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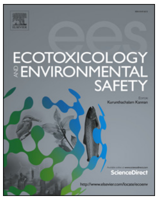

Impact of particulate matter accumulation on the photosynthetic apparatus of roadside woody plants growing in the urban conditions

Robert Popek[a,b,*], Arkadiusz Przybysz[a], Helena Gawrońska[a], Krzysztof Klamkowski[c], Stanisław W. Gawroński[a]

[a] Laboratory of Basic Research in Horticulture, Faculty of Horticulture, Biotechnology and Landscape Architecture, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland
[b] Laboratory of Ecology, Institute of Dendrology Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland
[c] Research Institute of Horticulture, Pomologiczna 18, 96-100 Skierniewice, Poland

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ABSTRACT

Particulate matter (PM) is one of the most harmful inhaled pollutants. When pollutants are emitted into the atmosphere, the only possible method for cleaning the air is through phytoremediation, where plants act as biological filters for pollutants. However, PM also has negative impacts on plants, although knowledge concerning the effects of PM on vegetation remains limited. In this work, an attempt was therefore made to define the amount of PM and waxes on foliage, and to evaluate the efficiency of the photosynthetic apparatus in seven plant species (three trees, three shrubs and one climber) grown in two locations (centre and suburbs of Warsaw) that differed in their level of PM pollution in the air.

More PM and waxes accumulated on the foliage of plants grown in the highly polluted location. These plants also exhibited a lowered efficiency of their photosynthetic apparatus, manifested by a lower photosynthesis rate that corresponded with an increased stomatal resistance. Plants grown in the more polluted environment also showed decreased values of F_v/F_m parameter and no statistically significant trend to increase total chlorophyll content. Among the tested species, *Betula pendula* Roth accumulated the greatest amount of PM and *Physocarpus opulifolius* L. showed no weakening of its parameters of photosynthesis in a more contaminated environment.

1. Introduction

In urban areas, which cover ca. 0.5% of the Earth's land area (United Nations, 2014), air pollution is an increasing threat to both human and ecosystem health (European Environment Agency EEA, 2015). One of the dangerous inhaled pollutants is particulate matter (PM) (Salvi, 2007), which consists of liquid and solid particles composed of different organic and inorganic compounds (Bell et al., 2011) with an aerodynamic diameter in the range of 0.001–100 µm (Farmer, 2002). As an aerosol, PM can be suspended in the air for weeks and can be transported long distances from the source of emission (Farmer, 2002). Inhaled PM can cause hypertension, heart diseases, allergies and asthma (Atkinson et al., 2001) and worldwide causes approximately 2.1 million premature deaths annually (ca. 154 thousand in Europe) (Silva et al., 2013).

If pollutants have been released into the atmosphere, the only possible way to remove them is via phytoremediation. This involves growing plants in urban areas that accumulate PM on their surface, allowing them to act as biological filters (Popek et al., 2013, 2017a; Ram et al., 2014; Sæbø et al., 2012) and to significantly limit the amount of PM suspended in the air (McDonald et al., 2007). Due to their large surface area, deciduous trees and shrubs possess the greatest ability to accumulate PM on foliage (McDonald et al., 2007), but species differ in their potential to remove PM from the air (Dzierżanowski et al., 2011; Popek et al., 2013, 2017a, 2017b; Ram et al., 2014; Sæbø et al., 2012). Morphological structures on the surface of the leaf, such as hairs and waxes, may increase PM accumulation (Jouraeva et al., 2002; Leonard et al., 2016).

Urban vegetation is constantly subjected to anthropopressure, limited sunlight, contamination, restricted space, increased temperature and soil compaction (Ferrini et al., 2014). These factors negatively affect every level of plant biological organisation, including efficiency of photosynthesis, thus they decrease plants' productivity (Hunt, 2003). The negative impact of urban stress on the photosynthetic apparatus is

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[*] Corresponding author at : Laboratory of Ecology, Institute of Dendrology Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland.
E-mail addresses: robert.popek@gmail.com (R. Popek), arkadiusz_przybysz@sggw.pl (A. Przybysz), helena_gawronska@sggw.pl (H. Gawrońska), krzysztof.klamkowski@inhort.pl (K. Klamkowski), stanislaw_gawronski@sggw.pl (S.W. Gawroński).

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well described, but PM is not considered as a key factor reducing its efficiency (Hanslin et al., 2017; Naidoo and Chirkoot, 2004; Przybysz et al., 2014a). PM accumulated on the foliage may absorb and scatter light rays, preventing its free access to the chloroplasts (Hirano et al., 1995). Small-diameter PM can clog the stomata, thereby reducing gas exchange (Cape, 2009), while chemically-active PM e.g., trace elements, organic pollutants and Cl− and Na+ , that, depending on the type and environmental conditions, can affect the physiological processes of plants (Chauhan, 2010). In an environment with high levels of PM pollution, the amount of chlorophyll may also be reduced (Van Heerden et al., 2007), and the values of fluorescence of chlorophyll *a* parameters may be decreased (Hanslin et al., 2017; Naidoo and Chirkoot, 2004). Moreover PM with pH values of ≥ 9, may cause direct injury to leaf tissues or indirectly through changing of soil pH (Vardaka et al., 1995). PM leaf cover can also increase leaf temperature (Sharifi et al., 1997). On the other hand there are differences between species. Some authors show that for tree species e.g. *Ilex rotunda* Thunb. the opposite effect was observed. This was explained by a possible protective role of PM by firstly, reducing photoinhibition and secondly, by buffering PM-induced oxidative stress (Takagi and Gyokusen, 2004).

Plant selection in cities is related to their decorative and functional role in the urbanized environment as well as their tolerance to various abiotic stresses. With recent discoveries about phytoremediation another criterion for selecting plants is also being taken into account – their ability to clear air of PM. Nevertheless selection of plants for PM phytoremediation should be a compromise between the amount of accumulated PM and the functioning of the plant in an environment with a raised PM level. A number of studies show the short term effect of PM on different species but environmental studies on the real impact of PM on plants grown in urban conditions are still rare and are needed. Therefore, the aim of this study was to determine the long term effect of PM on the efficiency of the photosynthetic apparatus of seven deciduous plant species from different types (trees, shrubs and climbers) recommended for planting in urban areas and expected to be good candidates for PM accumulation in a temperate climate. Plants were grown in the Polish capital city, Warsaw, in two locations that differed in levels of PM in the air. An attempt was made to: (1) determine the accumulation of surface PM, in-wax PM (in three different size fractions) and (2) wax deposition on foliage, (3) assess the efficiency of the photosynthetic apparatus and (4) correlate the efficiency of the photosynthetic apparatus with the amount of accumulated PM.

2. Materials and methods

2.1. Plant material and study area

The plants selected for this study included three tree species – silver birch (*Betula pendula* Roth), Swedish whitebeam (*Sorbus intermedia* Ehrh.) and London plane (*Platanus × hispanica* Münchh.); three shrub species – border forsythia (*Forsythia ×intermedia* ZAB.), common ninebark (*Physocarpus opulifolius* [L.] Maxim.) and Japanese spiraea (*Spiraea japonica* L.); and one climber, common ivy (*Hedera helix* L.). Plants had already been growing in vivo in the selected locations for several years and were approximately the same age and size. Studies were conducted in 2008, 2009 and 2010 in Warsaw (52°14'N, 21°00'E), the capital of Poland, in two locations with different levels of PM air pollution (Fig. 1). The site with the higher concentration of PM was located in the city centre. In this location, the average concentrations of PM_{10} and $PM_{2.5}$ for three vegetation seasons (2008 – 2010) amounted to 44.0 $\mu g/m^3$ and 25.5 $\mu g/m^3$, respectively (Chief Inspectorate for Environmental Protection, 2015). The area with lower PM pollution was located on the Warsaw University of Life Sciences campus situated in the suburban of southern part of the city, and possessed PM_{10} and $PM_{2.5}$ concentrations of 25.5 $\mu g/m^3$ and 19.6 $\mu g/m^3$, respectively (Chief Inspectorate for Environmental Protection, 2015). The main source of PM in city centres is car exhausts because of heavy traffic. The suburban PM is a mixture of car exhausts and particulates from heating in houses from burning wood and coal, which is a still big problem in Poland especially in winter, spring and late autumn.

Fig. 1. Study locations differing in level of air pollution.

2.2. Evaluation of efficiency of photosynthetic apparatus

Measurements were carried out four times in every vegetative season, in June, July, September and October. All parameters/processes were always measured on the same plants, which were selected in the first year of the study. For all tested species, leaves selected for measurements were chosen from the middle part of annual twigs located on the side of the plant facing the road, 1.0 – 1.7 m above ground level (the height of a human face). All leaves were undamaged, healthy and free of pests. The following parameters describing the efficiency of the photosynthetic apparatus were measured in vivo.

2.2.1. Plant gas exchange

As part of the gas exchange measurements using an infra-red gas analyser method, (i) rate of photosynthesis and (ii) stomatal resistance were evaluated via the LICOR 6200 Photosynthesis System (Li-6200) in 2008 and via the LICOR 6400 Photosynthesis System (Li-6400) in 2009 and 2010 (Lincoln, Nebraska, USA). The Li-6200 was equipped with the standard leaf chambers, whereas the Li-6400 possessed a 6400-40 Leaf Chamber Fluorometer and a 6400-01 CO_2 mixer. The light intensity for all measurements was 800 – 1000 $mmol\ m^{-2}\ s^{-1}$, provided by natural light (Li-6200) or a red-blue (10% blue) light source (Li-6400). The CO_2 concentration in the chambers was adjusted to a constant 400 $\mu mol\ mol^{-1}$, while relative humidity was approximately 30 – 35%. Measurements were always conducted under the same weather conditions (cloudless, sunny days), between 9 a.m. and 6 p.m. Leaves were placed separately in the chamber of the LICOR photosynthesis system and measured for 2 – 3 min until the photosynthesis rate stabilised, and then results were recorded. Despite the fact that two different Licor systems were used in this experiment, obtained results were very similar between subsequent years of measurements. For each location and term, 12 technical measurements (mean of them were used for

Table 1
Amounts of different particulate matter (PM) categories and waxes on the foliage of seven plant species. Results are averaged over three years, data are means ± SE.

PM category and size fractions	Location of measurement	Species						
		Betula pendula	*Forsythia ×intermedia*	*Hedera helix*	*Physocarpus opulifolius*	*Platanus ×hispanica*	*Sorbus intermedia*	*Spiraea japonica*
$_{T}$PM (µg cm^{-2})	City centre	38.2 a [*] ± 0.53	22.6 a ± 0.39	20.1 a ± 0.39	19.5 a ± 0.46	21.6 a ± 0.52	21.4 a ± 0.36	28.2 a ± 0.93
	Suburbs	27.9 b ± 0.44	19.8 b ± 0.23	12.3 b ± 0.20	12.9 b ± 0.44	13.5 b ± 0.59	18.2 b ± 0.33	23.1 b ± 0.70
$_{S}$PM (µg cm^{-2})	City centre	14.0 a ± 0.28	12.8 a ± 0.32	16.3 a ± 0.32	13.0 a ± 0.34	11.7 a ± 0.32	11.5 a ± 0.26	18.6 a ± 0.49
	Suburbs	10.1 b ± 0.26	10.0 b ± 0.20	10.3 b ± 0.17	7.8 b ± 0.30	7.1 b ± 0.29	8.3 b ± 0.18	14.0 b ± 0.45
$_{W}$PM (µg cm^{-2})	City centre	24.2 a ± 0.45	9.8 ± 0.18	3.8 a ± 0.14	6.5 a ± 0.20	9.9 a ± 0.23	10.0 ± 0.27	9.6 ± 0.50
	Suburbs	17.8 b ± 0.27	9.8 ± 0.12	2.0 b ± 0.05	5.1 b ± 0.20	6.4 b ± 0.32	9.9 ± 0.17	9.1 ± 0.32
Large (µg cm^{-2})	City centre	25.6 a ± 0.36	13.1 a ± 0.21	16.1 a ± 0.33	13.2 a ± 0.32	16.2 a ± 0.45	15.4 a ± 0.30	20.8 a ± 0.63
	Suburbs	17.7 b ± 0.33	11.7 b ± 0.15	9.4 b ± 0.19	8.2 b ± 0.45	10.0 b ± 0.45	13.5 b ± 0.28	15.4 b ± 0.51
Coarse (µg cm^{-2})	City centre	6.9 ± 0.19	6.9 ± 0.22	3.2 a ± 0.11	4.8 a ± 0.009	4.5 a ± 0.15	4.4 a ± 0.12	5.6 ± 0.27
	Suburbs	6.7 ± 0.22	6.3 ± 0.13	2.3 b ± 0.05	3.2 b ± 0.010	2.9 b ± 0.14	3.5 b ± 0.13	5.9 ± 0.26
Fine (µg cm^{-2})	City centre	5.7 a ± 0.22	2.6 a ± 0.09	0.8 a ± 0.02	1.5 ± 0.07	0.9 a ± 0.03	1.6 a ± 0.01	1.8 ± 0.14
	Suburbs	3.5 b ± 0.12	1.8 b ± 0.06	0.6 b ± 0.02	1.5 ± 0.14	0.6 b ± 0.02	1.2 b ± 0.04	1.9 ± 0.07
Waxes (µg cm^{-2})	City centre	871.1 a ± 20.2	195.4 a ± 4.41	38.3 a ± 1.50	56.2 a ± 1.83	56.4 a ± 1.62	73.7 a ± 2.78	127.0 a ± 3.05
	Suburbs	643.0 b ± 26.2	126.7 b ± 2.50	14.3 b ± 0.49	34.5 b ± 2.00	41.2 b ± 0.54	64.9 b ± 1.87	98.2 b ± 5.07

[*] Case letters indicate significant differences between sites determined using Tukey's HSD test with P = 0.05.

single plant) were made on four plants (biological replications) of every species (n = 3 years × 4 terms of measurement × 2 locations × 7 species × 4 plants = 672).

2.2.2. Total chlorophyll content

Measurements of the total chlorophyll content, expressed as a chlorophyll content index (CCI), were carried out using a Chlorophyll meter CCM-200 (OPTI-SCIENCES, USA). Before each set of measurement (single species in single location) chlorophyll meter was auto calibrated. Twenty technical measurements were carried out on four individuals (biological replications) from each species (n = 3 years × 4 terms of measurement × 2 locations × 7 species × 4 plants = 672).

2.2.3. Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence was measured using a continuous excitation fluorometer (Handy PEA, Hansatech, UK). Before every round of measurements, dark adaptation leaf clips were placed on the leaves and shutters were closed. After 45 min of adaptation to darkness, measurements of minimal (F_o), maximal (F_m) and variable ($F_v = F_m - F_o$) fluorescence of chlorophyll *a* were taken using a pulse of high light intensity (3000 µmol m^{-2} s^{-1}). Maximum quantum efficiency of photosystem II (F_v/F_m, where $F_v = F_m - F_o$) was calculated by the fluorometer software. Measurements were performed in four biological replications with 12 technical measurements each, when for single plant we used mean of them (n = 3 years × 4 terms of measurement × 2 locations × 7 species × 4 plants = 672).

2.3. Accumulation of PM and epicuticular waxes on foliage

2.3.1. Sample collection

At the end of each growing season, leaves of each species were harvested from the same four individuals and from the same side of the plant on which physiological measurements had been performed (n = 3 years × 2 locations × 7 species × 4 plants = 168). In order to obtain sufficient material to determine the fine fraction of PM while avoiding filter blockage by particles during filtration, the leaf area per sample ranged between 300 and 400 cm^2. Leaf samples were always collected in October, at the end of the growing season, from branches located 1.0 – 1.7 m above ground level, depending on the plant species. Leaf samples were placed in paper bags, labelled and kept at ambient temperature until analysis.

2.3.2. Quantitative analysis of PM and epicuticular waxes

Masses of PM and epicuticular waxes were determined gravimetrically, as described in detail elsewhere (Dzierżanowski et al., 2011). Total amount of PM ($_{T}$PM) was determined in two categories: washed off with water - surface PM ($_{S}$PM), and washed off with chloroform - in-wax PM ($_{W}$PM). Both categories were measured in three size fractions (10–100, 2.5–10 and 0.2–2.5 µm). Each leaf sample was washed with water and then with chloroform. The liquids were then filtered using three types of filters - Type 91 and Type 42 paper filters, and PTFE membrane filters (all Whatman, UK) with pore sizes of 10 µm, 2.5 µm and 0.2 µm, respectively. Before and after filtration, all filters were stabilised for humidity, passed through a deioniser gate (HAUG, Switzerland) and weighed (XS105DU balance, Mettler–Toledo International Inc., Switzerland). The quantity of wax was determined by weighing after the evaporation of chloroform collected (after filtration) in pre–weighed beakers. The amounts of PM and wax were then recalculated to µg cm^{-2} after the leaf area of samples was measured for analysis (Image Analysis System, Skye Instruments Ltd, UK).

2.4. Statistical analysis

Analysis of variance (ANOVA) was used to assess the statistical significance of differences and interaction between the amount of PM (total and according to size category), the amount of epicuticular waxes, the photosynthetic rate, stomatal resistance, the amount of chlorophyll and F_v/F_m. A Tukey's HSD test ($P = 0.05$) was then employed to assess the significance of differences between the two variants. Correlations between the amount of PM and epicuticular waxes on leaves, photosynthesis rate, stomatal resistance, total chlorophyll content, F_v/F_m were calculated using Pearson's correlation coefficient. The presented data are given as means with their standard error (± SE). All calculations were performed using JMP Pro 12.1.0 software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Accumulation of PM and wax deposition

Since PM accumulation on the foliage of the trees, shrubs and climber was similar in all three years of the study, irrespective of the category and size fraction, results presented in this work are the mean of the three years. A significantly higher amount of $_T$PM was accumulated by all species growing in the city centre, as the amount of $_T$PM in the suburbs was 26% lower (Table 1). In both locations, the greatest amount of $_T$PM was found on the leaves of *B. pendula.* The smallest amount of $_T$PM was observed on *P. opulifolius* in the city centre and on *H. helix* in the suburbs. All tested species accumulated both surface PM and PM stabilised in waxes (Table 1). The percentage ratio of $_S$PM to $_W$PM was strictly related to species, not to location. In the case of *B. pendula*, the amount of $_W$PM present was greater than $_S$PM ($_W$PM amounted to about 63.5% of $_T$PM in both locations), while in the *S. intermedia* the amount of $_w$PM was higher in the suburban location (54%) but not in the city centre (47%). In the other five species, however, the amount of $_S$PM present was greater than that of $_W$PM, ranging from 51% in *F. ×intermedia* to 84% in *H. helix.* Among size fractions, the greatest proportion was represented by large PM (70% and 67% respectively in city the centre and suburbs), followed by coarse PM (21% and 24%) and then fine PM (9% in both locations; Table 1). Regardless of location, the greatest amount of all fractions was found on *B. pendula* leaves. The lowest amounts of large PM were found on leaves of *F. ×intermedia* and *P. opulifolius* grown in the city centre and suburbs, respectively. In both locations, the lowest amount of coarse and fine PM was found on the leaves of *H. helix* (Table 1).

All plant species grown in the city centre were characterised by an increased amount of waxes on the surface of their leaves (Table 1); on average, they exhibited 28% more wax than did plants in the suburbs location. *B. pendula* was the species with the greatest amount of waxes in both locations, with wax levels that were, on average, nearly 30-fold higher than those recorded on the leaves of *H. helix*, which had the lowest amount of waxes in both locations. The calculated correlation coefficient for all tested plants showed a positive significant correlation between the amount of waxes and both $_T$PM ($r = 0.83$; $P < 0.0001$) and $_W$PM ($r = 0.92$; $P < 0.0001$).

3.2. Efficiency of photosynthetic apparatus

The rate of photosynthesis of plants grown in the city centre was, on average, significantly lower (by 13%) than that of plants in the suburban area (Fig. 2A), which most likely resulted from the significantly higher (by 32%) stomatal resistance in the city centre plants (Fig. 2B). In case of two species *S. intermedia* and *S. japonica* there was no significant differences in photosynthesis rate. The reduction in photosynthetic rate and the corresponding increase of stomatal resistance were greatest in *H. helix* (by 36% and 60%, respectively). Only in the case of *P. opulifolius* the average of three years measurements shows a 31% increase in the photosynthetic rate but no significant differences in stomatal resistance. Besides *H. helix* the only other significant increase in stomatal resistance was found in *S. japonica.*

Even though in most tested species the total chlorophyll content did not differ significantly between locations, *F. ×intermedia* and *P. ×hispanica* plants grown in the city centre showed a greater chlorophyll content (by 34% and 17%, respectively; Fig. 2C).

In this work, F_v/F_m was evaluated, as a parameter of chlorophyll *a* fluorescence that is commonly used to monitor overall plant performance under abiotic stresses. Plants grown at the two sites significantly differed in values of F_v/F_m, which were on average lower in plants grown in the highly polluted location (Fig. 2D). When comparing individual species, significantly lower values of F_v/F_m were recorded in the city centre for *H. helix* only.

The correlation coefficient for all species tested reached a low, but statistically significant negative value between $_T$PM and photosynthesis rate ($r = -0.32$; $P = 0.0384$), between $_T$PM and F_v/F_m ($r = -0.48$; $P = 0.0127$), and between large PM fraction and photosynthesis rate ($r = -0.43$; $P = 0.0491$). There was also a positive significant correlation between the fine PM fraction and stomatal resistance ($r = 0.47$; $P = 0.0281$). No other significant correlations between the amount of PM and the measured parameters were found.

4. Discussion

4.1. PM accumulation and dust deposition

PM suspended in the air is a major threat to human health, (Silva et al., 2013), but its importance is often underestimated. Fortunately, numerous studies has proven that vegetation can reduce the concentration of PM in ambient air (McDonald et al., 2007; Sæbø et al., 2012). Also in this work, all tested species accumulated PM on their foliage, but the amount of PM deposited on leaves' surfaces strongly depended on its concentration in the air and was always greater in the more polluted location, as has been previously shown by Popek et al. (2017a) and Przybysz et al. (2014a). The examined species considerably differed in their accumulation of total PM. *B. pendula* exhibited the greatest ability to accumulate PM, irrespective of location. This could be explained by the fact that this species possessed the greatest amount of epicuticular waxes on its foliage, and as a consequence a greater proportion of the particulates it accumulated were $_W$PM. Similar data were obtained by Sæbø et al. (2012) for *B. pendula* trees grown in Norway, where high levels of waxes resulted in an increased amount of $_W$PM, up to 83% of total PM. It should be emphasised that the wax layer is just one of several factors affecting PM accumulation. Many other morphological characteristics, such as surface roughness and presence of trichomes, have also been shown to be positively associated with the amount of PM on foliage (Jouraeva et al., 2002; Leonard et al., 2016). Plants with the smallest amount of PM accumulated on their leaves in this study were *P. opulifolius* and *H. helix.* The low accumulation of PM on these species is likely also caused by the morphology of their leaves, which are smooth and lack hairy structures.

The species examined in this work also differ in their potential to accumulate different PM size fractions (Table 1). The highest amount of every PM fraction in both locations was recorded on *B. pendula*. The lowest amounts of large PM were recorded on leaves of *F. ×intermedia* in the city centre and *P. opulifolius* in suburbs, while the smallest quantities of coarse and fine PM were found on leaves of *H. helix.* Accumulation levels of PM of different sizes can be shaped by the diverse leaf morphology of the tested species and likely by the composition of PM in the air, which can changed locally. In this work, the ratio of measured PM fractions for all species was 65% (large PM): 25% (coarse PM): 10% (fine PM). The same proportions between size fractions were also recorded by Dzierżanowski et al. (2011), Popek et al. (2013) and Sæbø et al. (2012). It should be highlighted, however, that our method depends of weighing and large PM are likely the heaviest, even if their absolute number present is typically much lower than that of smaller PM (Janhall, 2015).

In the present study, all tested species showed a tendency for higher wax deposition when growing in a more polluted area. Increased wax

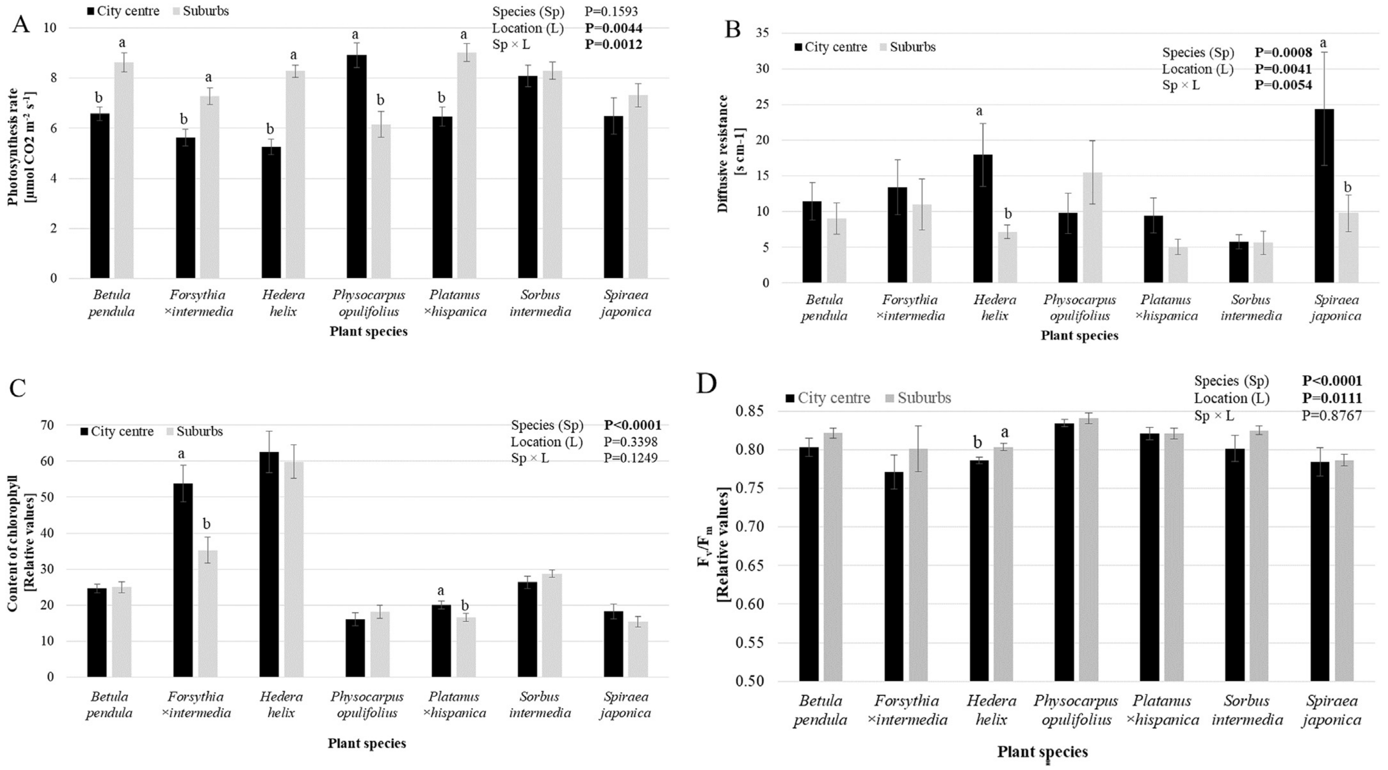

Fig. 2. Rate of photosynthesis (A), stomatal resistance (B), total chlorophyll content (C) and maximum quantum efficiency of photosystem II (F_v/F_m; D) in seven plant species growing at two sites differing in levels of particulate matter (PM) in the air. Results are averaged over three years, data are means ± SE. Case letters indicate significant differences between locations for each species determined using Tukey's HSD test with P = 0.05.

production is an acclimation mechanism to abiotic stresses (Kosma et al., 2009; Steinmüller and Tevini, 1985). Pollutants may also affect the structure of waxes (Jouraeva et al., 2002). Pal et al. (2002) demonstrated that, for eight plant species growing along roadsides, exposure to diesel exhaust resulted not only in lower wax content, but also in disorganisation and modification of wax composition. In contrast, Hanslin et al. (2017) showed that diesel exhaust may mitigate the negative effects of abiotic stress on wax production, as in *Pinus sylvestris* L. plants exposed to diesel, salt stress did not change, although drought decreased wax quantity. It appears, therefore, that the effects of abiotic stresses on wax production are likely species- and stress- (type, level, duration) dependent. In addition to its protective function, the wax layer usually also contributes to greater PM accumulation on foliage (Sæbø et al., 2012). In the current work, it was also shown that the thicker wax layer observed on the leaves of plants grown in the highly polluted city centre led to greater PM accumulation, as a significant positive correlation was found between the amount of wax on leaves, and the accumulation of $_T$PM and $_W$PM. The proportion of PM retained in the wax layer is critical for successful phytoremediation, because PM is immobilised in wax for a long period of time and cannot be washed off by rain or re-suspended by wind, as shown by Przybysz et al. (2014b) and Van Heerden et al. (2007). It should be pointed out, however, that plants, especially when grown in adverse conditions, can differ in the structural and chemical characteristics of their wax layer (Jouraeva et al., 2002). Thus, based on the results of this work, it cannot be clearly stated whether increased PM accumulation on the foliage of plants grown in a highly polluted location results from a thicker wax layer, the changed structure and composition of the wax layer, or both. It appears, however, that a combination of mild urban stresses may stimulate PM phytoremediation by broadleaf plants, as was not true in the case of pine (Hanslin et al., 2017).

4.2. *Efficiency of photosynthetic apparatus*

Plants grown in the polluted city centre were typically characterised by a decreased efficiency of their photosynthetic apparatus. Of the seven species tested, tree exhibited a significantly lowered photosynthesis rate and two no significant decrease in the highly polluted location. Only in the case of *P. opulifolius* a positive effect of urban conditions on performance was recorded. The rate of photosynthesis in urbanised areas may be decreased by many factors, including pollution and compaction of soil, deficit and contamination of water, limitation of light and space, and high temperature (Hanslin et al., 2017). PM is not typically considered as a factor affecting photosynthesis, and even when it is, it is not listed among the most important ones. In this work, we showed that the lowered rate of photosynthesis in the city centre was significantly negatively correlated with the amount of $_T$PM and large PM fraction accumulated on foliage. A negative effect of PM on rate of photosynthesis has previously been shown in plants dusted with various compounds (Hirano et al., 1995; Kuki et al., 2008; Vardaka et al., 1995). Popek et al. (2017b) also showed that the reduction of photosynthetic rate in two *Prunus* species corresponds well with their ability to accumulate PM, as the efficiently-accumulating *Prunus padus* L. had a lowered photosynthesis rate, whereas the intensity of this process in the poorly-accumulating *Prunus serotina* Ehrh. was not reduced. Similarly, in the current work, the species with highest rate of photosynthesis in the more polluted location (*P. opulifolius*) also possessed the lowest accumulation of PM. This strengthens our hypothesis that high levels of PM accumulation may decrease a plant's performance and should therefore be considered an important factor affecting the functionality of plants in urban areas. Lowering the rate of photosynthesis due to the presence of PM accumulated on the foliage could occur for one of several reasons. PM may, for instance, change the optical properties of leaves and decrease the level of radiation reaching the chlorophyll antenna due to its absorption or reflection (Hirano et al., 1995; Naidoo and Chirkoot, 2004; Nanos and Ilias, 2007).

The strong, negative effect on gas exchange is most likely due to fine and coarse PM. In most plants, the diameter of stomata ranges from 8 to 12 µm (Krajickova and Mejstrik, 1984), so coarse and fine PM can easily disturb stomatal functions by physically blocking them (Ram et al., 2014). Clogging stomata can lower stomatal conductance and consequently reduce transpiration rate and the assimilation of CO_2, which causes a reduction in the rate of photosynthesis (Chauhan, 2010). These findings were also confirmed in the current work, in which two species (significantly) and four (not significantly) showed greater stomatal resistance in the more polluted city centre. Only the *P. opulifolius* showed a decrease in stomatal resistance but this was not significant. Moreover, a significant positive correlation was found between stomatal resistance and fine PM accumulated on the plant surface. Reduced stomatal conductance (parameter directly opposed to stomatal resistance) after PM deposition on foliage was also recorded by Nanos and Ilias (2007) and Vardaka et al. (1995). Clogged stomata and decreased transpiration rate may also result in response to increased leaf temperature, as demonstrated by Flückiger et al. (1979) in *Populus tremula* L. trees treated with silica dust.

The potential negative effect of air pollutants, including PM, on a plant's performance, especially gas exchange, is also supported by the fact that plants that are better adapted to pollutants possess a number of modifications that allow them to maintain intense photosynthesis under such conditions. One of the most common adjustments is an alteration of the stomatal features on the leaves of plants exposed to a dusty environment (Siqueira-Silva et al., 2016). Another possibility is modification of stomatal frequency, size and localisation on leaves, as a greater number of smaller stomata located on the abaxial side of the leaf may result in a greater resistance of gas exchange to airborne PM (Gostin, 2009).

In our study, there were no statistical differences between locations when regarding total chlorophyll content, but there was a trend for this parameter to increase in the highly polluted for two plant species (*F.* ×*intermedia* and *P.* ×*hispanica*). Similar results were obtained by Vardaka et al. (1995), who showed that the concentration of chlorophyll in holm oak (*Quercus ilex* L.) did not differ significantly between plants growing near a limestone mine and those growing further away from the mine. Conversely, Kuki et al. (2008) found an increase in chlorophyll content in *Sophora tomentosa* L. and *Schinus terebinthifolius* Radii, and Van Heerden et al. (2007) showed a decrease in chlorophyll content in the desert shrub *Zygophyllum prismatocarpum* E.Mey. ex Sond. in response to the accumulation of PM on leaves. According to our data, we believe that chlorophyll content is likely not a limiting factor for photosynthesis. Even when the amount of chlorophyll decreases, if other parameters important for efficient photosynthesis, particularly gas exchange, remain at optimal levels, the rate of photosynthesis rate will typically be sufficient. We hypothesise that the negative effect of PM on photosynthesis is primarily associated with decreased gas exchange and disturbed light accumulation.

In this work, the maximum quantum efficiency of photosystem II (F_v/F_m) did not differ significantly between the two tested locations for every species in exception of *H. helix*, which exhibited significantly lower values of the F_v/F_m in plants grown in the city centre. When averaged data from all study years and all species were analysed, however, a significant 3% decrease in F_v/F_m was recorded. These results correspond well with data obtained by Naidoo and Chirkoot (2004) and Vardaka et al. (1995), who reported a decrease in F_v/F_m as a plant response to increased dust deposited on foliage. Similarly, Van Heerden et al. (2007) reported a 4% reduction in F_v/F_m in *Zygophyllum prismatocarpum* Sond. exposed to limestone dust. When the limestone PM was washed from the leaves, however, values of this parameter rapidly achieved the levels recorded under control conditions (Van Heerden et al., 2007). Przybysz et al. (2014b) reported that as much as one third of PM present in nature can be relatively easily washed from the leaf surface by rainfall. We therefore suggest that after a precipitation event, the effect of PM on the photosynthetic apparatus is likely less pronounced, as some of the stomata may be unclogged and light may be more efficiently absorbed. This also suggests that the photosynthetic apparatus in plants that accumulate PM may not be permanently damaged, and under favourable conditions it may be able to return to optimum parameters. This possibility is supported by the relatively low changes in values of F_v/F_m observed in the current study.

Our study shows, that plants reacts differently to air PM pollution in urban conditions. *Physocarpus opulifolius* turn out to be the most resistant to PM, which can be explained by the fact that it has the lowest efficiency of PM accumulation, resulting most probably from the specific defense mechanisms e.g. morphological features like smooth leaves or lack of hairs. On the other hand, species that accumulated high amounts of PM, such as *B. pendula*, were PM sensitive. These results support therefore the thesis that species selection for air phytoremediation purposes most be a compromise between tolerance to PM and potential for PM accumulation. For plantings in most polluted areas selected should be plants from generas tolerant to air pollutants, while more sensitive species should be planted in an adequate distance from the emission source.

5. Conclusions

Plants, especially trees, shrubs and climbers, play an important role in the urban environment. Through the accumulation of air contaminants on their foliage, plants can successfully reduce health risks for humans. Nevertheless, the urban environment is also harmful for greenery. This work shows that the amount of accumulated PM is negatively correlated with the rate of photosynthesis and F_v/F_m, and positively correlated with stomatal resistance. This may indicate that, together with other urban conditions, PM negatively affects the photosynthetic process for most plant species, which can reduce their productivity and functionality.

It is extremely important to extend our knowledge and understanding of the complex way in which city conditions affect plants hopefully allowing these organisms to be used in the future as a very efficient tool to reduce threats to human health.

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