

Short Communication

Influence of Particulate Matter Accumulation on Photosynthetic Apparatus of *Physocarpus opulifolius* and *Sorbaria sorbifolia*

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Abstract

One of the most dangerous pollutants in the atmosphere is particulate matter (PM), which can be suspended in the air for weeks and has a negative impact on human health when inhaled. The most effective method for clearing the air of PM is through phytoremediation, whereby plants act as biological filters. PM also has a negative impact on plants, but knowledge of the effects of PM on the photosynthetic apparatus is limited.

The aim of this study was to assess the accumulation of PM in the context of leaf morphology and the efficiency of the photosynthetic apparatus in two shrub species with different types of leaves – *Physocarpus opulifolius* and *Sorbaria sorbifolia* – both commonly planted along busy roads in urbanized areas. The study was conducted under controlled conditions using three variants of PM (construction, cement, and roadside PM).

Results showed that *S. sorbifolia* accumulated higher amounts of PM than did *P. opulifolius*, likely because of its more complex leaves. The higher presence of PM on leaves of *S. sorbifolia* resulted in a reduction of the efficiency of the photosynthetic apparatus and chlorophyll *a* fluorescence. A strong negative correlation was found between the amount of PM accumulation and the efficiency of the photosynthetic apparatus in *S. sorbifolia*, and a similar weak correlation was found in *P. opulifolius*.

Keywords: air pollution, common ninebark, false spiraea, phytoremediation, PM, shrubs

Introduction

Air pollution has become a major threat to human and ecosystem health [1]. One of the most harmful pollutants in the atmosphere is particulate matter

(PM) – microscopic dust particles with aerodynamic diameters ranging from 0.001 to 100 μm [2]. PM contains toxic compounds such as polycyclic aromatic hydrocarbons and heavy metals [3]. In the case of its uptake by humans, PM can lead to diseases like chronic obstructive pulmonary disease, respiratory and cardiac problems, and lung cancer [4]. Plants growing in urban environments can improve air quality and

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serve as natural bio-filters for PM [5-6]. Unfortunately, PM particles also have harmful effects on plants [7-9]. Most likely, the main reason for the decreases in plant condition caused by PM is the shading effect, whereby accumulated PM absorbs and scatters light rays [7]. Another reason is that PM can be sucked into the openings of the stomata and reduce the intensity of transpiration [10]. Chemically active particles can also disturb the photosynthesis and respiration process [11-12], negatively influencing the amount of chlorophyll [13] and the fluorescence of chlorophyll *a* [8]. Until now, only a few papers have described the impact of PM on photosynthesis processes.

In this paper, we assess: (i) the accumulation of PM (two types and three size fractions) on foliage, (ii) epicuticular waxes on the surfaces of leaves, and (iii) the efficiency of the photosynthetic apparatus in common ninebark (*Physocarpus opulifolius* [L.] Maxim.) and false spiraea (*Sorbaria sorbifolia* [L.] A. Braun), which were experimentally polluted with PM from different source variants (construction, cement, and roadside PM). Previously, it has been shown [14-15] that these species are PM accumulators, but based on field observations, they may differ in terms of urban conditions tolerance. We hypothesized that: (I) PM accumulation on leaves of *P. opulifolius* is lower than on leaves of *S. sorbifolia* because of differences in leaf structure and the amount of waxes on their surface; (II) PM has a negative impact on the photosynthetic apparatus, and the effect is weaker in *P. opulifolius* than in *S. sorbifolia*; and (III) there are differences in particle accumulation and plant stress reactions to PM with different origins.

Material and Methods

Plant Material and Study Area

Studies were conducted in two vegetation seasons of 2015 and 2016 using *P. opulifolius* 'Red baron' and *S. sorbifolia* 'SEM' growing in pots filled with the same substrate. Every shrub chosen for the experiment was the same age (5 years old) and had an average height of 80 cm. To protect plants from the accumulation of PM from the air, they were placed in greenhouses under appropriate growing conditions. Plants were divided into four groups of four plants of each species and placed in separate greenhouses. Shrubs from the first three groups were dusted with equal volumes of (i) construction PM, (ii) cement PM, or (iii) roadside PM, consisting of particles less than 100 μm in diameter. Cement PM consisted of sieved Portland cement particles (Cemmas A.S., Poland); roadside PM was collected from the edge of a busy road (Matyi and Bolesława Krzywoustego) in Poznań, Poland (52°24'N, 16°56'E); and construction PM was collected from the debris of various demolished buildings in the city. The fourth group of shrubs, consisting of controls, was not subjected to any stress conditions.

Analysis of PM and Accumulation of Epicuticular Waxes on Leaves

For each variant, leaves were harvested from shrubs at the end of the growing season ($n = 4$ PM source variants \times 2 species \times 4 shrubs). Samples consisted of leaves gathered from different parts of the canopy.

Table 1. The amount of particulate matter (PM) from different sources divided into three size fractions and two categories, surface PM (μsPM) and in-wax PM (μwPM), at the end of the growing season. The amount of epicuticular waxes on leaves of *Physocarpus opulifolius* and *Sorbaria sorbifolia* is also presented. Analysis of variance (ANOVA) was used to assess the statistical significance and Tukey's HSD test ($P = 0.05$) was employed to assess the significance of differences among variants. P values < 0.05 are in bold.

Species	PM source	PM size fraction ($\mu\text{g cm}^{-2}$, mean \pm SE)						Total μsPM ($\mu\text{g cm}^{-2}$, mean \pm SE)		Total μwPM ($\mu\text{g cm}^{-2}$, mean \pm SE)		Epicuticular waxes ($\mu\text{g cm}^{-2}$, mean \pm SE)	
		10–100 (μm)		2.5–10 (μm)		0.2–2.5 (μm)							
<i>Physocarpus opulifolius</i>	Control	17.7e	(1.5)	6.4e	(0.4)	3.2d	(0.3)	17.6f	(1.2)	9.7e	(1.0)	103.7a	(4.0)
	Construction	75.5c	(3.6)	9.4d	(0.8)	4.9c	(0.4)	62.9d	(3.1)	26.9c	(1.0)	99.5a	(2.7)
	Cement	76.9c	(1.5)	17.0b	(0.8)	6.6b	(0.5)	71.8c	(0.8)	28.7c	(0.7)	104.9a	(3.2)
	Roadside	72.7c	(2.0)	9.6d	(0.6)	4.9c	(0.4)	61.9d	(1.8)	25.3c	(0.9)	101.6a	(1.4)
<i>Sorbaria sorbifolia</i>	Control	30.2d	(1.2)	7.3e	(0.5)	3.1d	(0.3)	26.7e	(0.8)	13.9d	(0.5)	89.6b	(1.1)
	Construction	89.9b	(2.0)	15.4c	(1.0)	5.4c	(0.5)	78.5b	(1.2)	32.2b	(1.1)	91.9b	(1.1)
	Cement	165.8a	(3.1)	26.7a	(1.3)	7.7a	(0.4)	152.1a	(1.1)	48.1a	(1.4)	93.8b	(0.5)
	Roadside	77.1c	(2.2)	14.5c	(0.6)	4.4c	(0.2)	65.2d	(1.4)	30.8b	(0.6)	90.0b	(1.0)
ANOVA													
Species		<0.0001		<0.0001		0.4333		<0.0001		<0.0001		<0.0001	
PM source		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		0.0878	
Species \times PM source		<0.0001		<0.0001		0.0477		<0.0001		<0.0001		0.0164	

The amount of PM was determined in two categories: surface PM ($_{s}PM$), which was washed off with water, and in-wax PM ($_{w}PM$), which was washed off with chloroform. Both categories were measured in three size fractions (10-100, 2.5-10, and 0.2-2.5 μm). Each leaf sample was washed, first with water and then with chloroform. The liquids were filtrated using pre-weighed type 91 and type 42 paper filters and PTFE membrane filters (Whatman, UK) with pore sizes of 10 μm , 2.5 μm , and 0.2 μm . After filtration, all filters were weighed again. The quantity of epicuticular waxes was weighed after evaporation of the chloroform collected in pre-weighed beakers. Afterward, the filtration area of leaves from each sample was measured using a scanner and WinFOLIA 2004 measuring software (Regent Instruments Inc., Canada). The amounts of PM from filters and waxes were then recalculated to $\mu g cm^{-2}$ of leaves.

Evaluating Photosynthetic Apparatus Efficiency

Measurements were carried out four times in each of the two years of the study after dust application, at monthly intervals throughout the vegetation season (June-September). The following parameters of efficiency of the photosynthetic apparatus were measured *in vivo*.

Plant Gas Exchange

As part of plant gas exchange, measurements of (i) photosynthetic rate and (ii) stomatal conductance were evaluated via the LI-6400 photosynthesis system using an infrared gas analyzer method (LI-COR Inc., Lincoln, Nebraska, USA). For each shrub, four separate measurements were conducted on each of five random leaves (biological repeat) selected from the middle parts of annual twigs. Each leaf was placed separately in the leaf chamber and maintained until the photosynthesis rate stabilised, at which point measurement was recorded. In every month of the study period, leaves were selected for measurement from the same part of the plant ($n = 4$ PM source variants \times 2 species \times 4 months \times 4 shrubs \times 5 leaves).

Chlorophyll *a* fluorescence determination

On the same leaf samples used for the gas exchange study, measurements of chlorophyll *a* fluorescence were performed using the portable chlorophyll fluorometer OS1p (Opti-Sciences, Inc., Hudson, New Hampshire, USA). Thirty minutes prior to measurement, dark adaptation clips were placed on the leaves and the shutter was closed. Minimal (F_0) and maximal (F_m) fluorescence of chlorophyll *a* was measured using a high-intensity light pulse ($3,000 \mu mol m^{-2} s^{-1}$). The maximum quantum efficiency of photosystem II (F_v/F_m , where F_v is the difference between F_m and F_0)

was calculated by the instrument's software ($n = 4$ PM source variants \times 2 species \times 4 months \times 4 shrubs \times 5 leaves).

Statistical Analysis

Analysis of variance (ANOVA) was used to assess the statistical significance of the differences between species and among PM sources regarding the amount of PM (total and according to size category), epicuticular waxes, photosynthetic rate, stomatal conductance, and maximum quantum efficiency of photosystem II. Relationships between the photosynthetic rate and total PM amount, and between stomatal conductance and the amount of fine PM (0.2-2.5 μm) fraction were evaluated using Pearson's correlation coefficient. The individual plant was considered a random effect. All calculations were performed using JMP Pro 12.1.0 software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

For both tested plant species at the end of the growing season, PM was found on the surface of leaves. Since PM accumulation on the tested shrub species was similar in both years of the study, irrespective of the category and size fraction, the results below are presented as the mean of the two years.

The results showed significant differences in PM accumulation between *P. opulifolius* and *S. sorbifolia* (Fig. 1). *S. sorbifolia* accumulated approximately 49% more PM in the control variant, 23% more in the construction variant, 99% more in the roadside variant, and 110% more in the cement variant than did *P. opulifolius*. These results are similar to previous observations of the PM accumulation pattern on these two species growing in a nursery [14-15], where the amount of PM on *P. opulifolius* leaves was more than twice as low as that on *S. sorbifolia*. The differences between these two species can be mostly explained

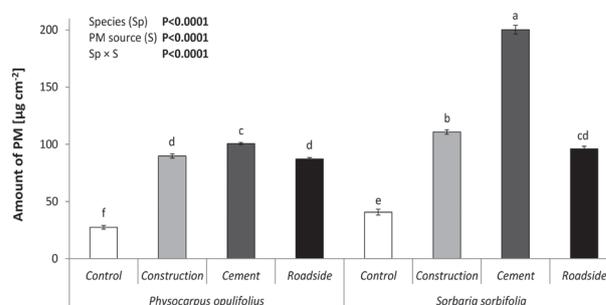


Fig. 1. Total amount of particulate matter (PM) from different sources deposited on leaves of *Physocarpus opulifolius* and *Sorbaria sorbifolia* at the end of the growing season. Analysis of variance (ANOVA) was used to assess the statistical significance and Tukey's HSD test ($P = 0.05$) was employed to assess the significance of differences among PM variants.

by leaf morphology. As *S. sorbifolia* leaves are composite with small leaflets and can have some stellate hairs on the lower surface [16], air turbulence between leaflets may increase the level of deposition of PM [12]. Moreover, high amounts of PM can become stuck between hairs [17]. The midribs and veins of the leaflets of *S. sorbifolia* are concave, which can also increase deposition because PM are often deposited on the leaf margins [18] or along the leaf nerves [19]. In contrast, the leaves of *P. opulifolius* are smooth, without hairs [20]. Moreover, there were no statistical differences between accumulation of construction and roadside PM for either species.

In both species, PM was found both on the surface ($_s$ PM) and in-wax ($_w$ PM). Similar to results of other studies [6, 14-15], the amount of $_s$ PM on both species was higher than $_w$ PM. In nature, $_s$ PM can be washed off foliage by rain or blown away by strong wind, whereas $_w$ PM is more important for phytoremediation because particles can stick to the surface or penetrate the wax layer and be phytostabilized [21]. In our study, *P. opulifolius* had a greater amount of waxes on leaves than did *S. sorbifolia*, but no statistical differences in the amount of waxes between the control and polluted leaves was found for either species. In this research, no statistical differences in the amount of waxes between the control and polluted leaves was found for either species.

In our research, despite of the origin of the PM and the plant species, the large size PM fraction (10-100 μm) comprised the largest portion of all accumulated PM, an average of 80% of the total amount for both plant species. Furthermore, greater deposition of this fraction was observed on *S. sorbifolia* than on *P. opulifolius*. Whereas there were no differences in PM accumulation on *P. opulifolius* in relation to the origin of PM, for *S. sorbifolia* the amount of cement dust accumulated on leaves was approximately two times higher than that of demolition and road dust. This PM fraction is not as dangerous to human health as is PM with a smaller diameter, but it can have a significant negative impact on plants by shading leaves and obstructing access to solar light [22].

Coarse PM (2.5-10 μm) and fine PM (0.2-2.5 μm) accumulation patterns on leaves are similar to those of large PM. In this case, however, both fractions from *P. opulifolius* had higher levels of cement PM than of the other two types of PM. The phytoremediation of these two PM fractions, especially in highly polluted areas, is gaining much attention because it can contribute to an improvement in the health of both people [4] and plants [6].

Our study showed that the efficiency of the photosynthetic apparatus was strongly negatively affected by the amount of PM in the case of *S. sorbifolia*, but only slightly negatively affected in the case of *P. opulifolius* (Fig. 2a). The reduction in efficiency of the former shrub was 38% on average, whereas reduction in the latter shrub was only 5% on

average. Additionally, a strong negative correlation was found between photosynthesis rate and total amount of PM accumulation in *S. sorbifolia* ($r = -0.71$; $P < 0.0001$), whereas the same correlation was weaker in *P. opulifolius* ($r = -0.35$; $P = 0.0256$). There were no significant differences in relation to sources of PM. The effects of air pollution on plants are reflected in terms of reduction in photosynthetic pigments, inhibition of certain physiological processes, and alteration in metabolic functions and enzyme activities [23]. A lower rate of photosynthesis due to PM coverage on the surface of leaves can also result from a decrease in radiation reaching the chlorophyll antenna due to absorption or reflection by PM [9]. A pattern very similar to that regarding the rate of photosynthesis was found in measurements of stomatal conductance of the studied shrubs (Fig. 2b). Whereas reduction in the stomatal conductance of *P. opulifolius* did not differ in relation to PM source and amounted to 7% on average, in the case of *S. sorbifolia*, conductance was highest in leaves polluted by cement PM (31%, in contrast to 26% and 28% for construction and road PM, respectively). This reduction could be due to very high accumulation of cement PM on leaves of *S. sorbifolia*. Grantz et al. [24] stated that cement dust contaminated

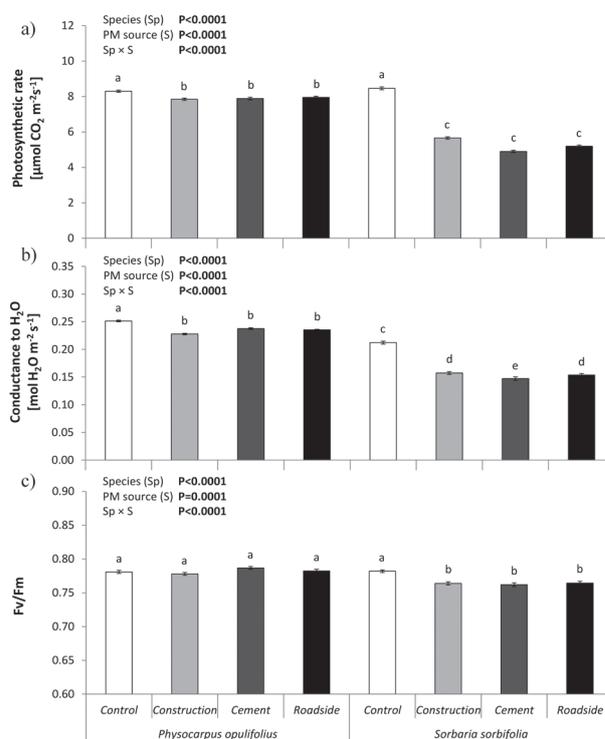


Fig. 2. Mean rate of photosynthesis, stomatal conductance and maximum quantum efficiency of photosystem II (Fv/Fm) in *Physocarpus opulifolius* and *Sorbaria sorbifolia* dusted by construction PM, cement particulate matter (PM) and roadside PM. Analysis of variance (ANOVA) was used to assess the statistical significance and Tukey's HSD test ($P = 0.05$) was employed to assess the significance of differences between PM variants.

with reactive constituents such as heavy metals can damage vegetation more than other types of pollutants. Furthermore, a strong negative correlation was found between stomatal conductance and the fine PM fraction in *S. sorbifolia* ($r = -0.77$; $P < 0.0001$), and a moderate negative correlation was found in *P. opulifolius* ($r = -0.51$; $P = 0.0063$). The reason for this is likely the fact that the smallest particles can block stomata and reduce the accessibility of CO_2 , independent of the light phase of photosynthesis and transpiration [8, 10-11].

The chlorophyll *a* fluorescence results obtained for *P. opulifolius* showed no significant differences between dusted and control plants based on the parameter FV/FM, which described the maximum quantum efficiency of photosystem II (Fig. 2c). Different values were obtained in *S. sorbifolia*, where the values of this parameter in all cases were reduced. These data correspond to research conducted by Heerden et al. [25], where *Zygophyllum prismatocarpum* responded to increased exposure to limestone dust by reducing its Fv/Fm. Additionally, Naidoo et al. [8] and Vardaka et al. [22] claimed that the decrease in the parameter Fv/Fm because of PM accumulation can be connected to the fact that photosystem II efficiency can be reduced via impaired electron transport, which can explain the previously reported decrease in the rate of photosynthesis. For *P. opulifolius*, a lower amount of PM on the leaves can be better tolerated in this case for this parameter.

Conclusions

1. Significantly greater amounts of particulate matter (PM) were found on the leaves of *Sorbaria sorbifolia* than on the leaves of *Physocarpus opulifolius*.
2. Both study species accumulated PM of both categories (surface and in-wax PM) and from all three size fractions on their leaves in different amounts.
3. The amount of PM that accumulated on the leaves of these plant species depended on the morphological and anatomical differences between the leaves of these species.
4. All three types of PM had a negative impact on photosynthesis and stomatal conductance in both tested species, although reduction was greater in *S. sorbifolia*.
5. There was reduction in maximum quantum efficiency of photosystem II in *S. sorbifolia*, but no negative impact was found in the case of *P. opulifolius*.
6. For both *S. sorbifolia* and *P. opulifolius*, a negative correlation was found between the rate of photosynthesis together with stomatal conductance and amount of PM on leaves.
7. We conclude that *P. opulifolius* is more tolerant to the stressful conditions of PM contamination in air than is *S. sorbifolia*.

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