 67.8% difference). The minimum value is comparable to the results of Petrova (2011), while the highest value significantly exceeds the results obtained in the above work (2.44). There are seasonal differences in the leaf of Chl *a*, Chl *b* content and the Chl *a*:*b* ratio. Research confirms that stress factors have different effects on trees from various zones. Stress factors affect the photosynthetic pigment content of birch leaves. The Chl *a*:*b* ratio may also indicate adaptation to light intensity, which is increased in spring and weakened in autumn. Our studies showed no significant differences in carotenoid levels between the zones. However, when comparing the objects tested by season, carotene content was significant (Mann–Whitney U test) (Tab. 2). The median for the spring season reached 18.05 $mg \cdot g^{-1}$ and for the autumn season, 25.76 $mg \cdot g^{-1}$. This may indicate some adaptation of the trees to the conditions where they grow. The difference between seasons may be an indicator of leaf ageing and the natural displacement of Chl *a* by carotene. The lack of differences between zones in carotenoid levels may suggest that birch trees are highly tolerant of air pollution.

THE RELATIONSHIPS BETWEEN STRESS AND LEAF PARAMETERS

The leaf is the active part of the tree, which relates its effectiveness to the size of its surface area. Increased leaf area affects water evaporation, absorption of solar energy and CO_2, and influences the biomass production of the tree through the production of carbohydrates by photosynthesis. The leaf area size is essential for both juvenile and mature individuals. The leaf area is crucial for the tree's growth and development, especially in the early stages. Leaf area size influences tree development, but leaf area can be determined by various environmental factors (Konôpka *et al.*, 2023). The research shows that the average leaf area is the most variable trait from site to site. The area of the birch leaf in forests and contaminated areas ranged between 11 cm^2 and almost 15 cm^2 (Kalashnikova *et al.*, 2001). Wojda (2007) reports that the average birch leaf area taken from some localities in Poland ranged from 583 to 738 mm^2. Franiel (2012), investigating the vegetative traits of the silver birch occurring on post-industrial sites (coal mine spoil heaps, lead and zinc wastelands), reported that the average leaf area was 10 cm^2. The results obtained in our research differ from those reported in the above mentioned papers. The lowest measured leaf area value was 5,734 cm^2. Similar results were also obtained by (Wojda, 2007). The mean leaf area, considering all zones, was 13,723 cm^2. The lowest leaves were recorded on trees in the third zone, whereas the highest leaf area was noted in the second zone. The growing season was not significant, considering the leaf area. The lower leaf area can be connected with stress response since the risk of damage and leaf surface exposure to pollution is lower. Relative water content (*RWC*) is a poorly described functional trait for the silver birch. Junttila *et al.* (2022), in a study on tree water status, found that spectral reflectance measurements can be related to leaf water content, driven by leaf transpiration and given in EWT units (equivalent water thickness) as an identity value for *RWC*. However, unlike *RWC*, this value is expressed in $g \cdot m^{-2}$. The equivalent water content of the leaf in the aforementioned paper mentions a reflectivity of up to 99%. The average *RWC* value in birch leaves in the Rybnik area was 77.92%. The minimum water content was just under 42% (41.991%), and the highest was 91.81%. The highest *RWC* values were found in zone 1 (91.81%). Significant differences occurred between the autumn and spring seasons (Tab. 2), with values of 82.505 (spring) and 79.147 (autumn). This could be due to climatic conditions, metabolic activity or the developmental state of the plant. The level of *RWC* will be higher when there are higher levels of precipitation and rising temperatures may favour the level of *RWC*, as plants take up more water as growth intensifies. In autumn, levels may be lower as plants go into dormancy in preparation for the winter period. Differences between zones may also be due to damage to the stomatal apparatus caused by pollution. In addition, pollution can cause a reduction in photosynthesis, leading to less water uptake. Specific leaf area (*SLA*) is a key functional trait (Westoby, 1998). *SLA* reflects the expected return on previously acquired food and energy resources. Leaves with a low *SLA* perform better in resource-poor environments where retention of acquired resources is a priority, whereas. Leaves with high *SLA* perform better in resource-rich environments. This value depends on leaf arrangement, thickness, and shading (Wilson, Thompson and Hodgson, 1999). A study on the functional characteristics of the leaves of the birch conducted in Punkaharju (Finland) established in 1999 to study within-stand differences in growth phenomena among birch genotypes, showed that the *SLA* index ranged from 3.520 to 5.842 $cm^2 \cdot g^{-1}$ dry weight (Possen *et al.*, 2014). In another research, 10 silver birch genotypes were grown in two adjacent, identical greenhouses at the Suonenjoki Research Nursery (Finnish Forest Research Institute, Suonenjoki Unit, Finland), the *SLA* values ranged from 47.4 to 55.0 $cm^2 \cdot g^{-1}$ (Possen *et al.*, 2015). The *SLA* values of the leaves of the birch trees in the study area range from 6.789 $mm^2 \cdot mg^{-1}$ (3 zone) to 28.622 $mm^2 \cdot mg^{-1}$. The *SLA* of silver birches in zone 1 is higher than in zone 3. This may mean that zone 3, although by design the cleanest and presumably with less environmental stress, may be poor in nutrient resources for tree growth (Westoby, 1998). Trees in zone three probably invest in leaf thickness rather than leaf area, and perhaps oxidative stress rather than air pollution is the key factor in this zone. A comparison of the spring and autumn seasons showed significant differences in the mean *SLA* of birch leaves (Tab. 2). Considering *SLA* and leaf area, the lowest values of both traits were found in zone three and the highest in zone two.

STRESS FACTORS AND REPRODUCTIVE EFFORTS OF *BETULA PENDULA*

Birch as a pioneer species is characterised by early flowering (spring), a rapid reproductive cycle and pollination by wind. This favours anemogamy and enables effective pollination of the female inflorescences. Together with the rapid germination of potentially germinable seeds, birch can appear in urbanised or post-industrial areas (Franiel, 2012). Silver birch can form mixed populations, together with wind-pollination and ease of inter-breeding, offspring with varying degrees of trait partitioning and thus high flexibility to environmental pressures can be obtained (Franiel, 2012).

In studies of Franiel and Kompała-Bąba (2021) recorded an average of 29 staminate inflorescences on lead and zinc mining and processing areas and in the area of the Katowice Forest Park an average of about 44 staminate inflorescences. In Rybnik, the lowest number of staminate inflorescences (about 45) was found in zone one and the highest in zone two (169 staminate inflorescences).

The number of fructifications varies between research areas. Franiel and Kompała-Bąba (2021) recorded an average of 253 fructifications in the zinc-lead area; about 327 in the coal mine area, and 474 in the Katowice Forest Park. In the Rybnik area, the number of fructifications was much lower. The highest number of fructifications was recorded in zone two, with 215 per tree, while the lowest number was 7 in zone three. The average number of fructifications per tree surveyed was approximately 51 pieces (50.09 pieces). The highest result represents only 45.3% of the value recorded in the Katowice Forest Park.

Franiel (2012), conducting a study of measurement fructification axes, noted that the length of the fructification axes measured from 1.89 cm to 4.42 cm. These values were not exceeded during the three-year study. In studies of (Franiel and Kompała-Bąba, 2021), the length of the fructification axes was 1.96 cm on zinc and lead-contaminated sites; at the coal mine site, the value was 3.51 cm, and in the sample from uncontaminated areas, the length of fructification axes was 4.36 cm. In Rybnik, the mean lengths of the fructification axes differed markedly between the studied zones. The shortest fructification axes were found in zone one, measuring from 2.08 to 3.70 cm. The length of the fructification axes were already longer in zones two and three, measuring from 2.90 to 4.02 cm. The average value for all zones is approximately 3.31 cm. These values are comparable to the data from the cited studies, both from the polluted sites and control plots.

Specific conditions in urban areas and their periphery, such as elevated temperatures, water scarcity or heavy pollution, significantly weaken the condition of trees. The life strategy of birch trees is primarily to maintain basic life processes. Birch individuals channel more energy into building vegetative organs than generative ones. As a result, this can lead to a large number of empty seeds, which has consequences for tree survival (Franiel, 2012). This statement was confirmed by our studies in Rybnik. Full seeds accounted for approximately 28.6% of all seeds, while empty seeds were found in 71.4% of the nuts. The highest value assigned to full seeds was 56.7, whereas the lowest was 11.3 in the first zone. In the work of (Franiel and Kompała-Bąba, 2021), empty seeds in the heavy metal-contaminated environment accounted for 188, in the mine site for 70 and in the control sample for 98 (Franiel, 2012) in study conducted at zinc-lead areas and control sites noted that the smallest number of full nut seeds was 55 and the highest number reached 406, respectively.

CONTAMINANTS ACCUMULATED ON THE SURFACE OF THE LEAF

Air pollution, including particulate matter (PM), is an increasing threat to both human and ecosystem health in urban areas (Popek *et al.*, 2018). PM often contains different organic and inorganic toxic compounds that can remain airborne for a long time and can be transported over long distances.

The pollutants collected on the leaves in the form of dust are from 2022/2023. In the General Directorate for Environmental Protection (Pol.: Główny Inspektor Ochrony Środowiska – GIOŚ) measurement data bank from the government station located at 37d Borki St. The value of PM_{10} during the 2022/2023 period ranged from 5.9 to 126.1 $\mu g \cdot m^{-3}$ (GIOŚ, 2024). The standards for airborne PM_{10} are 40 $\mu g \cdot m^{-3}$. This means that PM_{10} levels were exceeded almost three times.

Rai (2016) showed that 75% of the debris collected from the leaf surface represented fractions larger than 2.5 and less than 10 μm. It was also observed that particles larger than the opening of the stomata accumulate on the pore, while fine particles clog the stomata. This prevents gas exchange, photosynthesis, water retention, respiration and overall plant growth. Plants with settled dust on their leaves showed reduced growth and altered surface structures. The maximum dust capture capacity on leaves ranged from 0.8 to 38.6 $g \cdot m^{-2}$. Leaf morphology strongly influenced dust capture (Wang, Shi and Li, 2010). Moreover, leaf thickness can be changed comparing control samples (270.8 μm) with polluted areas (e.g. 243 μm at the railway station and 209 μm at the main road) (Gostin, 2009). Nevertheless, the plants she studied proved to be quite resistant to air pollution and, despite the observed modifications, continued to grow and reach the flowering stage. The results of Xiao-Yan *et al.* (2022) showed that the highest particular matter removed by *Osmanthus fragrans* Lour was 3.451 $g \cdot m^{-2}$, and the lowest PM removed by *Magnolia denudata* Desr. was 1.005 $g \cdot m^{-2}$. (Honour *et al.*, 2009) researched the effects of diesel exhaust emissions on 12 herbaceous species with respect to growth, flower development, leaf senescence and leaf surface wax characteristics. Their study showed clearly the potential for realistic levels of vehicle exhaust pollution to have direct adverse effects on urban vegetation.

In the study conducted in Rybnik, in autumn showed the PM value washed off from leaves in zone 3 was lower, then in zone 1, reaching 7.90 and 8.88 $g \cdot m^{-2}$ respectively.

Of the common air pollutants, airborne PM has shown greater potential for reduction by plants (Yli-Pelkonen, Setälä and Viippola, 2017). In addition, PM deposited on leaves is enriched in toxic or harmful substances such as heavy metals and polycyclic aromatic hydrocarbons Plants can remove PM from the air by dry deposition, a process involving gravitational sedimentation, particle impaction, interception. In a given area, plants are more effective at capturing PM from the surrounding air than concrete surfaces. PM adheres only temporarily to the leaf surface and were disturbed by wind or rain. Re-suspension of PM from leaves or from the ground after PM wash-off may lead to potential secondary pollution (Li *et al.*, 2022). Therefore, intensive research into the mechanisms of PM retention by plant leaves and the stability of leaf surface PM is needed to accurately assess the ability of plants to retain PM. Leaf surface microstructures provide a large deposition area for PM and influence the roughness and wettability of the leaf surface, which determines the strength of adhesion. However, the effect of different leaf characteristics on PM retention capacity remains uncertain, including the advantages and disadvantages of leaf size and surface microstructure types for PM retention (Saebø *et al.*, 2012). Leaf characteristics that affect PM retention of different tree species require further research.

CONCLUSIONS

The silver birch is a widespread species due to its wind-pollinated nature and tolerance to environmental stresses. Plants grown along urban air pollution gradient, developed a range of morphological and physiological adaptations, that allow them to cope with complex environmental stress: increase of chlorophyll *b* content, chlorophyll *a*:*b* ratio, specific leaf area (*SLA*), relative water content (*RWC*), while decrease the number and length of fructification axes. This made make them good indicators of air pollution. The study showed that zone 3 with low-density areas, allotment gardens with low-traffic and $PM_{2.5} = 26.87$, $PM_{10} = 23.00$ present the best conditions for birch growth. The study supports the view that silver birch can be a useful species for biomonitoring air pollution.

SUPPLEMENTARY MATERIAL

Supplementary material to this article can be found online at: https://www.jwld.pl/files/Supplementary_material_66_Franiel.pdf.

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CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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