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TITLE:

Assessing the Particulate Matter Removal Abilities of Tree Leaves

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KEYWORDS:

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SUMMARY:

The ultrasonic cleaning method was applied to elute the particulate matter (PM) retained on leaf surfaces after PM was eluted by the conventional cleaning methods (water cleaning only or water cleaning plus brush cleaning). The methodology can help to improve the estimation accuracy for PM retention capacity of leaves.

LONG ABSTRACT:

Based on the conventional cleaning methods (water cleaning (WC) + brush cleaning (BC)), this study evaluated the influence of ultrasonic cleaning (UC) on collecting various sized particulate matter (PM) retained on leaf surfaces. We further characterized the retention efficiency of leaves to various sized PM, which will help to assess the abilities of urban trees to remove PM from ambient air quantitatively.

Taking three broadleaf tree species (*Ginkgo biloba*, *Sophora japonica,* and *Salix babylonica*) and two needleleaf tree species (*Pinus tabuliformis* and *Sabina chinensis*) as the research objects, leaf samples were collected 4 days (short PM retention period) and 14 days (long PM retention period) after the latest rainfall. PM retained on the leaf surfaces was collected by means of WC, BC, and UC in sequence. Then, retention efficiencies of leaves (AE_{leaf}) to three types of the various sized PM, including easily removable PM (ERP), difficult-to-remove PM (DRP), and totally removable PM (TRP), were calculated. Only around 23%-45% of the total PM retained on leaves could be cleaned off and collected by WC. When the leaves were cleaned through

WC+BC, the underestimation of the PM retention capacity of different tree species was in the range of 29%-46% for various sized PM. Almost all PM retained on leaves could be removed if UC was supplemented to WC+BC.

In conclusion, if the UC was complemented after the conventional cleaning methods, more PM on leaf surfaces could be eluted and collected. The procedure developed in this study can be used for assessing the PM removal abilities of different tree species.

INTRODUCTION:

The abilities of different tree species to remove PM from ambient air can be assessed through quantifying the mass of PM retained on leaf surfaces. To achieve this objective, the subtraction method[1-2], the membrane filter method[3-5], and the elution-weighing method coupled with particle size analysis[6] have been applied to quantitatively estimate the mass of $PM_{2.5}$ (diameter ≤ 2.5 µm), PM_{10} (diameter ≤ 10 µm) or total suspended particulate (TSP) retained on leaves. However, the accuracy of these methods basically depends on their performance in collecting PM retained on the leaf surfaces. At present, the conventional leaf cleaning method used in related studies often includes one or two steps, namely only water washing (soak and rinse leaves using deionized water)[3,7] or plus brushing[5,8-9]. However, some studies[10-11] have demonstrated that PM on leaf surfaces could not be completely eluted by the conventional cleaning method. As ultrasonic cleaning has the advantages of high speed, high quality, and little damage to the surface of the object, it has great potential to be used to collect the PM retained on leaf surfaces with complex microstructures. At present, ultrasonic cleaning has been applied in some studies to collect PM retained on leaf surfaces (*i.e.*, put the leaves into deionized water, and use the ultrasonic cleaner to elute PM)[12-13]. However, this method is only used as a supplement to a leaf cleaning method, while it is not known whether the ultrasonic cleaning has a positive effect on collecting PM from leaf surfaces and its optimum operating parameters are also not clear. Our previous research has shown that the PM retained on *Ginkgo biloba* leaf surface could be completely eluted without destroying the leaf surfaces, if a proper ultrasonic cleaning procedure was supplemented to the conventional cleaning method[11]. However, the stability and general applicability of the ultrasonic cleaning parameters (ultrasonic power, time, and other information) to different plant species experiencing different dust retention periods are still not clear.

Currently, the mass of $PM_{2.5}$, PM_{10}, or TSP on unit leaf area has often been utilized to evaluate the abilities of different tree species to remove PM from ambient air[14-15]. Under the natural condition, the PM retained on leaf surfaces can be classified into two parts: the first part is the PM that can fall off leaves due to the effects of wind and rainfall, while the other part is the PM that is tightly adhered to leaf surfaces and cannot be easily washed off by rainfall. However, few studies have focused on the mass of both types of PM on leaf surfaces. In addition, the PM retention periods of leaves in different studies differ enormously. Thus, the comparability of the results of these studies will be poor, if the mass of PM retained on unit leaf area is adopted to assess the PM removal abilities of trees[16]. Consequently, the PM retention efficiency (the mass of PM retained on unit leaf area per unit time), as an alternative, was proposed to evaluate the PM purification effects of urban trees[5,17]. In general, there is still a lack of research in this aspect. It is extremely necessary to carry out relevant studies for different tree species to provide methodological basic and data support for assessing the PM removal abilities of different tree species accurately.

Here, three broadleaf tree species (*G. biloba*, *Sophora japonica*, and *Salix babylonica*) and two needleleaf tree species (*Pinus tabuliformis* and *Sabina chinensis*) were selected to evaluate their PM removal abilities under two PM retention periods. The leaf sampling site was in Xitucheng Park (39.97° N, 116.36° E), located in an area with heavy pollution in Beijing. The three specific objectives of this study were: (1) to assess the efficiency of different leaf cleaning methods (water cleaning (WC), brush cleaning (BC), and ultrasonic cleaning (UC)) in eluting the PM on leaves, (2) to verify the effect of ultrasonic cleaning on eluting PM, and (3) to assess the retention efficiency of different tree species to PM_1, $PM_{2.5}$, PM_5, PM_{10}, and TSP.

PROTOCOL:

1. Leaf Collection, Elution and Mass Measurement of PM

1.1. Select five healthy individual trees (*i.e.,* five replicates) of each tree species with similar diameter at breast height. Collect four larger branches randomly from four directions of the outer canopy in the middle canopy layer and cut off all intact leaves.

Note: All plants for leaf sampling should be located closely in a greening strip with length and width of about 250 and 60 m, respectively, to ensure that the environment conditions (wind, light, and rain) of these trees are similar. The leaves used in the protocol were collected on October 15th (short dust retention (SDR) period) and October 25th (long dust retention (LDR) period) in 2014, which were 4 and 14 days after the latest rainfall (> 15 mm), respectively. The average levels of PM under the short and long dust retention period (*i.e.,* the duration between the last rainfall and the leaf sampling time) in our experiment were 26 ($PM_{2.5}$), 57 (PM_{10}), and 111 ($PM_{2.5}$), 160 μg/m^3 (PM_{10}), respectively.

1.1.1. Place the sampled leaves in labeled valve bags and transport the bags to the laboratory immediately. Store the leaf samples in the fridge.

1.2. Wash and dry the beakers in the 80 °C oven. Equilibrate the beakers to room temperature and humidity and weigh the empty beakers (W_1).

1.3. Randomly select a certain amount of leaves from leaf samples and put the leaves in a 1000 mL beaker (Beaker A).

Note: The leaf area is about 2000 cm^2, which can guarantee all the leaves can be immersed in the water completely and the eluted dust has sufficient weight to be weighed accurately.

1.4. Add 270 mL of deionized water to the Beaker A and immerse the leaves in water completely.

1.4.1. Stir the water for 60 s with a glass rod in one direction (frequency: 2 seconds for one rotation). Afterwards, pour the eluent into three 100 mL small beakers (Beaker a) evenly.

1.4.2. Wash the leaves using a fine tipped squeeze bottle with 30 mL of deionized water and transfer the washed leaves to a 1000 mL beaker (Beaker B). Pour the eluent into three 100 mL small beaker (Beaker a) evenly.

1.5. Add 270 mL of deionized water to Beaker B and immerse the leaves in water again. Then use a nylon brush to scrub the leaf surface (placing on flat thin plastic plate) with deionized water and avoid destroying the microstructure of leaf surface. Pour the eluent into three 100 mL small beakers (Beaker b).

1.5.1. Wash the leaves using the squeezable bottle with fine tip with 30 mL of deionized water and transfer the leaves into a 1000 mL beaker (Beaker C). Pour the eluent into three 100 mL small beaker (Beaker b).

1.6. Add 270 mL of deionized water to Beaker C and immerse the leaves in water again.

1.6.1. Put the glass container into the ultrasonic cleaning machine. Using an ultrasonic power of 500 W, clean for 3 min and 10 min for leaves of the broadleaf and needleleaf tree species, respectively. Stir the leaves with a glass rod in one direction (frequency: 2 seconds for one circle) simultaneously.

1.6.2. Wash the leaves using the squeezable bottle with fine tip with 30 mL of deionized water and pour the eluent into three 100 mL small beakers (beaker c).

1.7. Cover a piece of clean filter paper (diameter = 11 cm, area = 94.99 cm^2) on each beaker (a, b, c) and dry the beakers in the 80 °C oven for approximately 5 days until the mass of the beakers becomes constant.

1.7.1. Put the beakers in a balance chamber to equilibrate the temperature and humidity for 30 min, and weigh the mass of each 100 mL beakers (W_2). Calculate the mass of PM eluted by each cleaning step by W_2-W_1.

2. Measurement of PM Size Distribution and Leaf Area

2.1. Add 50 mL of deionized water to each weighed beaker (a, b, c) mentioned above and place these beakers in an ultrasonic cleaning machine for 30 min until the PM disperses in deionized water.

2.2. Add the supernatant in beaker (a, b, c) to the laser granularity instrument and measure the size distribution of PM eluted by different cleaning steps.

2.2.1. Assume the measured volume percentages to be mass percentages (Q) of different-sized particles. Calculate the proportion of different-sized particles eluted by each cleaning step by equation (1):

$$P_{i,j} = \frac{W_i \cdot Q_{i,j}}{\sum_i W_i \cdot Q_{i,j}} \quad (1)$$

where $P_{i,j}$ represents the mass proportion (%) of the particles within the *j* diameter class eluted from the leaf surfaces by the cleaning step *i*; W_i represents the total mass (g) of all sized particles eluted by the cleaning step *i*; $Q_{i,j}$ represents the mass percentage (%) of the particles within the *j* diameter class in the total PM mass eluted by the cleaning step *i*; *i* is the cleaning step (*i.e.,* WC, BC, and UC); and *j* is the diameter class, which was set to d ≤ 1 µm (PM_1), 1 < d ≤ 2.5 µm ($PM_{1-2.5}$), 2.5 < d ≤ 5 µm ($PM_{2.5-5}$), 5 < d ≤ 10 µm (PM_{5-10}), d > 10 µm ($PM_{>10}$) in the present study.

2.3. Spread leaves on the plastic board and scan the leaves with a high-quality scanner. Use automatic image analysis software to estimate the surface area and projected area of leaves.

Note: The protocol can be paused here.

3. Data Presentation and Analysis

3.1. Calculate the total removable particulate matter (TRP) as the sum of the ERP and DRP that can be eluted by WC + BC + UC.

3.2. Under different dust retention periods, calculate the total mass of the PM within a specific diameter class retained on leaves as the sum of the mass of the PM within corresponding diameter class eluted by the different cleaning steps (*i.e.,* WC, BC, and UC).

3.2.1. Using these data and the leaf area data, calculate the retention efficiency (AE_{leaf}) of the various sized particles on unit leaf surface area using equation (2):

$$AE_{leaf} = \frac{LZ_j - SZ_j}{LT - ST} \quad (2)$$

where LZ_j and SZ_j are the mass (g) of the particles within the *j* diameter class retained on unit leaf area under the periods of LDR and SDR, respectively; *LT* and *ST* are the numbers of days in the periods of LDR and SDR, respectively.

3.3. Conduct all the statistical analyses with SPSS software.

3.3.1. Use the Kolmogorov-Smirnov test and the Levene test to verify the ANOVA assumptions of normality and the homogeneity of variances, respectively, for the elution percentages of the different-sized particles and the PM retention capacity data.

3.3.2 Apply one-way ANOVA to investigate the effects of the different cleaning steps on the elution percentages of the different-sized particles under various dust retention periods. Use Duncan's test ($P = 0.05$) to detect the significant differences among different cleaning steps.

REPRESENTATIVE RESULTS:

The PM retained on leaf surfaces had two types under natural conditions. The PM falls off easily by rainfall and wind under natural conditions is defined as the easily removable particulate matter (ERP). This type of PM was represented by the PM eluted by WC in this study. The PM that tightly adheres to leaf surfaces and cannot be easily washed off by BC and UC is defined as the difficult-to-remove particulate matter (DRP). This kind of PM cannot be eluted by natural rainfall and wind.

There was a significant difference among the five tree species in the mass proportions of various-sized PM eluted by different cleaning steps. The result showed that a large number of different sized PM were eluted from leaf surface by WC (**Figures 1-5**). The average eluted proportions (ERP) of various-sized PM of the five tree species were 31% and 35% under the SDR and LDR, respectively (**Figure 6**).

In addition, the WC showed a stronger effect on eluting PM on leaves of needleleaf tree species (*P. tabuliformis* and *S. chinensis*), especially for *S. chinensis* under the LDR period. As such, the elution percentage of WC was significantly higher than that of BC and UC ($P < 0.05$) for all sized PM except $PM_{>10}$. After cleaning the leaves by BC, large fractions of various sized PM were also eluted, which were 28% and 29% under the SDR and LDR periods, respectively. Similar to WC, the most distinct elution effect of BC was observed for *S. japonica*. The elution percentage of BC was significantly higher than that of WC ($P < 0.05$) for all fractions of PM under both the SDR and LDR periods. Moreover, the elution effect of BC was significantly higher than that of UC ($P < 0.05$) except PM with diameter < 5 µm (**Figure 6**). Although a large fraction of PM could be eluted from leaf surface by WC + BC, some PM with smaller diameters still adhered on leaf surfaces. Subsequently, when UC was applied to clean leaves, the residual PM retained on leaf surfaces were eluted completely (**Figures 1-5**), and the elution proportions were 41% under SDR and 36% under LDR (**Figure 6**). In addition, the elution percentage of small sized PM was higher when the UC was applied. Consequently, the mass of PM would be underestimated apparently, if the conventional elution method was only adopted to elute the PM on leaves. Especially for *S. babylonica*, the average eluted proportions of PM in all diameter classes would be underestimated by 46%, which was higher than that for *P. tabuliformis* (43%), *G. biloba* (42%), *S. japonica* (31%), and S. *chinensis* (29%).

The AE_{leaf} of different types of PM of the five tree species is shown in **Table 1**. There was a great difference in the retention efficiency calculated by two different methods. Compared with the result estimated by equation (2), the AE_{leaf} calculated by equation (3): retention efficiency ($mg/m^2 \cdot d^{-1}$) = the mass of the PM on a unit of leaf area (mg/m^2) / the dust retention duration (d) was about 5 times higher. Especially for *S. japonica*, the PM_1 of ERP calculated by equation (3) was 18.94 times higher than that calculated by equation (2). In this study, for ERP, the TSP AE_{leaf} of different tree species varied between 12.69 and 34.69 $mg \cdot m^{-2} \cdot d^{-1}$ and decreased in the following order: *P. tabuliformis* > *S. babylonica* > *G. biloba* > *S. japonica* > *S. chinensis*. While in a previous study, the TSP AE_{leaf} of different tree species varied between 35.27 and 85.79 mg·m^{-2}·d^{-1} and decreased in the following order: *S. japonica* > *S. chinensis* > *S. babylonica* > *P. tabuliformis* > *G. biloba*. The retention efficiency of different tree species in retaining various sized PM of different types (ERP, DRP, TRP) could also vary. In this study, *S. japonica* exhibited the highest AE_{leaf} in retaining the TRP of PM_1 and $PM_{2.5}$, which were 4.3 and 21.91 mg/m^2·d^{-1}, respectively. *S. babylonica* had the highest AE_{leaf} in retaining the TRP of PM_5 (40.98 mg/m^2·d^{-1}) and PM_{10} (62.01 mg/m^2·d^{-1}). In addition, *S. chinensis* could retain more ERP of PM_1, $PM_{2.5}$, and PM_5 than the other tree species.

FIGURE AND TABLE LEGENDS:

Figure 1: The particulate matter residue on leaves of *Ginkgo biloba* after different elution steps. A and **B** stand for the upper and lower sides of leaves of *Ginkgo biloba*. Different numbers stand for different elution steps. (1: without cleaning; 2: single water cleaning; 3: water cleaning + brush cleaning; 4. water cleaning + brush cleaning + ultrasonic cleaning).

Figure 2: The particulate matter residue on leaves of *Pinus tabuliformis* after different elution steps. A and **B** stand for the concave and convex sides of leaves of *Pinus tabuliformis*. Different numbers stand for different elution steps. (1: without cleaning; 2: single water cleaning; 3: water cleaning + brush cleaning; 4. water cleaning + brush cleaning + ultrasonic cleaning).

Figure 3: The particulate matter residue on leaves of *Sophora japonica* after different elution steps. A and **B** stand for the upper and lower sides of leaves of *Sophora japonica*. Different numbers stand for different elution steps. (1: without cleaning; 2: single water cleaning; 3: water cleaning + brush cleaning; 4. water cleaning + brush cleaning + ultrasonic cleaning).

Figure 4: The particulate matter residue on leaves of *Salix babylonica* after different elution steps. A and **B** stand for the upper and lower sides of leaves of *Salix babylonica*. Different numbers stand for different elution steps. (1: without cleaning; 2: single water cleaning; 3: water cleaning + brush cleaning; 4. water cleaning + brush cleaning + ultrasonic cleaning).

Figure 5: The particulate matter residue on leaves of *Sabina chinensis* after different elution steps. A and **B** stand for the conical and scale-form leaves of *Sabina chinensis*. Different numbers stand for different elution steps. (1: without cleaning; 2: single water cleaning; 3: water cleaning + brush cleaning; 4. water cleaning + brush cleaning + ultrasonic cleaning).

Figure 6: **The mass proportions of the various sized particulate matter retained on the leaves of different tree species. A** and **B** stand for short (SDR) and long (LDR) dust retention periods, respectively. WC, BC, and UC stand for the single water cleaning, brush cleaning, and ultrasonic cleaning, respectively. Data are mean ± SE. Different letters (a, b, c) above the data bar indicate significant difference ($P < 0.05$) among the different cleaning steps on the elution percentages of the different-sized particles under different dust retention periods, according to the Duncan test.

Table 1: The mass of different types of PM retained on unit leaf area. ERP, DRP, and TR stand for the easily removable dust retention capacity, the difficult-to-remove dust retention capacity, and the total dust retention capacity, respectively. Equation (2): retention efficiency ($mg/m^2 \cdot d^{-1}$) = the subtract mass of the PM on unit leaf area under the LDR and SDR periods (mg/m^2) / the dust retention duration between the LDR and SDR periods (d); Equation (3): retention efficiency ($mg/m^2 \cdot d^{-1}$) = the mass of the PM on a unit of leaf area (mg/m^2) / the dust retention duration (d).

DISCUSSION:

Accurate and proper collection of the PM retained on leaf surfaces is the basis for assessing the PM removal abilities of different tree species. However, the conventional cleaning method (WC or plus BC) cannot completely remove the dust on leaf surfaces, which has been confirmed by scanning electron microscopy[10]. This was further demonstrated clearly by the present study (**Figures 1-5**). Our study shows that, if only WC was applied to clean leaves, the PM on leaf surface would be underestimated by about 69% and 65% under the SDR and LDR periods, respectively. That is to say, a short period of rainfall with a certain intensity could only elute 31% and 35% of PM from leaf surfaces. In addition, previous studies have shown that a short and heavy rainfall could only elute 50% and 62% of the PM from the leaves of *Ligustrum lucidum* and *Viburnum odoratissimum*, respectively[18]. However, influence of rainfall on eluting the PM retained on leaf surfaces of *P. tabuliformis* was not evident. Consequently, under natural conditions, only a small part of PM on leaf surface could be eluted by rainfall. When both WC and BC were applied to clean leaves, the elution percentage of PM would also be underestimated by about 41% and 36% under the SDR and LDR periods, respectively. However, more PM could be eluted and collected from leaf surface after supplementing UC to WC+BC for cleaning leaves. Thus, for unbiased and accurate quantification of PM retained by leaves, it is necessary and crucial to add UC to the conventional leaf cleaning method.

At present, most studies using the retention capacity of PM retained on leaf surfaces to assess the particulate matter removal abilities of trees. Although this indicator is convenient for assessing the PM removal abilities under the same retention duration, there will be great difference in the retention capacities of the same tree species under different dust retention durations. Thus, it was proposed in some studies that the retention efficiency (the mass of PM retained on a unit of leaf area per unit time) should be applied to assess the plant PM removal abilities, as this can eliminate the evaluation deviation of PM removal ability due to the difference in dust retention duration. However, these studies neglected the fact that only a small part of PM on leaf surface can be eluted by rainfall. In addition, this method could result in five times overestimation of the PM removal abilities of trees, according to the results of this study (**Table 1**). For this reason, the calculation method of equation (2) should be applied to accurately assess the PM removal abilities of trees.

When PM elution method proposed in this study was used to determine the mass of PM on leaf surfaces, each experimental step must be accurate and avoid the errors caused by human factors as much as possible. For example, the number of leaves for each experiment depends on the specific situation, and it should be determined by the specification of experiment instrument, the dust retention duration, ultrasonic parameter, and other factors. Taking ultrasonic parameter as example, the ultrasonic cleaning duration and the centrifuge speed should be determined by multiple preliminary experiments to ensure the experimental error is within the acceptable range. In addition, the amount of deionized water used for elution should also be adjusted according to the mass of PM. Besides, each small beaker filled with eluent, while it is drying in the oven, should be covered with a piece of clean filter paper to prevent dust pollution. In brief, each step in the experiment must be carefully operated in order to repeat the proposed method accurately.

It is extremely necessary and crucial to supplement the ultrasonic cleaning procedure to the conventional leaf cleaning method, so that the PM removal abilities of trees can be assessed more accurately and quantitatively. In order to accurately compare the PM removal abilities of different tree species experiencing different dust retention durations, the retention efficiency should be calculated using the method (equation (2)) proposed in this study. Our proposed comprehensive protocol will be useful for evaluating the PM purification abilities of urban trees and forests in a precise, unbiased, and accurate manner.

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DISCLOSURES:
The authors have nothing to disclose.

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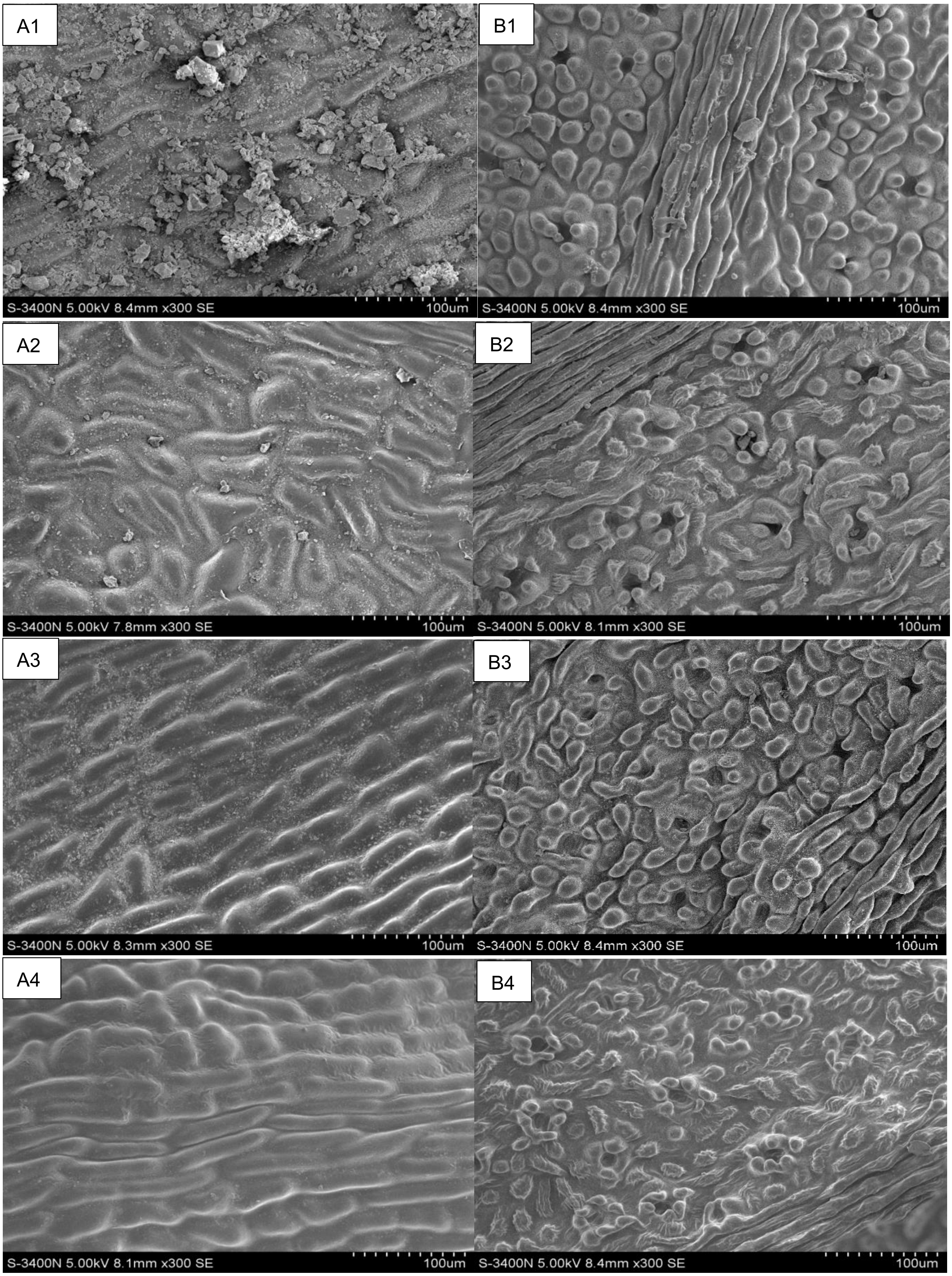
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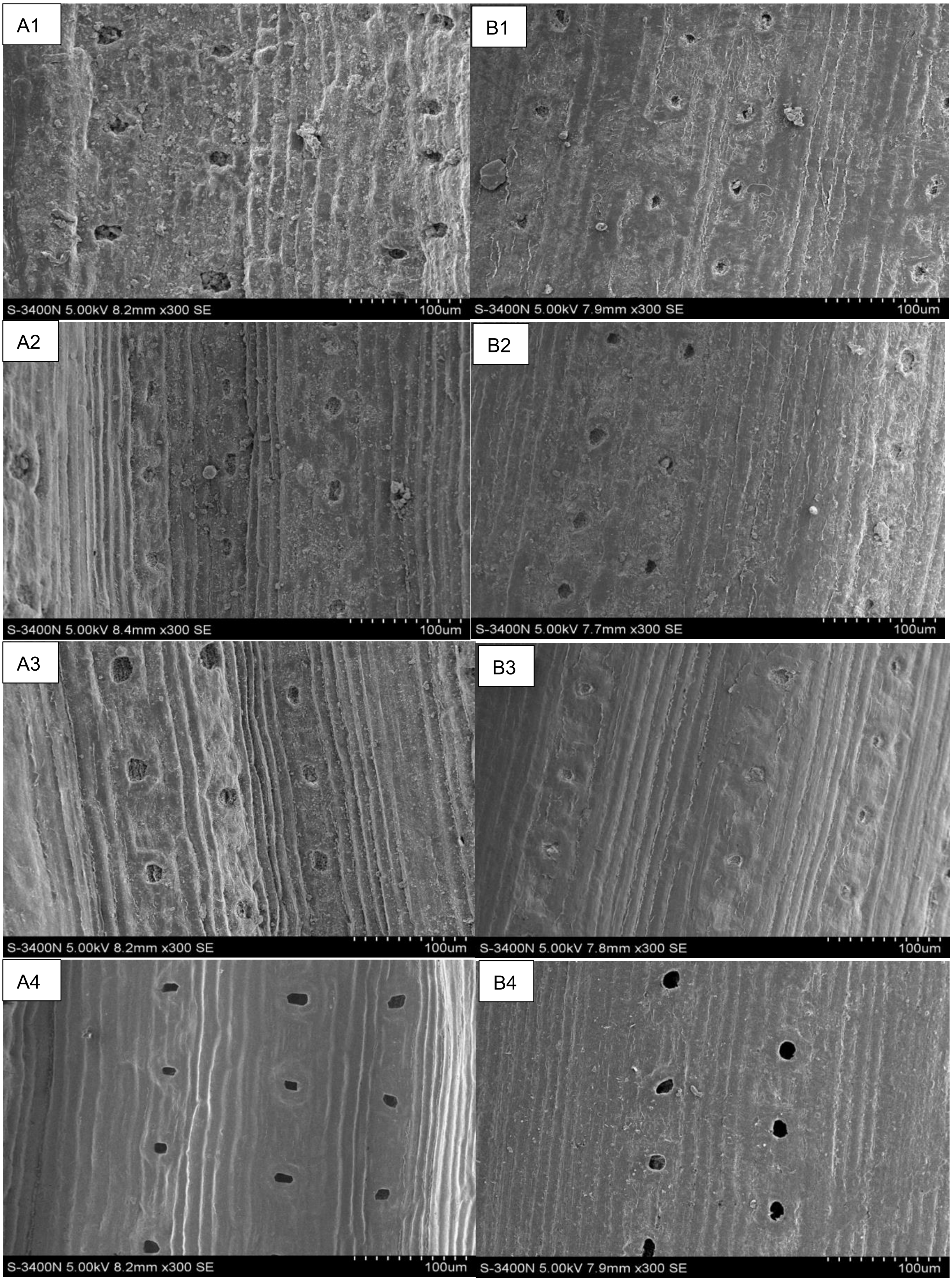
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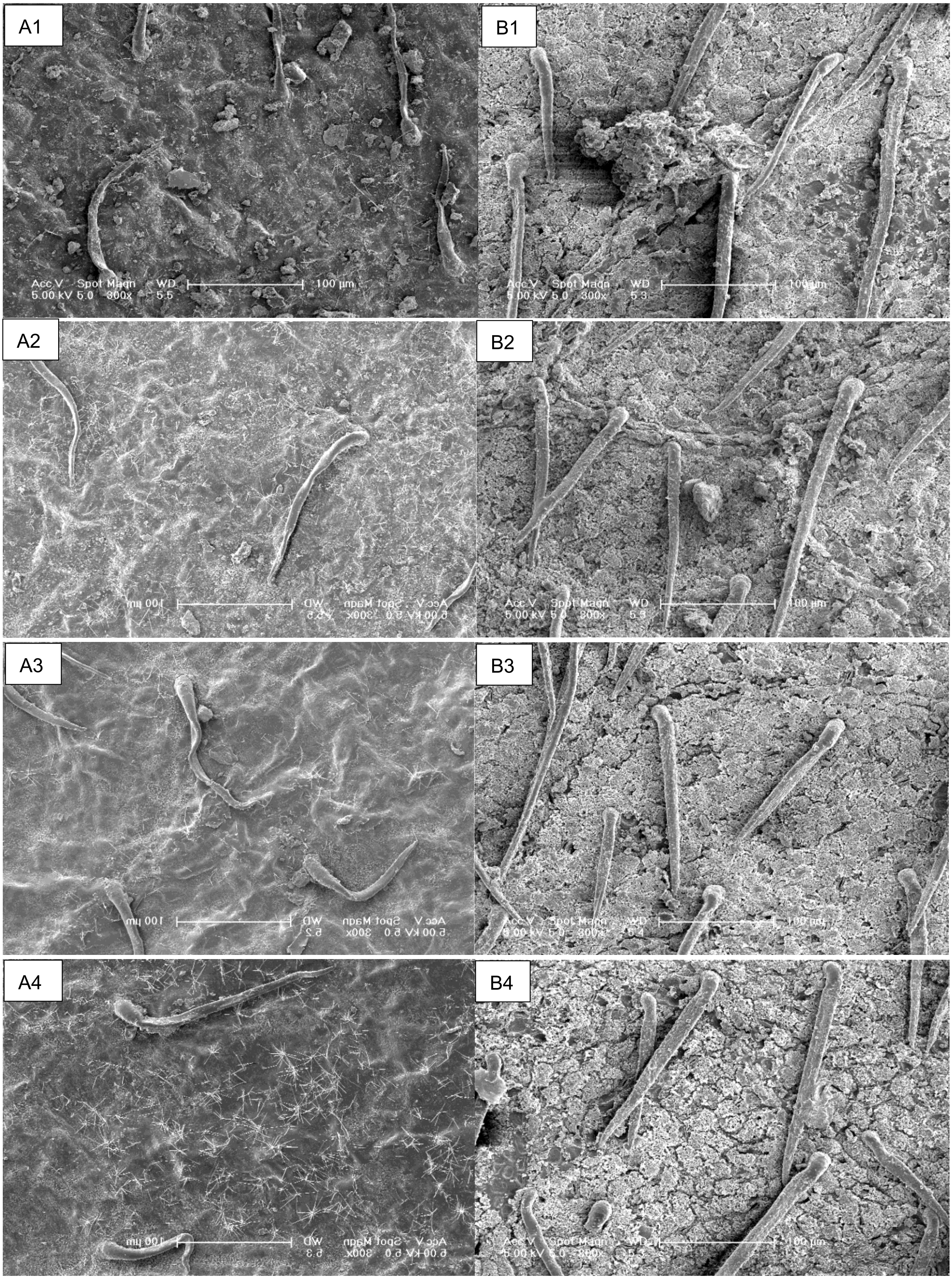
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B1
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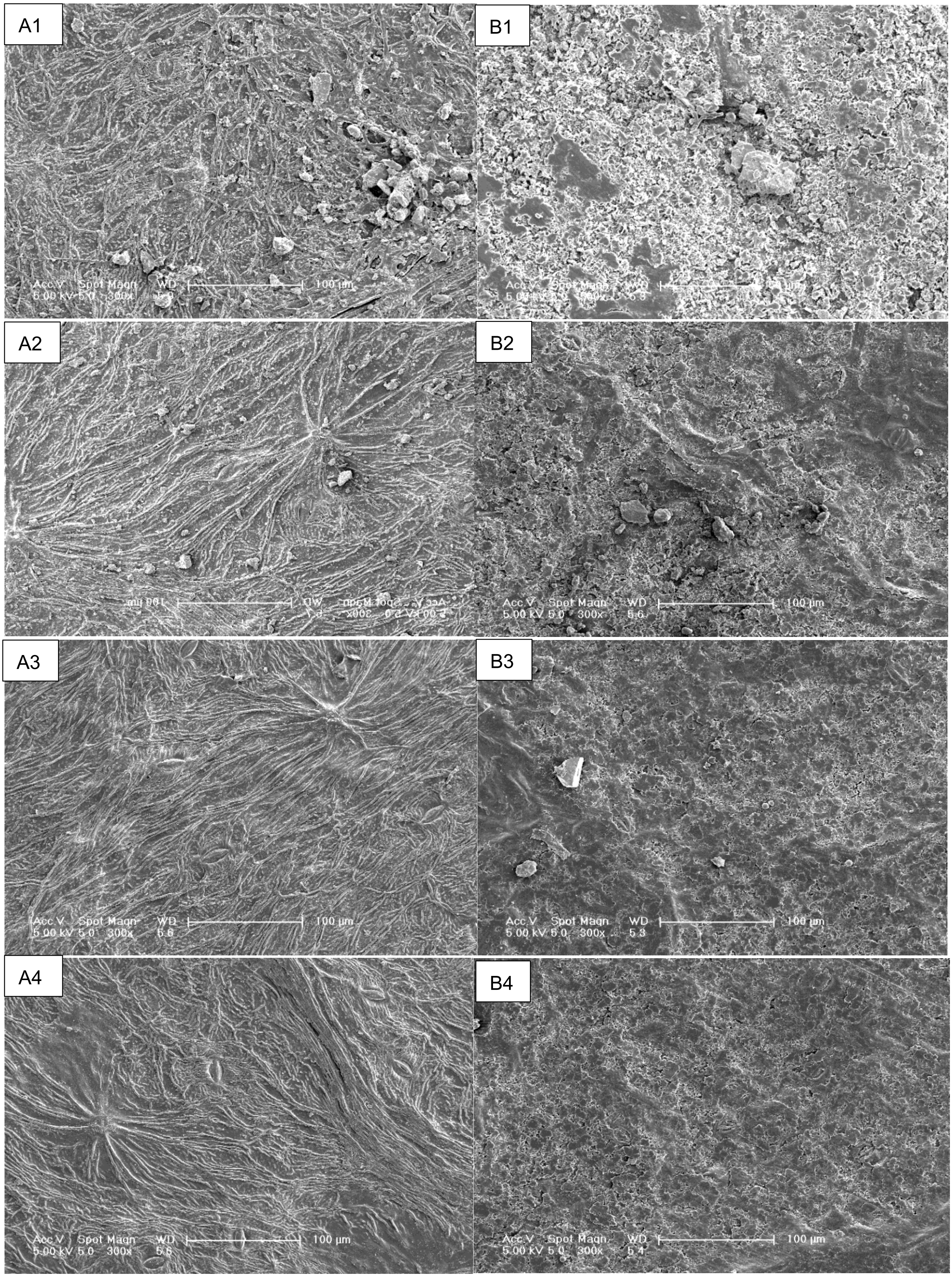
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A4
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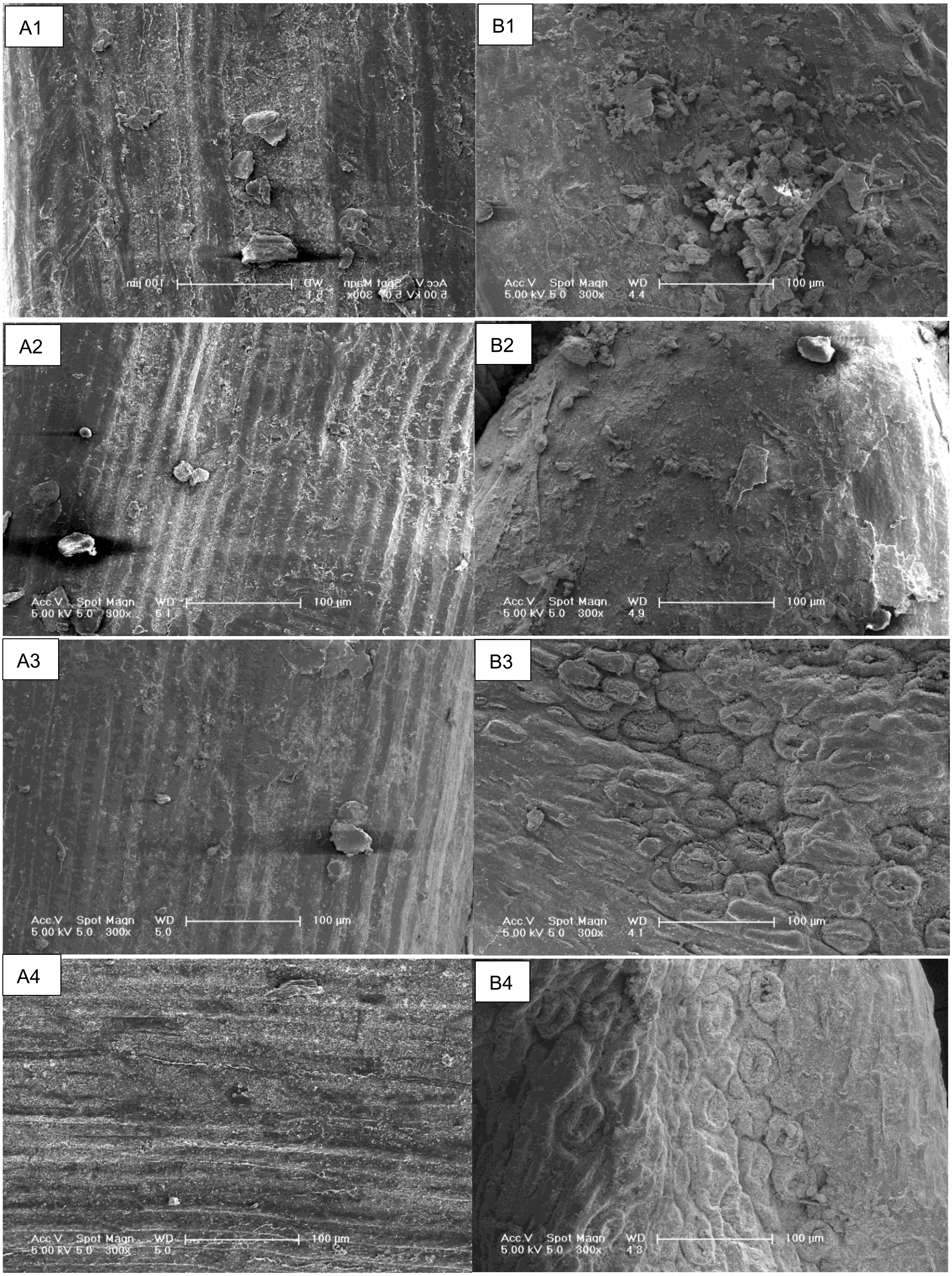
100 µm


A1
B1
A2
B2
A3
B3
A4
B4
Acc.V Spot Magn WD 100 µm
5.00 kV 5.0 300x 4.4

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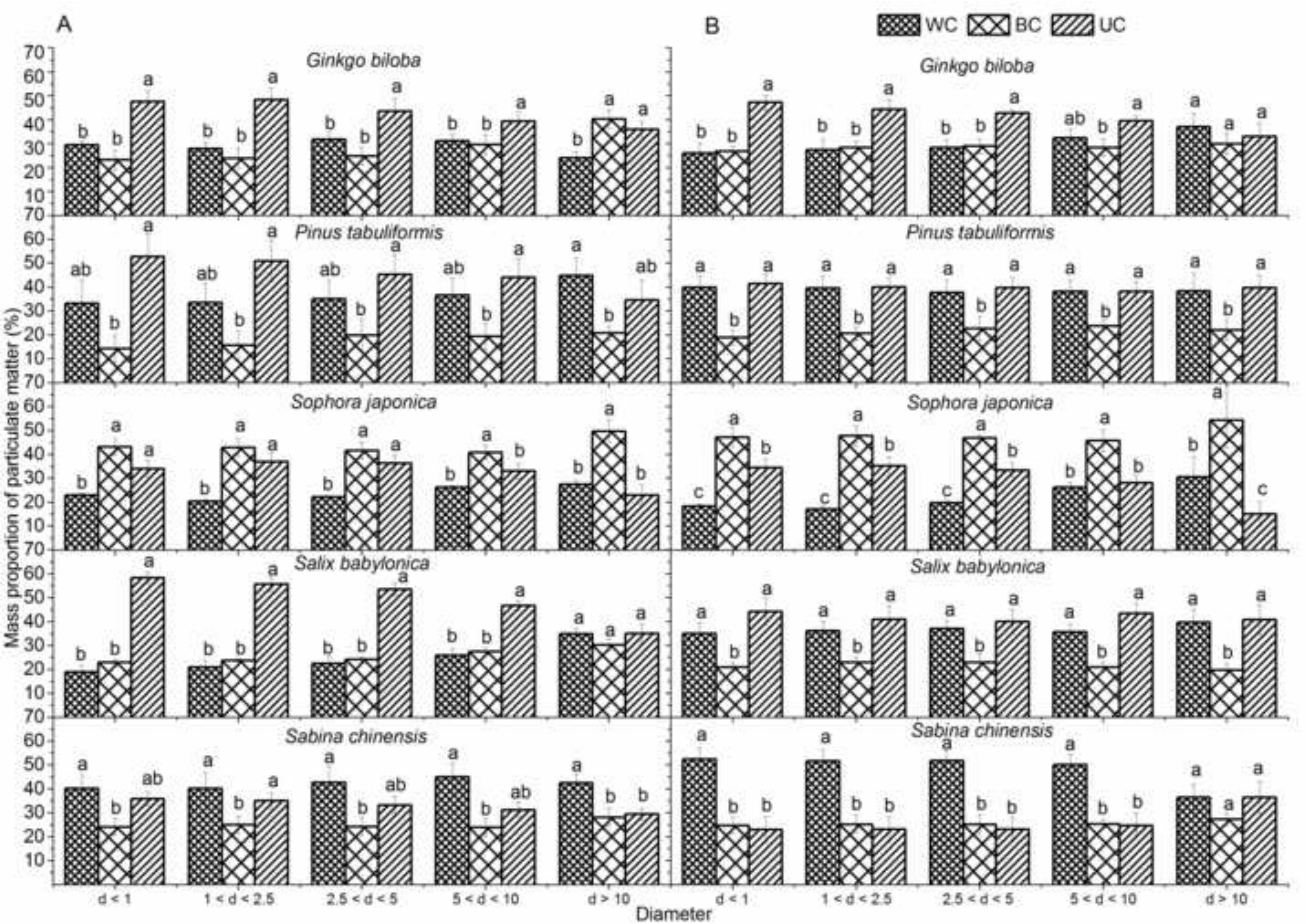

Table

Click here to download Table Table 1.xlsx

Tree species	The different type of particulte matter	The mass of PM retained on unit leaves area (mg.m^{-2}.d^{-1}) (Equation 2)					The mass of PM retained on unit leaves area (mg.m^{-2}.d^{-1}) (Equation 3)				
		PM_1	$PM_{2.5}$	PM_5	PM_{10}	TSP	PM_1	$PM_{2.5}$	PM_5	PM_{10}	TSP
G. biloba	ERP	0.25	0.1	0.2	3.41	19.01	1.87	4.43	7.46	14.25	35.27
	DRP	2.06	4	7.14	13.75	16.23	5.31	12.11	19.78	34.02	69.78
	TRP	2.31	4.1	7.34	17.16	35.24	7.18	16.53	27.24	48.28	105.04
P.tabuliformis	ERP	1.15	2.57	3.43	6.62	34.69	2.12	5.20	8.29	14.53	51.28
	DRP	0.82	1.65	2.37	6.9	60.84	3.20	7.91	13.02	23.13	82.23
	TRP	1.97	4.22	5.8	13.52	95.54	5.31	13.10	21.31	37.67	133.51
S. japonica	ERP	0.2	1.42	3.44	7.39	18.67	3.99	12.87	24.27	44.88	85.79
	DRP	4.1	20.48	36.16	47.04	55.18	17.81	60.98	107.25	165.16	257.95
	TRP	4.3	21.91	39.6	54.43	73.85	21.79	73.85	131.53	210.04	343.74
S. babylonica	ERP	1.76	10.24	21.46	32.57	33.04	2.84	11.27	22.30	37.20	62.62
	DRP	-1.31	7.28	19.52	29.44	17.76	5.28	20.16	38.88	65.74	104.58
	TRP	0.45	17.51	40.98	62.01	50.8	8.12	31.43	61.17	102.94	167.20
S. chinensis	ERP	2.53	11.39	21.68	31.96	12.69	4.24	14.22	26.01	43.58	63.22
	DRP	0.32	5.76	12.72	19.68	4.45	3.87	13.19	24.15	41.65	76.06
	TRP	2.85	17.15	34.4	51.64	17.14	8.11	27.41	50.15	85.23	139.28

Materials Table

Click here to download Table of Materials Materials.xls

Name of Material/ Equipment	Company	Catalog Number
MSA2258-1CE-DU ten-thousandth scale	Sartorius Scientific Instruments (Beijing) (	MSA2258-1CE-DU
The IS13320 laser granularity instrument	Beckman Coulter, Brea, USA	IS13320
Epson Twain Pro high-quality scanner	Seiko Epson, Nagano, Japan	expression1680
Automatic image analysis software WinRHIZ	Regent Instruments Inc., Quebec, Canada	WinRHIZO Pro 201

Comments/Description

precision: 0.01 mg

working conditions: liquid/power samples; particle size range of measurement: 0.017-2000 μm

3a

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Author(s): Jinqiang Liu, Ruina Zhang, Huanhuan Liu, Jie Duan, Jia Kang, Zemin Guo, Benye Xi, Zhiguo Cao

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CORRESPONDING AUTHOR:

Name: Benye Xi， Zhiguo Cao

Department: Forestry， Environment

Institution: Beijing Forestry University, Henan Normal University

Article Title: Methodology for assessing the air purification abilities of tree species quantitatively and accurately

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June 26, 2018

Nam Nguyen, Ph.D.

Review Editor,

JoVE58026R2

Title: Assessing the Particulate Matter Removal Abilities of Tree Leaves

Dear Nam Nguyen,

We appreciate the reviewers' valuable remarks, which considerably contributed to the improvement of our manuscript. According to the suggestions and comments from the reviewers, we have made revisions and corrections in our revised manuscript. Please find our point-to-point responses to the comments as follows. Thank you very much for your consideration and please contact us if you have any queries.

Best regards,

Dr. Benye Xi

Communicating editor and reviewers:

1. Please break up Figure 1 into separate Figures. Please provide a legend for each.

According to the reviewer's opinions, we have broken up the Figure 1 into a separate Figure. And the legend of it was also provided in a separate document.

2. Please obtain explicit reprint permissions for usage of Figure 1.

Based on the editor's opinions, we have already obtained explicit reprint permission from Elsevier (Fig. 1). In addition, the pictures of all scanning electron microscope in Figure 1 have been replaced by other similar pictures (we have taken a lot of SEM pictures when we were doing the experiments) in order to avoid repetition with published pictures.

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Reviewers' comments:

Reviewer #1:

Manuscript Summary:

Ok

Major Concerns:

No mention of statistical procedures used to compare your data.

According to reviewer's opinions, the statistical analysis used in our research has been rewritten in the revised manuscript. It has been changed to:

3.3. Conduct all the statistical analyses with SPSS (v. 22.0, SPSS Inc., Chicago IL, USA) software.

3.3.1. Use the Kolmogorov-Smirnov test and the Levene test to verify the ANOVA assumptions of normality and homogeneity of variances, respectively, for the elution percentages of the different-sized particles and the PM retention capacity data.

3.3.2. Apply one-way ANOVA to investigate the effects of the different cleaning steps on the elution percentages of the different-sized particles under various dust retention periods. Use Duncan's test (P = 0.05) to detect the significant differences among different cleaning steps.

Reviewer #2:

Manuscript Summary:

Manuscript entitled "Methodology for assessing the air purification abilities of tree species quantitatively and accurately" assessed three different extraction techniques to evaluate the accuracy of each method in quantifying dust retention capacity of leaves in 5 different tree species exposed to natural particulate matter for short and long duration. The manuscript is sound and relevant but lacks depth and several important information are missing.

Major Concerns:

The title should be changed as tree leaves of the middle canopy is only used in the experiment and not the whole tree is considered. Term "air purification" should be changed to "particulate matter removal".

Suggested title: "Methodology for assessing the particulate matter removal abilities of tree leaves quantitatively and accurately"

We completely agree with the reviewer's opinions, the term "air purification" in the title has been changed to "particulate matter removal". Thus, the title was changed to "Assessing the Particulate Matter Removal Abilities of Tree Leaves".

Authors in the long abstract mentioned "Leaf area index is also an important factor for assessing the PM retention efficiency in tree scale" but apart from abstract and final part of discussion section role of Leaf area index (LAI) is not mentioned in the manuscript. If LAI has any relationship with present work it should be measured and discussed if data is not available why to involve LAI at all in the manuscript.

We completely agree with the reviewer's opinions. Thus, we have deleted all mentions about leaf area index (LAI) in the manuscript. The reason why we refer to the LAI is that the original purpose of our experiment is to assess the particulate matter removal abilities in tree scale. After obtaining the reviewers' valuable remarks, I realize the innovation point in this manuscript was the elution method and the retention efficiency on leaf surfaces, and the LAI is only a further way to calculate the particulate matter removal abilities in tree scale. After careful consideration, we decided to deleted relevant parts of LAI in manuscript and it has already been modified in the manuscript.

Introduction: Introduction part is well structured with relevant information on the concerned topic.

Protocol:

Where is this experiment performed and what is the ambient level of PM in this area? Are all the trees are sampled from one location and exposed to similar PM?

1. Our experiment (leaf sampling) was conducted in the Xitucheng Park (39.97° N, 116.36° E), which is near the three ring road (Fig. 1) and thus a very heavily polluted area in Beijing, China (Fig. 2). The average levels of PM under the short and long dust retention period (i.e. the duration between the last rainfall and the leaf sampling time) in our experiment were 26 ($PM_{2.5}$), 57 (PM_{10}), and 111 ($PM_{2.5}$), 160 $\mu g/m^3$ (PM_{10}), respectively. We have already added this information in revised manuscript.

Fig.1 The location of Xitucheng Park

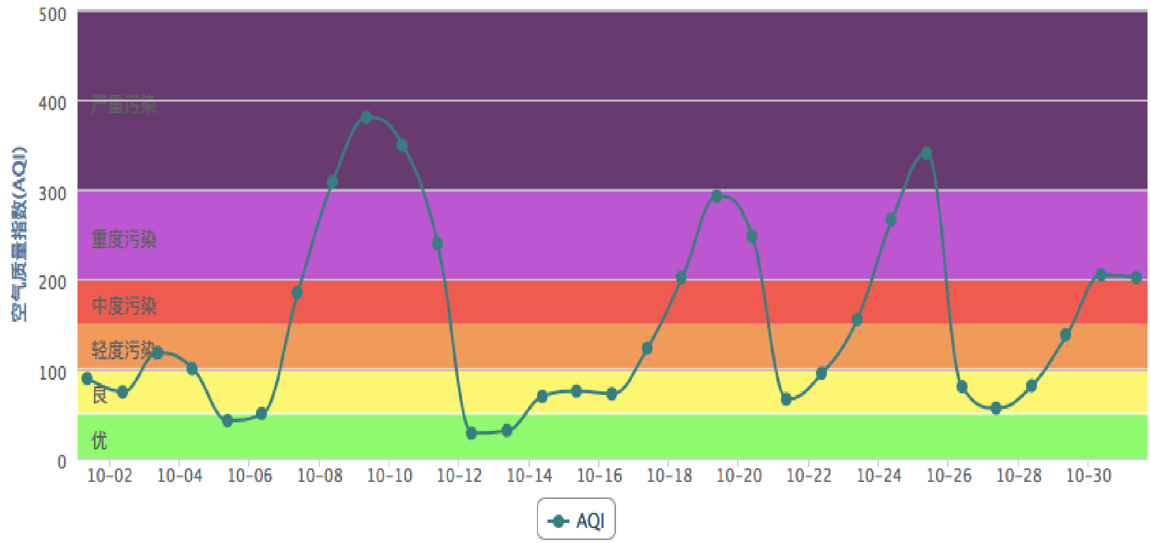


Fig.2 The AQI (Air Quality Index) during sampling period in Beijing at Oct, 2014.

2. All the trees for leaf sampling are located closely in a greening strip with length and width of about 250 and 60 m, respectively. So, the environment conditions (wind, light, and rain) of these trees were similar. The distances between sampling trees and the pollution source (road) are similar (about 10-20 m). We have already added this information in the revised manuscript.

Lines 98-99. Why authors collected leaf from the middle of the canopy, it is known that outer canopy leaves are more exposed to air pollutants. The authors should also mention how many leaves were collected for each plant species.

We completely agree the review's opinion that outer canopy leaves are more exposed to air pollutants. And actually the leaves sampled in our experiments were from the outer canopies. The middle of the canopy in manuscript refers to the sampling height above the ground. The reviewers may produce misunderstanding because of our inaccurate expression in this manuscript. So "the middle of the canopy" has been changed to "the outer canopy in the middle canopy layer" in the manuscript so that it could be understand clearly. And it has been modified in the manuscript.

On what basis authors selected dust accumulation period of 14 days for long dust retention? Is dust accumulation after this period is constant?

The "long" dust retention (14 days after rainfall) was just an expression relative to the "short" dust retention period (4 days after rainfall) used in our experiment. It does not mean "14 days" is definitely a "long time" for leaves to retain dust. The sampling time in our experiments (4 or 14 days after rainfall) was determined according to the weather and the air quality. Due to the air pollution in the sampling area (near a very busy ring road in Beijing) is very heavy and in order to avoid the influence of rainfall and obtain a dust retention duration gradient, we have to arbitrarily determine when we should go to sample the leaves according to the weather forecast.

Lines 114. Authors mentioned that "Randomly select a certain amount of leaves (the leaf area is about 2000 cm^2) from leaf samples". Why authors selected 2000 cm^2 leaf area for different plants. As the weight of leaves will also influence the sonication process. Authors should give rational reasons for such selection for other workers to repeat the experiment for other plants in same and different regions of the world.

When determining the PM quantity on leaf surface, the size of a replicated leaf sample of each tree species was to guarantee the total leaf sample area was about 2000 cm^2, which was determined according to our preliminary experiment (Liu et al., 2016). The 2000 cm^2 leaf area selected in present study was due to two reasons:

1) According to the volume of the 1000 ml beaker and 270 ml water we used, the leaves can be immersed in water exactly when the leaf area was about 2000 cm^2. If the leaf area was larger than 2000 cm^2, it could not be immersed in the water completely, and the elution effect will be bad.

2) Under the short and long dust retention period, the mass of particles could be measured accurately when the leaves area was about 2000 cm^2. If the leaf sample area was smaller than 2000 cm^2, the dust eluted from leaf surface will be so light that we could not weigh it accurately.

Based on the above two points, about 2000 cm^2 leaf area was selected in this research. We have added the reason for this in the revised manuscript, i.e. "Randomly select a certain amount of leaves (the leaf area is about 2000 cm^2, which can guarantee all the leaves can be immersed in the water completely and the eluted dust has enough weight so that we can weigh it accurately) from leaf samples".

References:

Liu, H.H., Cao, Z.G., Jia, L.M., Li, X.Z., Hao, L.F., Liu, J.Q., Wang, H., Xi, B.Y. Analysis of the role of ultrasonic cleaning in quantitative evaluation of the retention of tree leaves to atmospheric particles: a case study with Ginkgo biloba. Sci. Silvae Sin. 52 (12), 133–140 (2016).

Most important factor that determines particulate matter accumulation is surface characteristics of leaves. Are all the trees selected for the study have similar or different surface characteristics? I have one query hear that all the three methods used in this experiment for extraction have any influence of leaf surface characteristics.

Consistent with the reviewer's opinion, we also agree that leaf surface characteristic plays an important factor in determining particulate matter accumulation. From the observation by scanning electron microscope, the leaf surface microstructures of different tree species in this study are very different (Fig. S1-5, attached at the end of the response letter). The methods used in this study did not have influence on the leaf surface characteristics, and this has also been investigated in detail in our previous study (Liu *et al*., 2016). From the Fig. S1-5 at the end of the response letter, it can also been that the leaf surfaces microstructures were not destroyed by our cleaning methods.

References:

Liu, H.H., Cao, Z.G., Jia, L.M., Li, X.Z., Hao, L.F., Liu, J.Q., Wang, H., Xi, B.Y. Analysis of the role of ultrasonic cleaning in quantitative evaluation of the retention of tree leaves to atmospheric particles: a case study with Ginkgo biloba. Sci. Silvae Sin. 52 (12), 133–140 (2016).

Line 138-139. How author determines the ultrasonic time of 3 and 10 min. What was the frequency of ultrasonic pulse? Is it same for both species or different? Have authors tried to vary the pulse rate?

1. The ultrasonic time of 3 and 10 min was determined based on our preliminary study (Liu et al., 2016).
2. The power of ultrasonic pulse was 500 W.
3. And for different tree species, the power of ultrasonic pulse was same, all of it was 500 W
4. In order to obtain the optimal ultrasonic cleaning parameters, taking *Ginkgo biloba* as examples, after the leaves were cleaned by soak, stirring and scrubbing, we set 24 ultrasonic cleaning treatments which were a factorial combination of six ultrasonic cleaning time (1, 2, 3, 5, 10, 20 min) and four ultrasonic power [200(40%), 300(60%), 400(80%), 500(100%) W]. Then, it was sequentially conducted to leaf samples to collect particles retained on leaves. 1). The initial ultrasonic time (T_1) was determined by the time before chlorophyll a could be observed obviously (the concentration of the chlorophyll a and the color of eluent) (Fig. 3&4). And different T_1 could be considered as the optimal ultrasonic cleaning time without damaging the leaf microstructure under different ultrasonic powers, respectively. 2). Taking the mass of particulate matter elution as the mass of total particulate matter till the ultrasonic cleaning time (T_1), then, the eluting portion of particulate matter retained on leaves in different eluting steps (water cleaning, brush cleaning, and ultrasonic cleaning) was calculated (Fig. 5). 3). Comparing the eluting portion of different ultrasonic powers under the optimum ultrasonic cleaning duration (T_1), the final optimal ultrasonic cleaning time (T_2) and ultrasonic cleaning power were determined comprehensively. 4). The Scanning Electron Microscope was used to observe the particulate residue on leaf surface in order to examine the elution effect of the optimal ultrasonic parameters.

The result showed that after conventional cleaning procedures, further cleaning with ultrasonic under power of 500 W could make the leaves more clean, and wash off at the maximum about 27% of the particles retained on leaves. Meanwhile, the parameter (500 W for 3 min) could avoid the exudation of chlorophyll a from leaves obviously.

The ultrasonic time of the other tree species was also determined by this method, but its stability and general applicability to different plant species under different dust retention period should be further investigated when it was applied to use in similar studies.

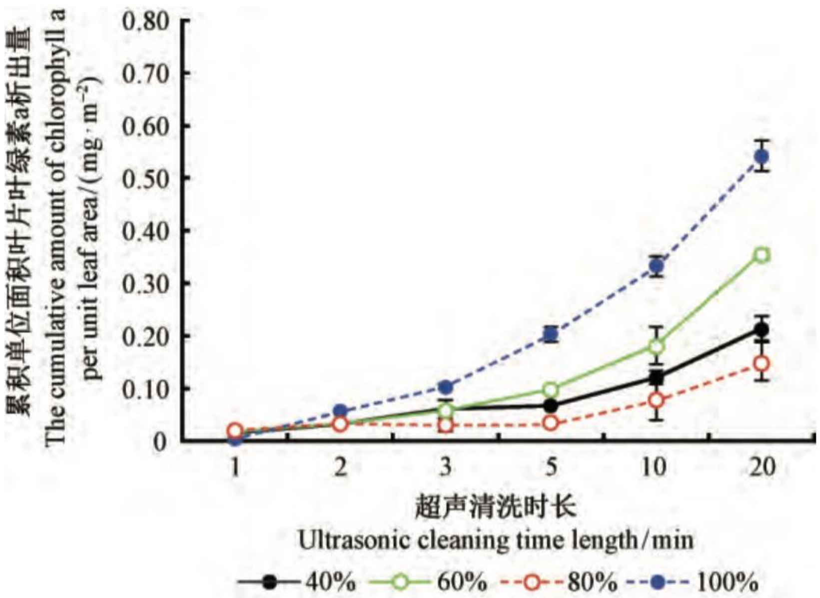


Fig.3 The cumulative amount of chlorophyll a exuded from leaves of *G. biloba* under different combinations of ultrasonic cleaning power and time length

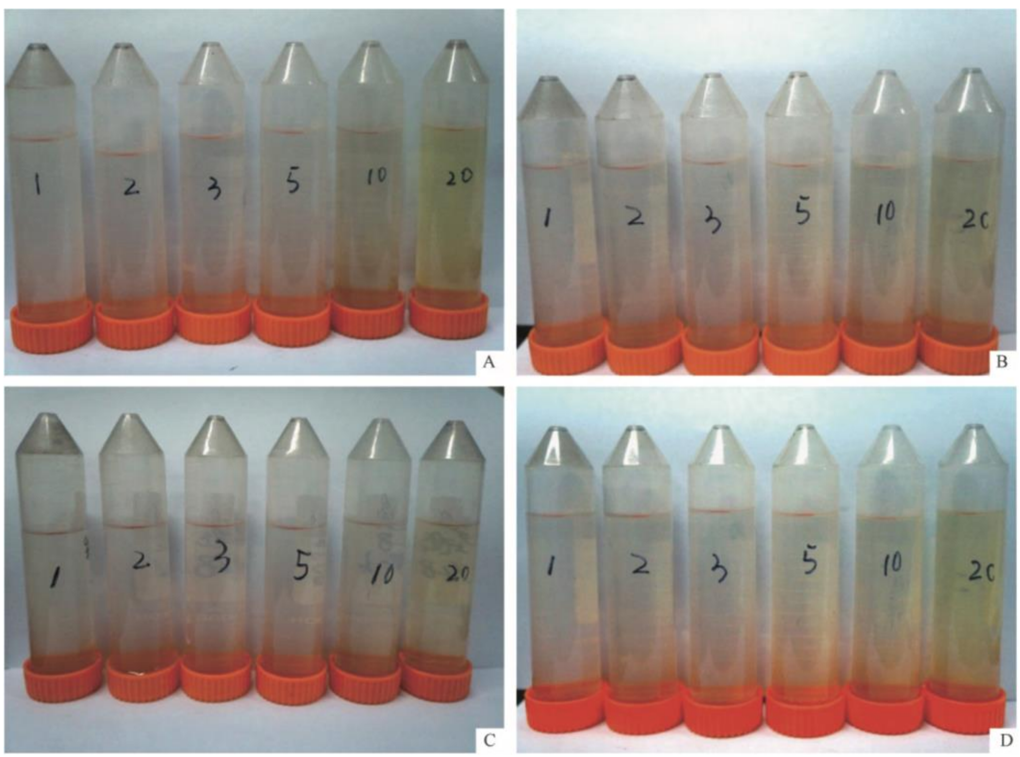


Fig. 4 Color variation of leaf eluent under different ultrasonic cleaning time length and power for *G. biloba*. (A: 200 W; B:300 W; C:400 W; D: 500 W). The number written on the centrifuge tubes in each picture is the ultrasonic cleaning time (min).

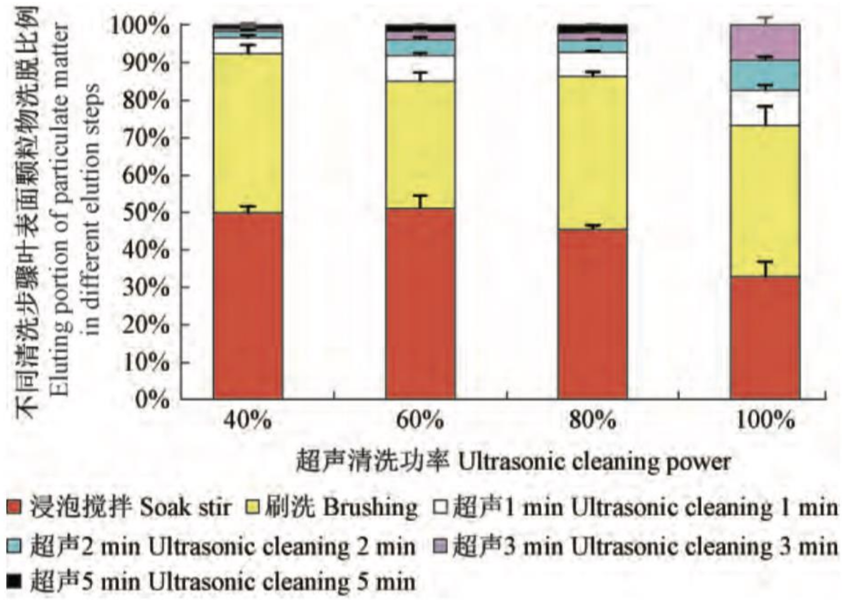


Fig.5 The eluting porting of particulate matter retained on leaves of *G. biloba* in different eluting steps

References:

Liu, H.H., Cao, Z.G., Jia, L.M., Li, X.Z., Hao, L.F., Liu, J.Q., Wang, H., Xi, B.Y. 2016. Analysis of the role of ultrasonic cleaning in quantitative evaluation of the retention of tree leaves to atmospheric particles: a case study with Ginkgo biloba. Sci. Silvae Sin. 52 (12), 133–140.

Authors did not perform any statistical analysis of the observed data. It will be better if statistical significance of observed methods were performed. How many times did authors repeat the experiment?

Based on reviewer's opinion, the data analysis was added in revised manuscript. And we set 5 replicates for each tree species in order to guarantee the precision of experiment.

Minor Concerns:

Abstract: Abbreviations must be defined first. In long abstract WC, BC and PM are not defined.

Based on reviewer's opinion, the WC, BC and PM has been defined in long abstract. And it has been modified in the manuscript.

Reviewer #3:

Manuscript Summary:

Accept.

Reviewer #4:

Manuscript Summary:

Although the manuscript focuses on an important topic, there is not a sufficient introduction for the protocol, furthermore, it needs major rewriting for sense and flow.

According to reviewer's opinion, the introduction for the protocol has already been rewritten in manuscript.

Major Concerns:

Insert data analysis

Page 6 line 199: The statistical significance of findings is not stated.

According to review's opinions, the statistical significance of findings was stated at the part of data presentation and analysis in the revised manuscript.

Page 4 line 114: sample size?

When determining the PM quantity on leaf surface, the size of a replicated leaf sample of each tree species was to guarantee the total leaf sample area was about 2000 cm^2, which was determined according to our preliminary experiment (Liu et al., 2016). The 2000 cm^2 leaf area was selected in present study might be due to two reasons:

1) According to the volume of the 1000 ml beaker and 270 ml water we used, the leaves were immersed in water exactly when the leaf area was 2000 cm^2. If the leaf area was larger than 2000 cm^2, it could not be immersed completely under the surface of water. So it might not have a good elution effect that was limited by experimental instrument.

2) Under the short and long dust retention period, the mass of particles could be measured accurately when the leaves area was about 2000 cm^2. In other words, only the leaf area about 2000 cm^2 that we could guarantee that enough PM could be eluted under this dust retention period. Thus, we selected enough leaves (the leaf area is about 2000 cm^2) to reduce experimental errors as much as possible.

Based on above two points, about 2000 cm^2 leaf area was selected in this research.

References:

Liu, H.H., Cao, Z.G., Jia, L.M., Li, X.Z., Hao, L.F., Liu, J.Q., Wang, H., Xi, B.Y. Analysis of the role of ultrasonic cleaning in quantitative evaluation of the retention of tree leaves to atmospheric particles: a case study with Ginkgo biloba. Sci. Silvae Sin. 52 (12), 133–140 (2016).

major rewriting

According to reviewer's opinions, the relevant part has been rewritten in manuscript.

Minor Concerns:

Title: rephrase your title; I would suggest to add air pollution removal and urban tree species.

We completely agree with the reviewer's opinions, considering the proposal of the editor and other reviewers simultaneously, the title has been changed to "Assessing the Particulate Matter Removal Abilities of Tree Leaves".

Page 3 line 53: add particulate matter (PM)

Based on reviewer's opinions, the particulate matter has been added in manuscript.

Page 3 line 54: delete greening

The word of "greening" has been deleted in manuscript.

Page 4 line 101: did you collect the samples from urban trees? Please describe the site.

Yes, I did.

1. Our experiment (leaf sampling) was conducted in the Xitucheng Park (39.97° N, 116.36° E), which is near the three ring road (Fig. 1) and thus a very heavily polluted area in Beijing, China (Fig. 2). The average levels of PM under the short and long dust retention period (i.e. the duration between the last rainfall and the leaf sampling time) in our experiment were 26 ($PM_{2.5}$), 57 (PM_{10}), and 111 ($PM_{2.5}$), 160 $\mu g/m^3$ (PM_{10}), respectively.
2. All the trees for leaf sampling are located closely in a greening strip with length and width of about 250 and 60 m, respectively. So, the environment conditions (wind, light, and rain) of these trees were similar. The distances between sampling trees and the pollution source (road) are similar (about 10-20 m). We have already added this information in the revised manuscript.

Fig.1 The location of Xitucheng Park

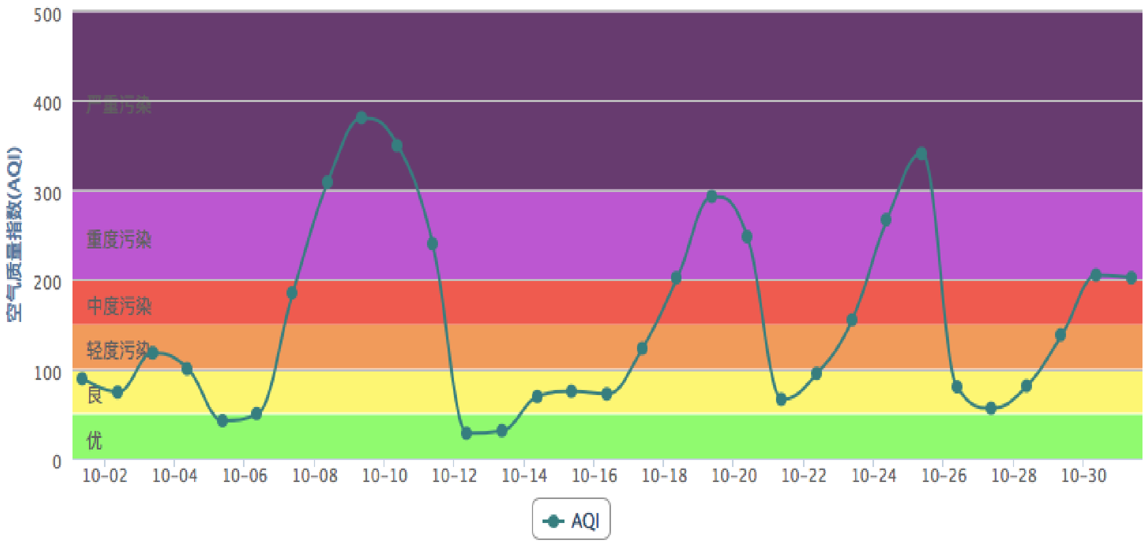


Fig.2 The AQI (Air Quality Index) during sampling period in Beijing

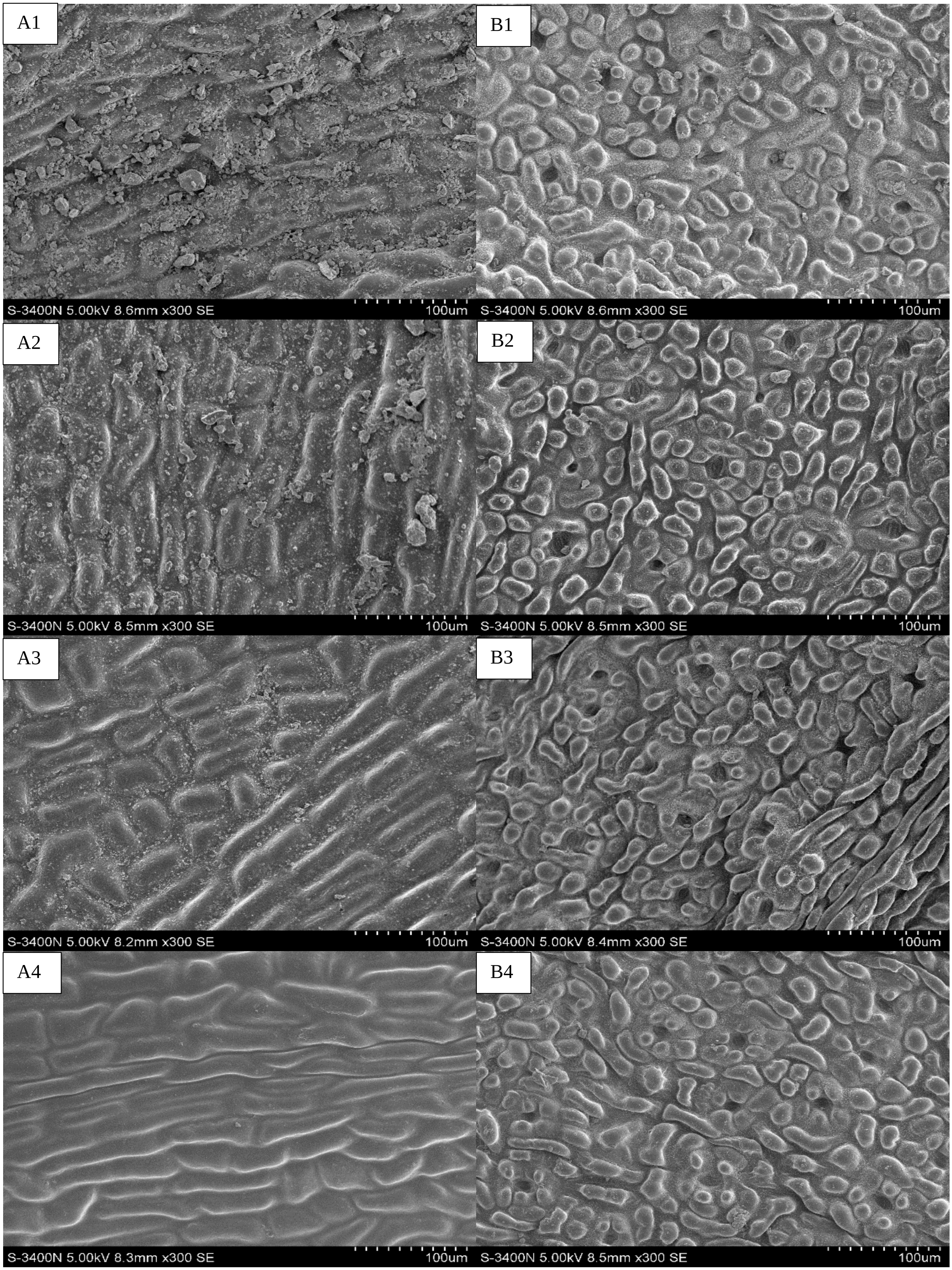


Fig. S1 The upper (A) and lower sides (B) of a *Ginkgo biloba* leaf subjected to different cleaning steps (1: without cleaning; 2: single water cleaning; 3: water cleaning + brush cleaning; 4. water cleaning + brush cleaning +

ultrasonic cleaning).

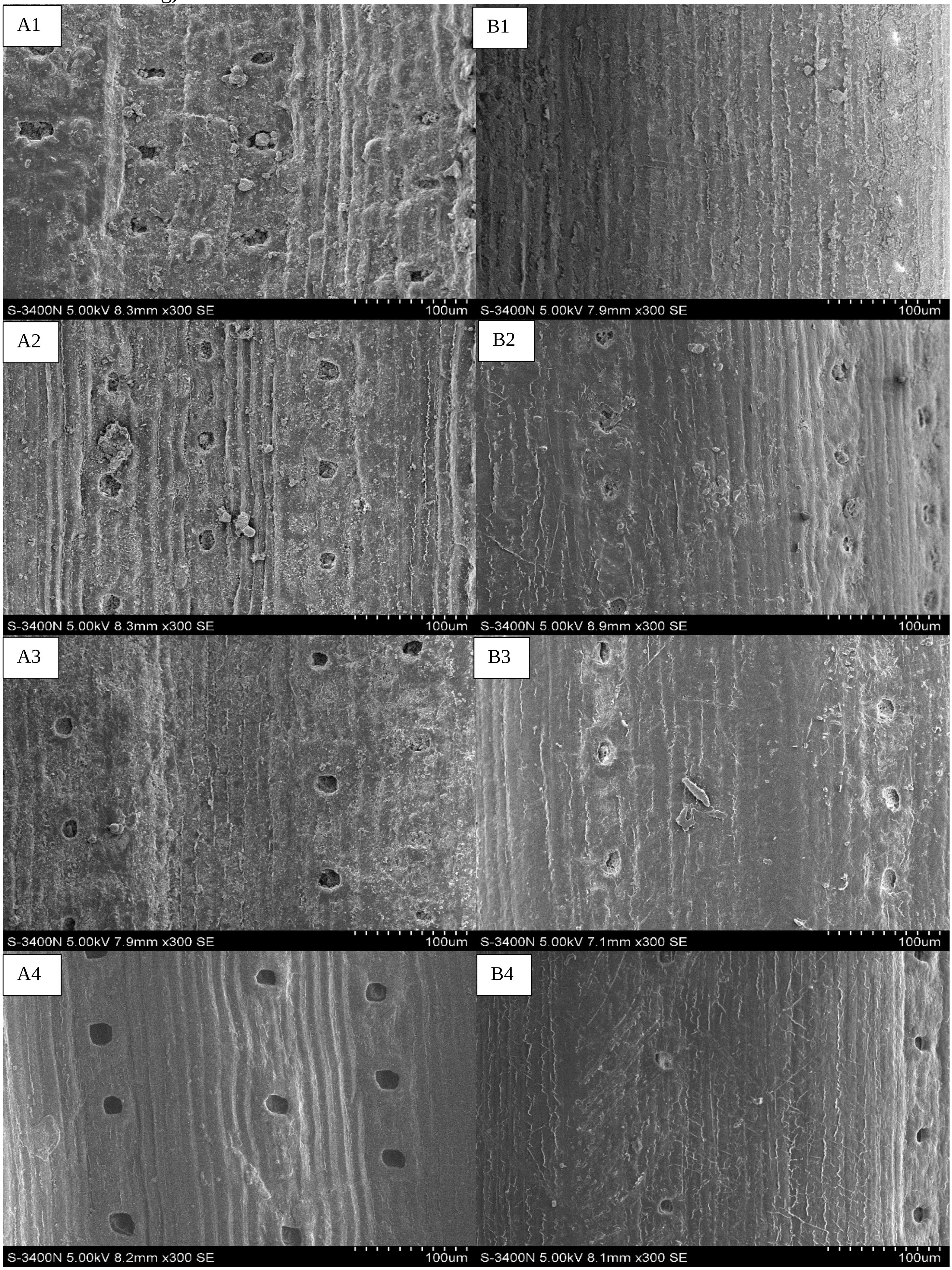


Fig. S2 The concave (A) and convex sides (B) of a *Pinus tabuliformis* leaf subjected to different cleaning steps

(1: without cleaning; 2: single water cleaning; 3: water cleaning + brush cleaning; 4. water cleaning + brush cleaning + ultrasonic cleaning).

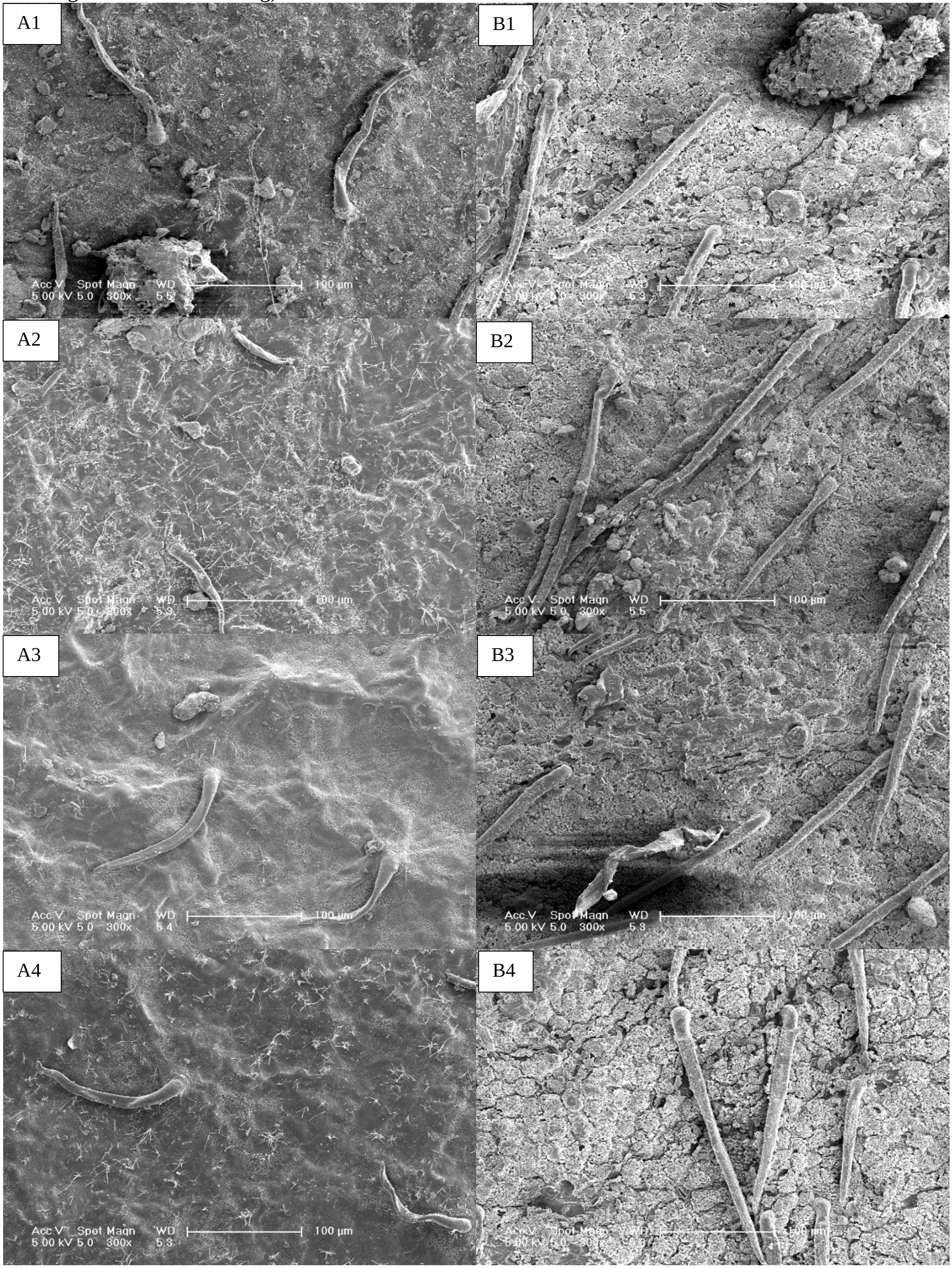
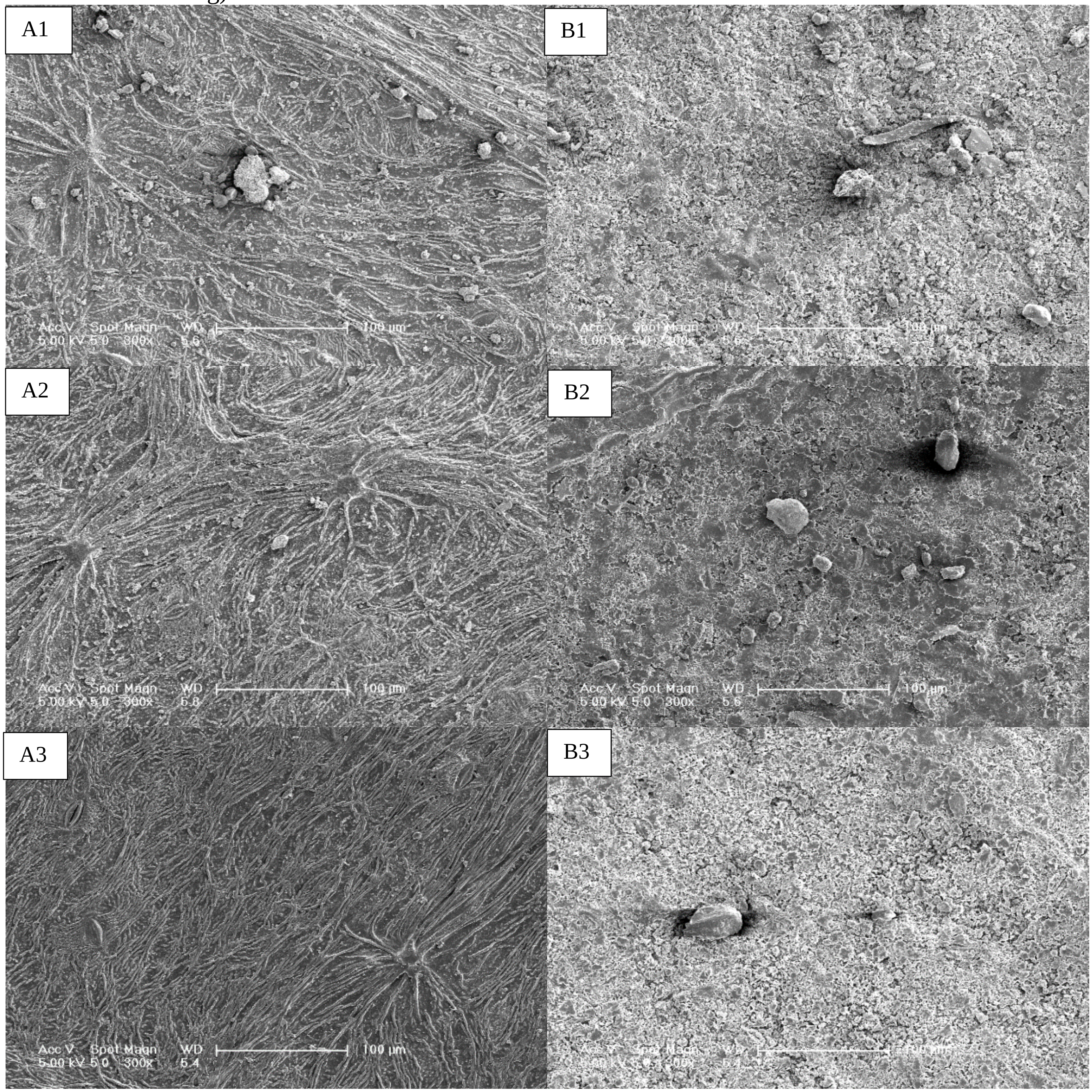


Fig. S3 The upper (A) and lower sides (B) of a *Sophora japonica* leaf subjected to different cleaning steps (1: without cleaning; 2: single water cleaning; 3: water cleaning + brush cleaning; 4. water cleaning + brush cleaning + ultrasonic cleaning).



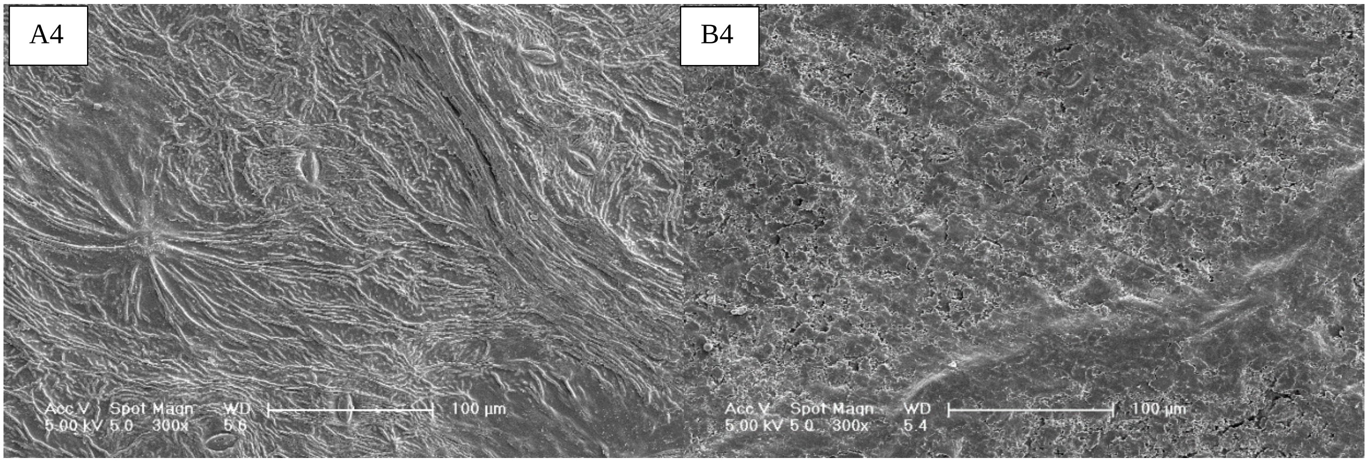


Fig. S4 The upper (A) and lower sides (B) of a *Salix babylonica* leaf subjected to different cleaning steps (1: without cleaning; 2: single water cleaning; 3: water cleaning + brush cleaning; 4. water cleaning + brush cleaning + ultrasonic cleaning).

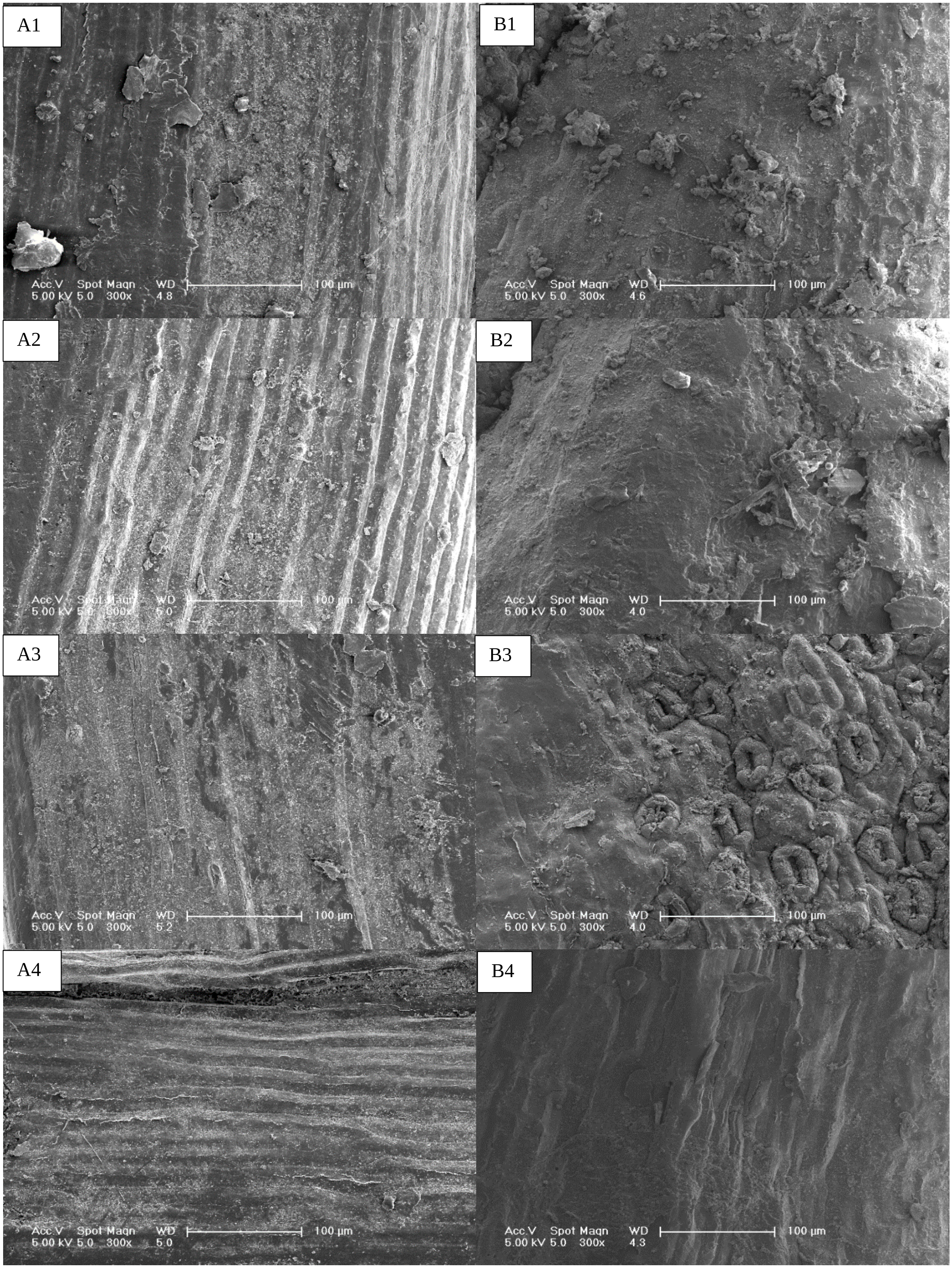


Fig. S5 The conical (A) and scale-form (B) leaves of *Sabina chinensis* subjected to different cleaning steps (1: without cleaning; 2: single water cleaning; 3: water cleaning + brush cleaning; 4. water cleaning + brush cleaning

+ ultrasonic cleaning).