




## Article

# Comparing Different Methodologies to Quantify Particulate Matter Accumulation on Plant Leaves

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**Abstract:** Urban air pollution poses a significant threat to human health, with metropolitan areas particularly affected due to high emissions from human activities. Particulate matter (PM<sub>x</sub>) is among the most harmful pollutants to human health, being composed of a complex mixture of substances related to severe pulmonary conditions. Urban green spaces play a vital role in mitigating air pollution by capturing PM<sub>x</sub>, and it is essential to select plant species with a high capacity for PM<sub>x</sub> accumulation to effectively enhance air quality. This study aimed to evaluate and compare the accuracy of two PM<sub>x</sub> quantification methods—light microscopy and filtration—which demonstrated a high correlation ( $R^2 = 0.72$ ), suggesting that both methods are reliable for assessing PM<sub>x</sub> accumulation on leaves. Light microscopy allowed for the visualization of PM<sub>x</sub> deposition, revealing the species warranting further analysis using the filtration method. Among the species analyzed, *Euonymus japonicus*, *Ligustrum lucidum*, *Alnus glutinosa*, *Rubus ulmifolius*, and *Laurus nobilis* demonstrated the highest total PM<sub>x</sub> accumulation, exceeding 50  $\mu\text{g cm}^{-2}$ , making them particularly valuable for air pollution mitigation. This study examined the correlation between leaf traits such as specific leaf area (SLA), leaf area (LA), leaf dissection index (LDI), and leaf roundness and PM<sub>x</sub> accumulation across the 30 different plant species. A multiple linear regression analysis indicated that these leaf traits significantly influenced PM<sub>x</sub> accumulation, with SLA and LA showing negative correlations and leaf roundness exhibiting a positive correlation with PM<sub>x</sub> deposition. In conclusion, this study highlights the importance of selecting plant species with specific leaf traits for effective air quality improvement in urban environments particularly in highly polluted areas, to enhance air quality and public health.

**Keywords:** PM<sub>x</sub> quantification; leaf traits; air pollution mitigation; urban green



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## 1. Introduction

Urban air pollution is a threat to human health. Air quality is generally worse in metropolitan areas due to high emissions from human activities [1]. A substantial portion of people live in cities where air quality limits are frequently exceeded, adversely affecting people's quality of life. Particulate matter (PM<sub>x</sub>), nitrogen dioxide (NO<sub>2</sub>), and ground-level ozone (O<sub>3</sub>) are the most harmful pollutants to human health in Europe. For instance, in Italy, air pollution is approximately related to 62,000 deaths yearly [2].

PM<sub>x</sub> is defined as a complex mixture of saturated solutions and solid substances, including heavy metals, unburned black carbon particles, polycyclic aromatic hydrocarbons (PAH), and other suspended constituents [3]. PM<sub>x</sub> can originate from natural or anthropogenic processes, with fossil fuel combustion from vehicles, building heating, and

industrial and agricultural activities being the primary sources in urban environments [4,5]. Atmospheric  $PM_x$  is classified based on the diameter of its particles:  $PM_{10}$  ( $\leq 10 \mu m$ ),  $PM_{2.5}$  ( $\leq 2.5 \mu m$ ), and  $PM_{0.2}$  ( $\leq 0.2 \mu m$ ) [6], with its accumulation on leaves depending on particle properties. Most of the total particle number accumulated on leaves is smaller than  $2.5 \mu m$ . These small particles are particularly harmful to human health, as they can penetrate the respiratory system, causing severe pulmonary conditions. Larger particles can absorb toxic materials due to their shape and size [7].

Urban green reduces air pollution by capturing  $PM_x$  [8], serving as biological barriers along roads, high-traffic avenues, and streets [9], providing an effective solution for reducing urban air pollution. Efforts have been made to estimate the amount of pollutants removed by plants globally. For example, one hectare of urban tree cover in the United States removes about 67 kg of pollution annually [10]. In Britain, it was estimated that natural urban green spaces removed 28,700 tonnes of pollutants ( $PM_{2.5}$ ,  $NO_2$ ,  $SO_2$ , and  $O_3$ ) in 2015, resulting in 900 fewer respiratory hospital admissions, 220 fewer cardiovascular hospital admissions, 240 fewer deaths, and 3600 fewer Life Years Lost [8].

Particle features such as size, shape, and composition influence the deposition mechanism on leaves and are crucial for predicting regional pollutant concentrations [11]. Understanding the impact of leaf morphology on  $PM_x$  accumulation is essential for elucidating the differential capacities among species to facilitate  $PM_x$  deposition [12]. Selecting plant species to improve air quality at vulnerable urban sites is fundamental before planning and designing efficient pollution-mitigating interventions [13]. Additionally, site-specific conditions such as traffic density, the presence of buildings, atmospheric stability, and wind angle must be considered for the effectiveness of urban green projects in improving air quality [11].

Several studies quantify  $PM_x$  accumulation on leaves using different approaches. Two common methods are (1) light microscopical analysis, which allows for an accurate visualization of  $PM_x$  deposition, and (2) filtration methods, which determine different classes of  $PM_x$ . However, the criteria for choosing between methods can be problematic, as the correlation between the results of different approaches is not often demonstrated or discussed.

There remains a significant need to identify and characterize plant species that are particularly efficient at accumulating  $PM_x$ , as these species could play a crucial role in mitigating urban air pollution. The efficiency of  $PM_x$  accumulation varies widely among plant species, and this variation can largely be explained by differences in specific leaf traits. Morphological characteristics, such as leaf shape, size, and surface roughness, have been shown to influence the ability of leaves to capture and retain particles. For instance, species with more complex leaf shapes and greater surface area often exhibit higher  $PM_x$  accumulation, as their surfaces provide more opportunities for particles to adhere [14,15].

In addition to morphology, structural features of the leaf, particularly the composition and thickness of epicuticular waxes, play a pivotal role in determining a plant's capacity to accumulate  $PM_x$ . Waxes can act as a sticky layer, trapping  $PM_x$  on the leaf surface and preventing it from being resuspended into the atmosphere. Research has demonstrated that species with thicker or more abundant wax layers tend to accumulate higher quantities of  $PM_x$ , making wax deposition a key trait in the identification of efficient accumulator species [15,16]. Furthermore, physiological traits such as stomatal density, distribution, and function can also impact  $PM_x$  capture, as stomata can either facilitate the internal deposition of smaller particles or be obstructed by  $PM_x$ , thereby affecting gas exchange processes.

Together, these leaf traits—morphological, structural, and physiological—offer a comprehensive framework for understanding the mechanisms behind  $PM_x$  accumulation and provide valuable criteria for selecting plant species that can effectively improve air quality in urban environments.

This study aimed to evaluate and compare two distinct  $PM_x$  quantification methods, hypothesizing a high correlation between them. To achieve this, we designed a comprehensive research framework focused on testing these methods' reliability and applicability in

investigating PM<sub>x</sub> accumulation on leaves. Furthermore, this study sought to determine the correlation between various leaf traits and PM<sub>x</sub> accumulation across different plant species. Our research questions centered on the following: (1) How accurate are the two selected PM<sub>x</sub> quantification methods? (2) Which method is more suitable for different research objectives related to PM<sub>x</sub> accumulation? (3) What is the correlation between leaf traits and PM<sub>x</sub> accumulation in diverse plant species?

## 2. Material and Methods

### 2.1. Study Area

The study was conducted in the province of Lucca, Tuscany, Italy. This region experiences high PM<sub>x</sub> levels due to a combination of factors, including the dispersion of air pollutants hindered by adverse weather conditions such as low wind regimes, stable atmospheric conditions, and shallow thermal inversions [17].

### 2.2. Plant Species

The thirty species chosen within the investigated area are listed in Table 1.

**Table 1.** Detailed classification of the 30 studied plant species.

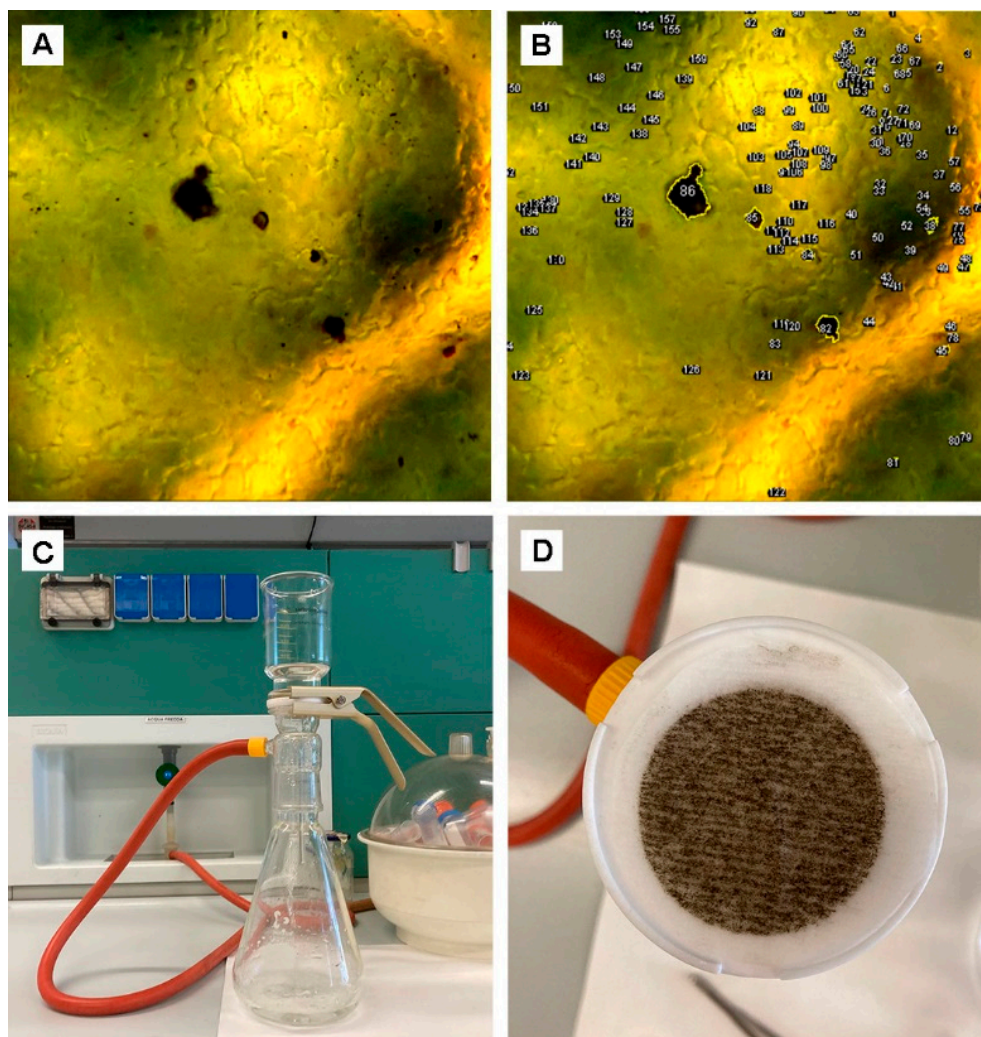
Species	Family Name	Type of Plant
<i>Acer campestre</i> L.	Sapindaceae	Tree
<i>Acer negundo</i> L.	Sapindaceae	Tree
<i>Acer saccharinum</i> L.	Sapindaceae	Tree
<i>Alnus glutinosa</i> (L.) Gaertn.	Betulaceae	Tree
<i>Celtis australis</i> L.	Cannabaceae	Tree
<i>Euonymus japonicus</i> Thunb.	Celastraceae	Shrub
<i>Forsythia viridissima</i> Lindl.	Oleaceae	Shrub
<i>Gaultheria procumbens</i> L.	Ericaceae	Shrub
<i>Ginkgo biloba</i> L.	Ginkgoaceae	Tree
<i>Impatiens walleriana</i> Hook.f.	Balsaminaceae	Herb
<i>Laurus nobilis</i> L.	Lauraceae	Shrub
<i>Lavandula latifolia</i> Medik.	Lamiaceae	Shrub
<i>Ligustrum lucidum</i> W.T. Aiton	Oleaceae	Tree
<i>Nerium oleander</i> L.	Apocynaceae	Shrub
<i>Olea europaea</i> L.	Oleaceae	Tree
<i>Populus alba</i> L.	Salicaceae	Tree
<i>Populus nigra</i> L.	Salicaceae	Tree
<i>Prunus domestica</i> L.	Rosaceae	Tree
<i>Prunus laurocerasus</i> L.	Rosaceae	Tree
<i>Prunus serrulata</i> Lindl.	Rosaceae	Tree
<i>Quercus ilex</i> L.	Fagaceae	Tree
<i>Rosa chinensis</i> Jacq.	Rosaceae	Shrub
<i>Rubus ulmifolius</i> Schott	Rosaceae	Shrub
<i>Salix cinerea</i> L.	Salicaceae	Shrub or tree
<i>Styphnolobium japonicum</i> (L.) Schott	Fabaceae	Tree
<i>Tilia x europaea</i> L.	Malvaceae	Tree
<i>Tilia cordata</i> Mill.	Malvaceae	Tree
<i>Ulmus minor</i> Mill.	Ulmaceae	Tree
<i>Ulmus glabra</i> Huds.	Ulmaceae	Tree
<i>Viburnum</i> spp.	Viburnaceae	Shrub

The individuals were dispersed across the different zones and were selected considering seasonal, climatical, and logistical parameters to represent the local species' biodiversity.

Sampling was conducted during the summer (July 2020), when the climate was dry, and leaves were exposed to environmental pollution for more than ten days without rain. Approximately 400 cm<sup>2</sup> of leaves per species were sampled, always selecting leaves fully exposed to the surrounding air. The samples were stored in paper bags and taken to the laboratory to be analyzed.

### 2.3. The Leaf Covering by $PM_x$ Using Light Microscopy

Two squares (1 cm) per leaf of each sample were analyzed adaxially under a light microscope (10 $\times$ ). The pictures were then processed using ImageJ software (<https://imagej.net/ij/>) [18] to verify the percentage of leaf surface covered with  $PM_x$  (Figure 1A,B). A threshold ( $\geq 1\%$  of the leaf surface covered with  $PM_x$ ) was used to select the species for further investigation.



**Figure 1.** (A) Leaf surface of *L. nobilis* covered with  $PM_x$  (black and brown spots). (B) Identification of  $PM_x$  with ImageJ. (C) Filtration system consists of glassware connected to pump. (D) Paper filter with high  $PM_x$  content after first filtration process.

To quantify particulate matter ( $PM_x$ ) using ImageJ, the following steps were carried out: First, the scale was set up to ensure accurate measurement. An image containing a scale bar was opened in ImageJ. Using the Zoom tool, the view was adjusted to focus closely on the scale bar. With the Line tool, a line was drawn along the length of the scale bar to match its size. The “Set Scale” option was then selected from the Analyze menu. In the “Set Scale” dialog box, the known distance of the scale was entered (0.5 in this study). The “Global” option was checked to apply this scale to all future analyses, and then the setup was confirmed by clicking “OK”. Next, the quantification of  $PM_x$  was performed. The image of interest was opened in ImageJ. From the Plugins menu, “Particle Analysis” and then “Grid” were selected. The grid type was set to “line” and the area per point was specified as 100,000  $\mu m^2$ . A random square area within the image was selected for analysis. The Zoom tool was used to increase the magnification of the selected area. In the

analysis phase, the “ROI Manager” was accessed by navigating to “Analyze > Tools > ROI Manager”. The PM<sub>x</sub> particles were identified and selected using the Magic Wand or Ellipse tool. Each selected PM<sub>x</sub> was added to the ROI Manager by clicking “Add” (or using the shortcut key “T”). This process was repeated for all visible PM<sub>x</sub> particles, ensuring that the zoom level remained at 200%. Once all PM<sub>x</sub> particles were selected and added to the ROI Manager, the “Measure” button in the ROI Manager window was clicked to generate the measurements. The resulting data were displayed in a Results window. Finally, the data were copied from the Results window and pasted into an Excel file for further analysis.

#### 2.4. The Filtration Methodology [9]

After measuring the leaf area, each leaf sample was agitated and washed for 60 s with 150 mL of deionized water. The washing solution was successively filtered using three filters with specific pore sizes (Sartorius FT-3-104-055 for PM<sub>x</sub> ≤ 10 μm, Sartorius FT-3-354-110 for PM<sub>x</sub> ≤ 2.5 μm, and Sartorius 11807-47-N PTFE membrane for PM<sub>x</sub> ≤ 0.2 μm). Each filter was dried for 30 min at 60 °C, stabilized at a constant relative air humidity (50%) for 60 min, and then pre-weighed. A 47 mm glass filter funnel connected to a vacuum pump was used to perform the filtration (Figure 1C,D). After filtration, filters were dried again, stabilized at a controlled temperature, and weighed again. The concentration of each PM<sub>x</sub> size was expressed in μg cm<sup>-2</sup>.

#### 2.5. Leaf Traits

High-resolution images of three leaves per sample were taken against a white background and processed using ImageJ to measure the following traits: leaf surface area (LA in cm<sup>2</sup>), specific leaf area (SLA in m<sup>2</sup> kg<sup>-1</sup>) calculated as the leaf area (m<sup>2</sup>) per unit leaf dry matter (kg<sup>-1</sup>), leaf dissection index (LDI = leaf perimeter/√leaf area), and leaf roundness (leaf roundness = (4 × leaf area)/[π × (major axis)]). Leaf mass per area (LMA) was determined using five discs (8 mm) per leaf of each sample, dried until constant weight, and used to calculate SLA.

#### 2.6. Statistical Analysis

The correlation between both methodologies was verified using linear regression, including the data from the 30 species studied. To ensure consistency in the comparison of methodologies, pooled leaf samples from each species were used for analysis rather than multiple samples per species, as the study’s primary focus was on evaluating methodological differences rather than inter-species variation.

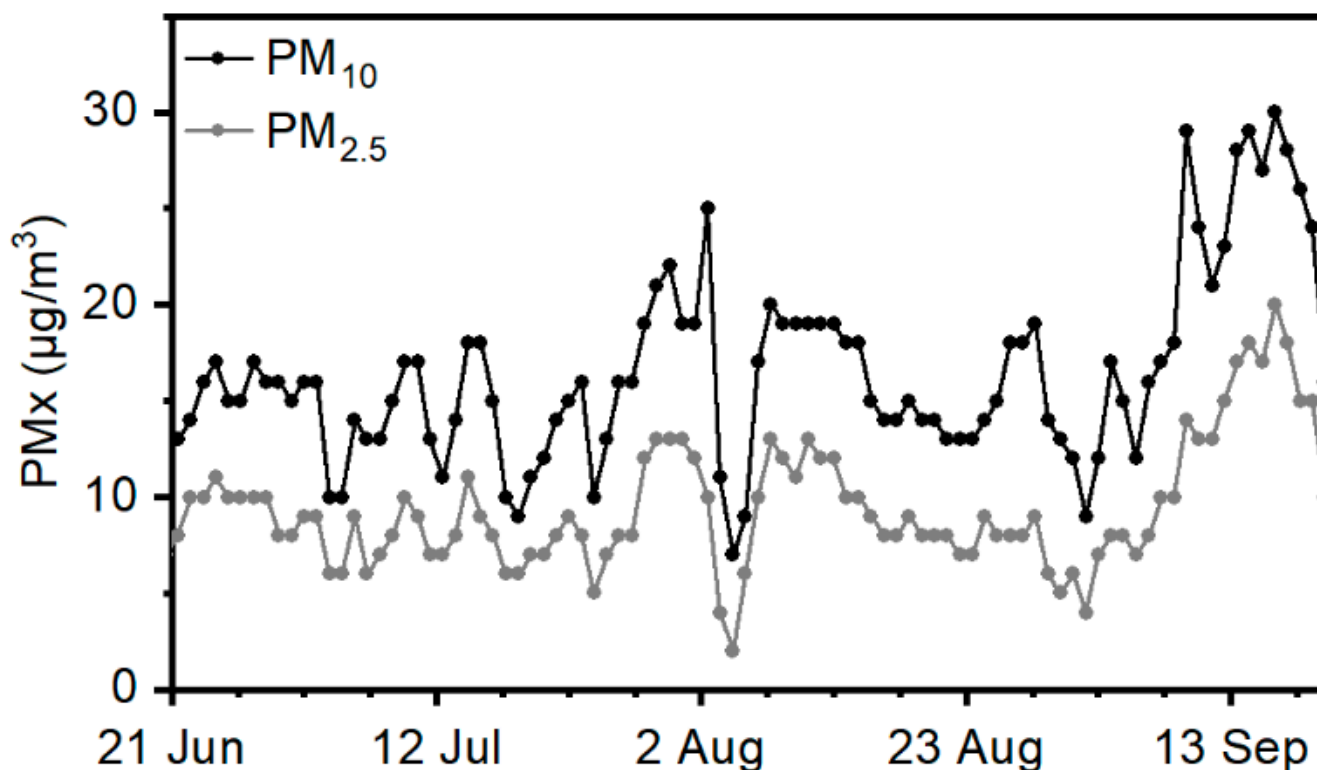
Eight species were selected to perform multiple linear regression (MLR) between the leaf traits and PM<sub>x</sub> accumulation factors to infer the contribution of leaf traits to the species’ capacity to accumulate PM<sub>x</sub>. These species were chosen because they were collected on the same day and in the same city zone (UT), ensuring exposure to similar levels of air pollution, which is necessary for predicting the plant species’ net particle accumulation ability [14]. The statistical analysis was performed using Origin (Pro), version 2023b (Origin Lab Corporation, Northampton, MA, USA).

### 3. Results

Figure 2 presents the values of PM<sub>10</sub> and PM<sub>2.5</sub> in the study region during the summer of 2020. The PM<sub>10</sub> reference limit of 50 μg/m<sup>3</sup> was not exceeded, and the PM<sub>2.5</sub> levels were considered low for the period.

Out of the 30 species collected, 18 had more than 1% of their leaf area covered with PM<sub>x</sub> and were selected for the filtration methodology (Figure 3B). The filtration method was applied to quantify the amount of PM<sub>x</sub> in the leaves of the 16 selected species: *E. japonicus*, *L. lucidum*, *A. glutinosa*, *R. ulmifolius*, *L. nobilis*, *O. europaea*, *T. x europaea*, *A. saccharinum*, *A. campestre*, *T. cordata*, *P. laurocerasus*, *P. nigra*, *S. cinerea*, *P. serrulata*, *Q. ilex*, and *R. chinensis*. Two species (*Viburnum* sp. and *U. minor*) presented unrealistic data (total PM<sub>x</sub> > 100 μg cm<sup>-2</sup>) and were excluded from the results. For *Viburnum* sp., the leaves were

sampled from an individual located very close to a high-traffic road, possibly leading to dust contamination and analytical errors. The *U. minor* samples were contaminated by a fungus, likely causing quantification errors.



**Figure 2.** Particulate matters  $PM_x$  in the study region for the summer season of 2020. Data are from ARPAT (Agenzia Regionale per la Protezione Ambientale della Toscana).

The 14 species not selected were *A. negundo*, *S. cinerea*, *A. campestre*, *G. biloba*, *P. domestica*, *I. walleriana*, *F. viridissima*, *N. oleander*, *S. japonicum*, *C. australis*, *G. procumbens*, *P. alba*, *U. glabra*, and *L. latifolia*.

The results of the filtration are presented in Figure 3A. The species with the highest total  $PM_x$  accumulation ( $>50 \mu\text{g cm}^{-2}$ ) were *E. japonicus*, *L. lucidum*, *A. glutinosa*, *R. ulmifolius*, and *L. nobilis*. Among these, *E. japonicus*, *A. glutinosa*, and *L. lucidum* also showed high values of  $PM_x > 10 \mu\text{m}$  ( $>50 \mu\text{g cm}^{-2}$ ). The highest accumulators for  $PM_x$  accumulation are between 2.5 and  $10 \mu\text{m}$  ( $>10 \mu\text{g cm}^{-2}$ ). The species with the highest  $PM_x$  accumulation between 2.0 and  $2.5 \mu\text{m}$  ( $>10 \mu\text{g cm}^{-2}$ ) were *E. japonicus*, *R. ulmifolius*, and *L. lucidum*.

The linear regression between the two analyses presented a high coefficient of determination ( $R^2 = 0.72$ ), with statistical significance ( $p > 0.001$ ) and linearity confirmed by a homogeneous distribution of the predicted and residual values (Figure 4).

Representative values of leaf traits for each species are presented in Table 2 (Part 1). The MLR analysis confirmed that a combination of leaf traits influenced  $PM_x$  accumulation (Table 2, Part 2). The percentage of  $PM_x$  was positively associated with leaf roundness but showed a negative relationship with both LA and SLA. Total  $PM_x$  exhibited a negative correlation with SLA, while  $PM_{10}$  did not correlate with any leaf trait.  $PM_{2.5}$  was positively associated with LDI and negatively with LA and SLA, whereas  $PM_{0.2}$  was negatively correlated with LA.

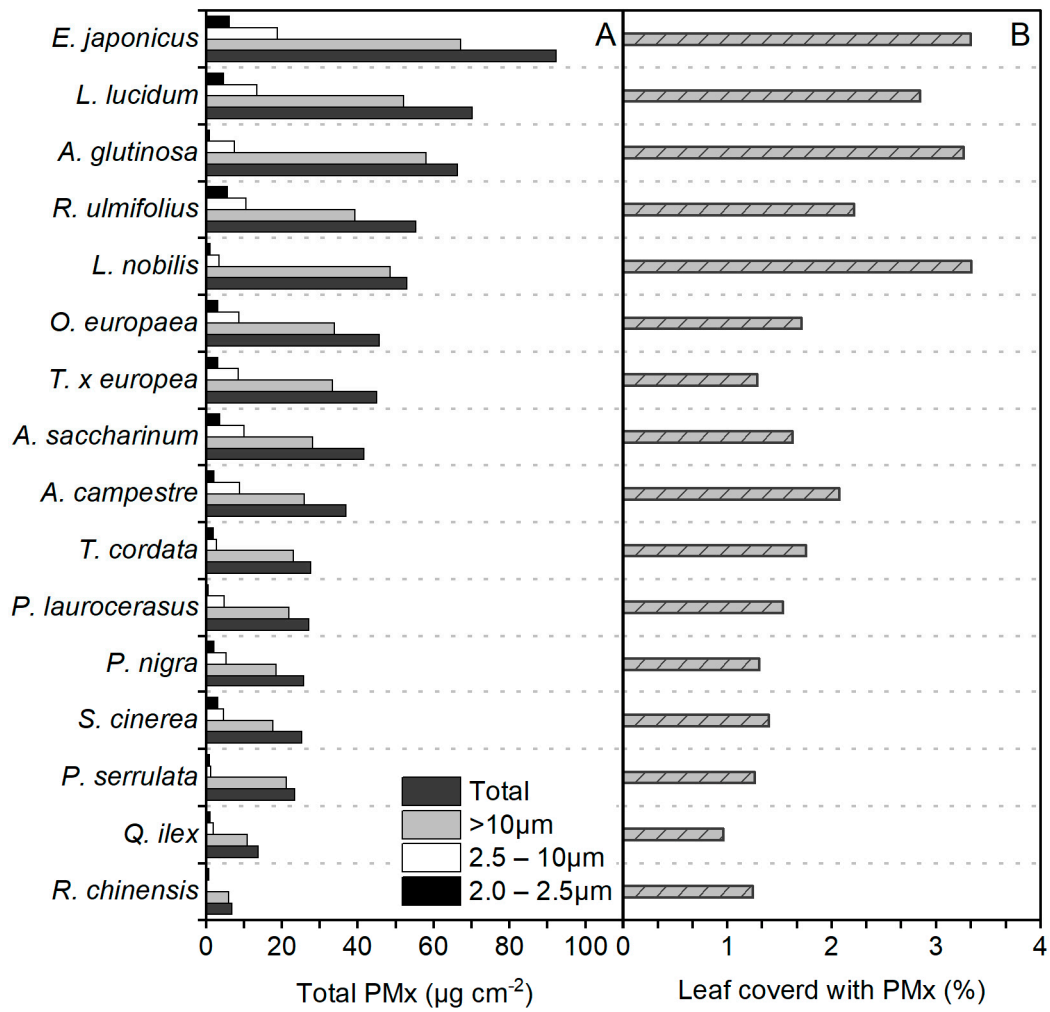


Figure 3. (A) The results of the filtration methods (total PM<sub>x</sub>) divided by the particle size and (B) leaf cover with PM<sub>x</sub> observed by light microscopical analysis.

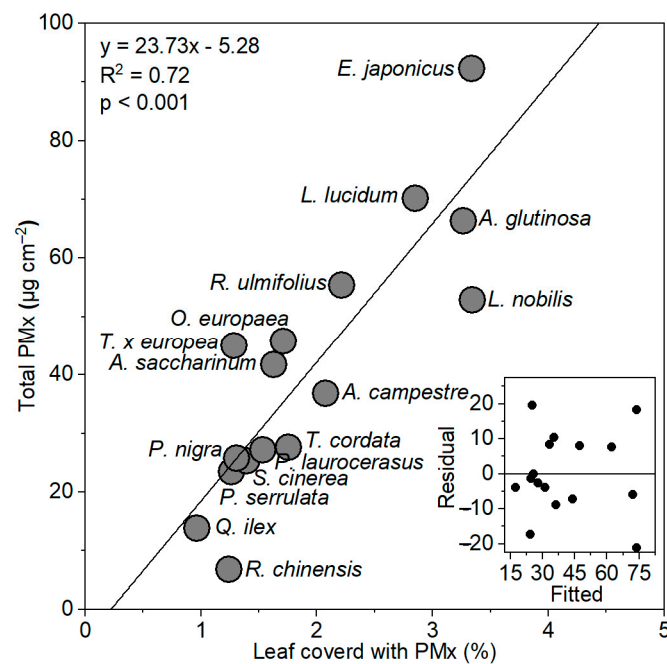


Figure 4. Regression analysis between filtration and light microscopy methodologies.

**Table 2.** Part 1. Leaf traits of the species analyzed: leaf area (LA cm<sup>2</sup>), specific leaf area (SLA m<sup>2</sup> kg<sup>-1</sup>), leaf dissection index (LDI, dimensionless), and leaf roundness (dimensionless). Species highlighted in bold are those selected for the multiple regression analysis. Part 2. Multiple linear regression (MLR) on total PM<sub>x</sub> (μg cm<sup>-2</sup>), leaf covered with PM<sub>x</sub> (%), PM<sub>10</sub> (μg cm<sup>-2</sup>), PM<sub>2.5</sub> (μg cm<sup>-2</sup>), and PM<sub>0.2</sub> (μg cm<sup>-2</sup>) indicating the effect of leaf traits: LA, SLA, LDI, and leaf roundness. Determination coefficient (R<sup>2</sup>); statistical significance (*p*-level); positive relationship (+); negative relationship (-); and variable not included in the linear model (ni). Significant effects (*p*-value < 0.1) are shown in bold.

Part 1	Species	LA	SLA	LDI	Leaf Roundness
	<i>E. japonicus</i>	<b>5.59</b>	<b>7.49</b>	<b>6.35</b>	<b>0.62</b>
	<i>L. lucidum</i>	<b>27.57</b>	<b>6.49</b>	<b>5.00</b>	<b>0.43</b>
	<i>A. glutinosa</i>	<b>54.81</b>	<b>14.03</b>	<b>5.53</b>	<b>1.00</b>
	<i>R. ulmifolius</i>	<b>6.37</b>	<b>9.29</b>	<b>4.97</b>	<b>0.39</b>
	<i>L. nobilis</i>	19.27	8.17	5.16	0.29
	<i>O. europaea</i>	5.19	7.15	6.60	0.17
	<i>Tilia x europaea</i>	<b>47.39</b>	<b>18.69</b>	<b>6.65</b>	<b>0.83</b>
	<i>A. saccharinum</i>	46.63	12.65	12.29	0.50
	<i>A. campestre</i>	<b>32.85</b>	<b>14.22</b>	<b>5.79</b>	<b>0.68</b>
	<i>T. cordata</i>	<b>75.56</b>	<b>15.95</b>	<b>6.13</b>	<b>0.99</b>
	<i>P. laurocerasus</i>	<b>85.48</b>	<b>8.52</b>	<b>5.09</b>	<b>0.36</b>
	<i>P. nigra</i>	39.02	12.08	7.29	0.77
	<i>S. cinerea</i>	33.44	9.72	6.04	0.45
	<i>P. serrulata</i>	29.42	8.17	4.85	0.56
	<i>Q. ilex</i>	12.11	9.57	5.42	0.41
	<i>R. chinensis</i>	8.21	21.20	4.71	0.60
Part 2		Variable	Correlation	R <sup>2</sup>	<i>p</i> -Level
	% PM <sub>x</sub>	LA	-	0.26	0.072
		SLA	-	0.69	0.022
		LDI	ni	0.52	0.422
		Leaf roundness	+	0.66	0.016
	Total PM <sub>x</sub>	LA	ni	0.26	0.104
		SLA	-	0.69	0.093
		LDI	ni	0.52	0.551
		Leaf roundness	ni	0.66	0.152
	PM <sub>10</sub>	LA	ni	0.26	0.188
		SLA	ni	0.69	0.112
		LDI	ni	0.52	0.809
		Leaf roundness	ni	0.66	0.121
	PM <sub>2.5</sub>	LA	-	0.26	0.034
		SLA	-	0.69	0.072
		LDI	+	0.52	0.099
		Leaf roundness	ni	0.66	0.745
	PM <sub>0.2</sub>	LA	-	0.26	0.054
		SLA	ni	0.69	0.541
		LDI	ni	0.52	0.347
		Leaf roundness	ni	0.66	0.655

#### 4. Discussion

The PM<sub>x</sub> levels in the sampling region during the summer of 2020 remained within the reference limits. This can be attributed to the expansion of the atmospheric boundary layer, which promotes aerosol dispersion [17]. Gualtieri et al. [19] also note that during summer, the accumulation of PM<sub>10</sub> is more influenced by the high variability of meteorological conditions than by anthropogenic emissions. Moreover, there is a significant decrease in combustion processes related to agricultural biomass and domestic heating during this season.

Light microscopy proved effective for visualizing PM<sub>x</sub> deposition on leaves. Although the procedure is simple and inexpensive, it is time-consuming. Its results are comparable to



those obtained by scanning electron microscopy (SEM), which is more complex, costly, and equally time-consuming, with similar values of leaf coverage (not exceeding 4%) reported by both methods [20].

The values obtained using the filtration methodology were consistent with the literature [13,21,22]. The high total  $PM_x$  values in this study may be attributed to the method being performed without prior filtration through a sieve to eliminate particles larger than 100  $\mu m$ . However, this approach might restrict the possibility of having reliable results regarding the accumulation of  $PM_{10}$ ; the decision to follow this method was made to ensure comparability between the methodologies.

The linear regression analysis confirmed that both methodologies (light microscopy and filtration) are comparable when leaves have more than 1% of their surface covered with  $PM_x$ . However, based on our results, we hypothesize that the regression might not be significant for leaves with less than 1% coverage. Light microscopy can underestimate values, as visualization might bias particle size identification if samples appear to have minimal  $PM_x$ .

Both methodologies have their pros and cons. Light microscopy may be inaccurate for species with trichomes obstructing visualization [21]. However, it is advantageous for identifying potential analytical errors, such as pathogen contamination, and for screening samples to select leaves with varying  $PM_x$  deposition levels.

The filtration method can overestimate  $PM_x$  accumulation in plant species that release leaf structures like trichomes during vigorous washing. Additionally, particles forming agglomerates might not accurately reflect particle size fractions (Hofman et al., 2014), and water-soluble particles are not accounted for [23]. This method may also lack sensitivity for quantifying  $PM_{0.2}$  due to its low weight; nevertheless, the methodology is fast, low-cost, and practical for processing many samples [21].

The plant community under investigation exhibited a wide range of foliar traits, and the accumulation of  $PM_x$  was negatively correlated with SLA and LA. Plant species with low SLA and high  $PM_x$  accumulation likely have more extended leaf longevity or higher leaf wettability (high wettability = high  $PM_x$  accumulation). SLA has been recommended as a crucial and easy-to-measure leaf trait for distinguishing between species that are low and high net particle accumulators [14]. Moreover, recent research in the same region has shown that species with lower LA [12] and more intricate branch architectures have a greater ability to entrap  $PM_x$  [24]. This finding is further supported by Manzini et al. [25], who reported that a complex shoot and leaf arrangement is a key factor in  $PM_x$  deposition.

Understanding the influence of leaf morphology on  $PM_x$  accumulation is crucial for elucidating species' differential capacities for  $PM_x$  deposition. On one hand, deciduous species with smaller foliar dimensions and more intricate branch architectures might entrap high levels of  $PM_x$  [24]. On the other hand, a recent comprehensive review synthesizing data on tree traits and  $PM_x$  accumulation across diverse species and geographical locations revealed that evergreen conifer needle leaves, as well as small rough leaves, waxy coatings, and high-density trichomes, are potentially beneficial for  $PM_x$  capture [26,27]. However, the study finds no consistent evidence to identify the most influential trait due to the diversity in sampling methods and other factors [27].

Additionally, environmental factors can influence leaf traits [28,29], and growth rate may significantly impact element deposition [20]. Specifically, the density of branches and leaves was found to be the most significant factor affecting  $PM_x$  retention, with negative effects, suggesting that crown morphological structure is more critical than leaf morphology in screening tree species for efficient PM retention [30].

## 5. Conclusions

This study investigated the effectiveness of various plant species in accumulating  $PM_x$  to mitigate urban air pollution. Our findings highlight significant differences in  $PM_x$  accumulation capacities among the studied species, emphasizing the importance of selecting appropriate plant species for urban green spaces to enhance air quality.

Our findings revealed significant disparities in PM<sub>x</sub> capture capabilities among different plant species, underscoring the importance of promoting diverse plant ecosystems within urban landscapes. Among the species analyzed, *Euonymus japonicus*, *Ligustrum lucidum*, *Alnus glutinosa*, *Rubus ulmifolius*, and *Laurus nobilis* demonstrated the highest total PM<sub>x</sub> accumulation, exceeding 50 µg cm<sup>-2</sup>. These species also exhibited high values of PM<sub>x</sub> > 10 µm, indicating their substantial role in capturing larger particulate matter. Specifically, *Euonymus japonicus* and *Ligustrum lucidum* were notable for their efficiency in capturing PM<sub>x</sub> between 2.0 and 2.5 µm. Additionally, the correlation between leaf traits and PM<sub>x</sub> accumulation was shown to be important when selecting plant species for PM<sub>x</sub> deposition.

Based on our comprehensive research framework and findings, we conclude that PM<sub>x</sub> accumulation on leaves is most accurately understood when multiple quantification methods are employed concurrently. Our hypothesis of a high correlation between the two methods was supported, demonstrating that different techniques elucidate various aspects of PM<sub>x</sub> deposition. By comparing data from these methods, researchers can select the most suitable technique based on laboratory capacity and sample size. For instance, filtration methods are particularly effective for large sample sizes, while light microscopy serves as an efficient screening tool for identifying target samples, as demonstrated in our study.

The next step in this research involves a more detailed investigation of the underlying mechanisms that contribute to the high PM<sub>x</sub> accumulation capacity of plant species. Future studies should focus on the physiological and morphological traits that enhance particulate matter capture. Additionally, long-term field studies are necessary to evaluate the effectiveness of these species in different environmental conditions and seasons.

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## References

1. Sawidis, T.; Breuste, J.; Mitrovic, M.; Pavlovic, P.; Tsigaridas, K. Trees as bioindicator of heavy metal pollution in three European cities. *Environ. Pollut.* **2011**, *159*, 3560–3570. [CrossRef] [PubMed]
2. European Environment Agency. 2023. Available online: <https://www.eea.europa.eu/themes/air/country-fact-sheets/2023-country-fact-sheets/italy-air-pollution-country> (accessed on 5 February 2024).
3. Bell, M.L.; Morgenstern, R.D.; Harrington, W. Quantifying the human health benefits of air pollution policies: Review of recent studies and new directions in accountability research. *Environ. Sci. Policy* **2011**, *14*, 357–368. [CrossRef]
4. Suzuki, K. Characterisation of airborne particulates and associated trace metals deposited on tree bark by ICP-OES, ICP-MS, SEM-EDX and laser ablation ICP-MS. *Atmos. Environ.* **2006**, *40*, 2626–2634. [CrossRef]
5. Lunghi, J.; Malpede, M.; Reis, L.A. Exploring the impact of livestock on air quality: A deep dive into Ammonia and particulate matter in Lombardy. *Environ. Impact Assess. Rev.* **2024**, *105*, 107456. [CrossRef]

6. Beckett, K.P.; Freer-Smith, P.H.; Taylor, G. Urban woodlands: Their role in reducing the effects of particulate pollution. *Environ. Pollut.* **1998**, *99*, 347–360. [[CrossRef](#)] [[PubMed](#)]
7. Lin, L.; Yan, J.; Ma, K.; Zhou, W.; Chen, G.; Tang, R.; Zhang, Y. Characterization of particulate matter deposited on urban tree foliage: A landscape analysis approach. *Atmos. Environ.* **2017**, *171*, 59–69. [[CrossRef](#)]
8. Jones, L.; Vieno, M.; Fitch, A.; Carnell, E.; Steadman, C.; Cryle, P.; Holland, M.; Nemitz, E.; Morton, D.; Hall, J.; et al. Urban natural capital accounts: Developing a novel approach to quantify air pollution removal by vegetation. *J. Environ. Econ. Policy* **2019**, *8*, 413–428. [[CrossRef](#)]
9. Mori, J.; Fini, A.; Galimberti, M.; Ginepro, M.; Burchi, G.; Massa, D.; Ferrini, F. Air pollution deposition on a roadside vegetation barrier in a Mediterranean environment: Combined effect of evergreen shrub species and planting density. *Sci. Total Environ.* **2018**, *643*, 725–737. [[CrossRef](#)]
10. Nowak, D.J.; Hirabayashi, S.; Bodine, A.; Greenfield, E. Tree and forest effects on air quality and human health in the United States. *Environ. Pollut.* **2014**, *193*, 119–129. [[CrossRef](#)]
11. Hashad, K.; Gu, J.; Yang, B.; Rong, M.; Chen, E.; Ma, X.; Zhang, K.M. Designing roadside green infrastructure to mitigate traffic-related air pollution using machine learning. *Sci. Total Environ.* **2021**, *773*, 144760. [[CrossRef](#)]
12. Moura, B.B.; Zammarchi, F.; Manzini, J.; Yasutomo, H.; Brilli, L.; Vagnoli, C.; Gioli, B.; Zaldei, A.; Giordano, T.; Martinelli, F.; et al. Assessment of seasonal variations in particulate matter accumulation and elemental composition in urban tree species. *Environ. Res.* **2024**, *252*, 118782. [[CrossRef](#)] [[PubMed](#)]
13. Sæbø, A.; Popek, R.; Nawrot, B.; Hanslin, H.M.; Gawronska, H.; Gawronski, S.W. Plant species differences in particulate matter accumulation on leaf surfaces. *Sci. Total Environ.* **2012**, *427–428*, 347–354. [[CrossRef](#)]
14. Muhammad, S.; Wuyts, K.; Samson, R. Atmospheric net particle accumulation on 96 plant species with contrasting morphological and anatomical leaf characteristics in a typical garden experiment. *Atmos. Environ.* **2019**, *202*, 328–344. [[CrossRef](#)]
15. Popek, R.; Przybysz, A.; Gawrońska, H.; Klamkowski, K.; Gawroński, S.W. Impact of particulate matter accumulation on the photosynthetic apparatus of roadside woody plants growing in the urban conditions. *Ecotoxicol. Environ. Saf.* **2018**, *163*, 56–62. [[CrossRef](#)]
16. Steinparzer, M.; Schaubmayr, J.; Godbold, D.L.; Rewald, B. Particulate matter accumulation by tree foliage is driven by leaf habit types, urbanization-and pollution levels. *Environ. Pollut.* **2023**, *335*, 122289. [[CrossRef](#)]
17. Brilli, L.; Carotenuto, F.; Andreini, B.P.; Cavaliere, A.; Esposito, A.; Gioli, B.; Martelli, F.; Stefanelli, M.; Vagnoli, C.; Venturi, S.; et al. Low-cost air quality stations' capability to integrate reference stations in particulate matter dynamics assessment. *Atmosphere* **2021**, *12*, 1065. [[CrossRef](#)]
18. Gualtieri, G.; Toscano, P.; Crisci, A.; Di Lonardo, S.; Tartaglia, M.; Vagnoli, C.; Zaldei, A.; Gioli, B. Influence of road traffic, residential heating and meteorological conditions on PM10 concentrations during air pollution critical episodes. *Environ. Sci. Pollut. Res.* **2015**, *22*, 19027–19038. [[CrossRef](#)] [[PubMed](#)]
19. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671–675. [[CrossRef](#)]
20. Mori, J.; Sæbø, A.; Hanslin, H.M.; Teani, A.; Ferrini, F.; Fini, A.; Burchi, G. Deposition of traffic-related air pollutants on leaves of six evergreen shrub species during a Mediterranean summer season. *Urban For. Urban Green.* **2015**, *14*, 264–273. [[CrossRef](#)]
21. Blanuša, T.; Qadir, Z.J.; Kaur, A.; Hadley, J.; Gush, M.B. Evaluating the effectiveness of urban hedges as air pollution barriers: Importance of sampling method, species characteristics and site location. *Environments* **2020**, *7*, 81. [[CrossRef](#)]
22. Przybysz, A.; Popek, R.; Stankiewicz-Kosyl, M.; Zhu, C.Y.; Małecka-Przybysz, M.; Maulidyawati, T.; Mikowska, K.; Deluga, D.; Grizuk, K.; Sokalski-Wieczorek, J.; et al. Where trees cannot grow—Particulate matter accumulation by urban meadows. *Sci. Total Environ.* **2021**, *785*, 147310. [[CrossRef](#)]
23. Beckett, K.P.; Freer-Smith, P.H.; Taylor, G. Particulate pollution capture by urban trees: Effect of species and windspeed. *Glob. Change Biol.* **2000**, *6*, 995–1003. [[CrossRef](#)]
24. Freer-Smith, P.H.; Beckett, K.P.; Taylor, G. Deposition velocities to *Sorbus aria*, *Acer campestre*, *Populus deltoides* × *trichocarpa* “Beaupré”, *Pinus nigra* and × *Cupressocyparis leylandii* for coarse, fine and ultra-fine particles in the urban environment. *Environ. Pollut.* **2005**, *133*, 157–167. [[CrossRef](#)]
25. Manzini, J.; Hoshika, Y.; Carrari, E.; Sicard, P.; Watanabe, M.; Tanaka, R.; Badea, O.; Nicese, F.P.; Ferrini, F.; Paoletti, E. FlorTree: A unifying modelling framework for estimating the species-specific pollution removal by individual trees and shrubs. *Urban For. Urban Green.* **2023**, *85*, 127967. [[CrossRef](#)]
26. Prigioniero, A.; Postiglione, A.; Zuzolo, D.; Niinemets, Ü.; Tartaglia, M.; Scarano, P.; Mercurio, M.; Germinario, C.; Izzo, F.; Trifuoggi, M.; et al. Leaf surface functional traits influence particulate matter and polycyclic aromatic hydrocarbons air pollution mitigation: Insights from Mediterranean urban forests. *J. Clean. Prod.* **2023**, *418*, 138158. [[CrossRef](#)]
27. Kwak, M.J.; Lee, J.K.; Park, S.; Kim, H.; Lim, Y.J.; Lee, K.A.; Son, J.-A.; Oh, C.-Y.; Kim, I.; Woo, S.Y. Surface-based analysis of leaf microstructures for adsorbing and retaining capability of airborne particulate matter in ten woody species. *Forests* **2020**, *11*, 946. [[CrossRef](#)]
28. Wuytack, T.; Wuyts, K.; Van Dongen, S.; Baeten, L.; Kardel, F.; Verheyen, K.; Samson, R. The effect of air pollution and other environmental stressors on leaf fluctuating asymmetry and specific leaf area of *Salix alba* L. *Environ. Pollut.* **2011**, *159*, 2405–2411. [[CrossRef](#)]

- 
29. Moura, B.B.; Alves, E.S. Climatic factors influence leaf structure and thereby affect the ozone sensitivity of *Ipomoea nil* “Scarlet O’Hara”. *Environ. Pollut.* **2014**, *194*, 11–16. [[CrossRef](#)]
  30. Xie, C.; Guo, J.; Yan, L.; Jiang, R.; Liang, A.; Che, S. The influence of plant morphological structure characteristics on PM2.5 retention of leaves under different wind speeds. *Urban For. Urban Green.* **2022**, *71*, 127556. [[CrossRef](#)]

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