Micro-morphological Analysis of Foliar Uptake and Retention of Airborne Particulate Matter (PM)-bound Toxic Metals: Implications for Their Phytoremediation

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Abstract

While airborne particulate matter (PM) pollution is a serious problem for urban environments, it can be reduced through uptake by plant leaves. In this study, we investigated and compared the uptake of PM-bound toxic metals by different plant species. Enrichment factor (EF) and correlation analyses across different sample types indicated anthropogenic origins of these toxic metals with airborne source signatures. Scanning electron microscopy (SEM) analyses of both leaf surfaces (adaxial and abaxial) suggested that the micro-morphological properties of the leaf surface (e.g., stomata, trichomes, epicuticular wax, and epidermal appendages) control the accumulation of PM and associated metals in plant leaves. *Senna siamea* leaves showed the most micro-morphological variation as well as the maximum concentration of toxic metals. It was found that foliar uptake of PM-bound toxic metals is affected by leaf surface morphological characteristics. Our results imply that plant speciation strategies can be used to help decrease PM pollution.

Novelty Statement

The collaborative study was intended to assess the effect of micro-morphological leaf surface properties on the accumulation of PM and associated toxic metals. To this end, accumulation potential of airborne toxic metals was assessed by plant leaves from distinct locations of an urban environment in India. The uptake pattern of toxic metals (PM bound) by plant leaves through the airborne route is considered as a relatively less common field. Our findings offer valuable insights into the role of plant species in controlling airborne PM pollution in urban environments.

Introduction

Air pollution is the most pressing problem in urban environments. Air pollutants are categorized into various components, of which PM is one of the most significant. PM is further categorized based on particle diameter as suspended/large PM (10 to 100 μm), respirable/coarse PM (2.5 to 10 μm), fine PM (0.1 to 2.5 μm), and ultrafine particles (UFPs) ($<0.1 \mu\text{m}$) (Popek et al. 2017). $\text{PM}_{2.5}$ (fine PM) was estimated to cause approximately 3.45 million premature deaths worldwide in 2007 (Zhang et al. 2017). Particles ranging in size from respirable to UFP can enter the human body and cause several chronic issues such as respiratory symptoms and cardiovascular disease. PM can also contain a number of toxic substances such as airborne toxic metals (Leonard et al. 2016). Transportation and industrialization activities are responsible for the emission of airborne toxic metals in association with PM of different sizes into the air.

Air pollution has become a serious health concern in developing Asian countries (HEI 2010; Kan et al. 2012; Deshmukh et al. 2013; Guo et al. 2016; Pant et al. 2016; Mannucci and Franchini 2017). According to a comprehensive study conducted in 230 Asian cities, about 80% of the studied cities had annual average PM_{10} level higher than the acceptable level as defined by the WHO guideline (Interim Target-2, $50 \mu\text{g m}^{-3}$). Moreover, only two of these cities [Pekanbaru and Palangkaraya (Indonesia)] had an annual average PM_{10} level within the WHO air quality guideline ($20 \mu\text{g m}^{-3}$) (CAI-Asia 2010; Ram et al. 2015). In recent years, the annual mean PM_{10} values measured in northern India were within the WHO and NAAQS standards, while most cities are critically polluted (Greenpeace India 2017). Moreover, Greenpeace India (2017) reported that 1.2 million people are dying annually due to air pollution in India. In India, the average respirable suspended particulate matter (RSPM) concentration is $160 \mu\text{g m}^{-3}$, which is higher than the NAAQ standards for many different categories (i.e., $120 \mu\text{g m}^{-3}$ for industrial, $60 \mu\text{g m}^{-3}$ for residential, and $50 \mu\text{g m}^{-3}$ for sensitive areas) (CPCB 2010).

This increase in airborne PM has been suggested to be due to various sources including vehicular emission, road dust resuspension, wear and tear of brakes and tires, industrialization, and dust from construction (Gajbhiye et al. 2016c, Adeniran et al. 2017). Since toxic/heavy metals (e.g., Pb, Cd, Cu, Zn, Cr, and Ni) bind to PM, their presence in PM can

increase exposure toxicity (Saeedi et al. 2012; Pourabadehei and Mulligan 2016; Ogundele et al. 2017). Hence, there is a pressing need to control the levels of PM and its associated toxic metals in urban environments.

In the urban environment, road side vegetation plays an effective role in reducing PM pollution (Gajbhiye et al. 2016a, b). Aerial plant parts such as branches, bark, stems, and especially leaves are able to reduce PM pollution via adsorption (Simon et al. 2016; Liang et al. 2017). Moreover, the large surface areas of leaves enable them to accommodate increased metal deposition on their surfaces (Sawidis et al. 2012; Leonard et al. 2016). However, the ability to accumulate metals can vary across different plant species (Deepalakshmi et al. 2014; Gajbhiye et al. 2019). This capacity can be determined by qualitative [epidermal cells, cuticular composition and structures, texture of both surfaces (adaxial and abaxial), and stomata type] and quantitative (numbers of stomata and trichomes) micro-morphological leaf features (Mulgrew and Williams 2000; Simon et al. 2014; Shi et al. 2017). Hence, micro-morphological studies of leaf surfaces can be employed to assess the PM pollution load in urban environments (Shi et al. 2017, Gajbhiye et al. 2019).

Although the accumulation of these toxic metals in plant roots via soil has been extensively studied, relatively limited research has focused on the uptake pattern of toxic metals by plant leaves through the airborne route. The aim of the present study was to assess the accumulation potential of airborne toxic metals by plant leaves from different locations of an urban environment in India. Plants naturally growing in six different sites of Bilaspur were selected for detailed study. The objectives of this study were (1) to assess the abundance of toxic metal content (Pb, Cd, and Cu) relative to Fe content in order to derive an enrichment factor in the leaves of the selected plants for foliar dust, road dust, and soil samples; (2) to explore the metal source processes; and (3) to assess the effect of micro-morphological leaf surface properties (adaxial and abaxial) on the accumulation of PM and associated toxic metals. Our findings offer valuable insights into the role of plant species in controlling PM pollution in urban environments.

Materials And Methods

Study site

The study was conducted in Bilaspur ($E = 82^{\circ}08'28.32''$, $N = 22^{\circ}09.6''$), which is located in the state of Chhattisgarh in central India. The selected area included the city center and covered both commercial and residential areas of the city. All sites are major squares of the city with strong traffic source activity (Fig. S1). Site 1 is the traffic square located near Govt. Girls Higher Secondary School. This site was also affected by transport activities of both light and heavy vehicles. Site 2 is a market square located near a bridge that connects the city. Site 3 and site 4 are located on National Highway (NH) 49, which has high traffic-related emission sources. Site 5 is located near Apollo Hospital Square, which connects the Rajkishor Nagar, Vasant Vihar, and Lingyadiah residential areas. Site 6 is located on a city bypass road that connects NTPC (National Thermal Power Corporation) Sipat to NH130 Raipur Road.

Selection of plant species

Six angiospermic plant species with different leaf surface characteristics were selected for this study (*Ficus religiosa*, *Mangifera indica*, *Butea monosperma*, *Alstonia scholaris*, *Azadirachta indica*, and *Senna siamea*). These species are widespread, remain green year-round, and are very common in subtropical areas.

F. religiosa (Family: Moraceae) is a species of large evergreen trees. Its leaves are generally 5 to 12 cm long and 5 to 8 cm wide, with alternate reticulate intercostae, a leathery appearance, an ovate-lanceolate (caudate) shape, and a green color when they are mature.

M. indica (Family: Anacardiaceae) is considered to be the most delicious Indian fruit plant. Its evergreen trees are erect with broad canopies, and its leaves are alternate and clustered at the tip of the branch. Its leaves are 12-20 cm long and 6-8 cm wide, pinnate, and have reticulate/intercostae venation.

B. monosperma (Family: Fabaceae) is a medium-sized deciduous tree with a bent trunk and irregularly arranged branches. The leaves are compound, alternate/spirally arranged on the stem, trifoliate, 8-12 cm long, and 5-7 cm wide. They also have an acute apex with an ovate shape, reticulate venation, and a prominent midrib.

A. scholaris (Family: Apocynaceae) is a tall ornamental tree with rough grey bark. The branches emerge from the same place in the main stem and are arranged in a whorled pattern. The leaves are leathery, and one whorl contains 4-7 leaves. Leaf shape is similar to that of leaves on the mango tree.

A. indica (Family: Meliaceae) is an evergreen tree that grows vertically erect and has greyish brown bark. Its leaves are imparipinnate and alternate with 12-20 cm long rachis. The leaflets generally contain 10 to 14 leaves, which are arranged in opposite/sub-opposite positions. The leaves are 4 to 6 cm long, 2 to 3 cm wide, and have a serrate margin. One pair contains 12-20 leaves. The leaves also display intercostae reticulate venation with a prominent midrib.

S. siamea (Family: Fabaceae) is a deciduous tree of medium height, up to 15-20 meters. It has a wide spreading canopy, and its leaves are pinnately compound, 25-35 cm long, and 10-15 cm wide. The leaflets are arranged in the opposite position in 10-14 pairs. The leaves are dark green and have an ovate shape, entire margins, and reticulate venation (Santapau 1966; Indian biodiversity portal: online).

In the present study, all plants showed similar phenological conditions. Therefore, at the time of collection, the leaves of the different plants were similarly matured. Special care was also taken at the time of sampling to select leaves equally in terms of morphological damage, bird feces, dynamics such as chlorosis and necrosis, and uneven dust deposition. Sampling of leaves, foliar dust, road dust, and soil samples and sample preparation

All samples were collected on the same day (January 6, 2014). Weather conditions were relatively stable within 2 weeks before sampling. The average temperature was 23.3°C and average relative humidity was 49.8%. Rainfall was not recorded prior to two weeks of sampling (Source: <https://www.worldweatheronline.com/>). Dusted leaves from each site were collected at a height of 2.5 meters above the ground. Ten leaves were collected each study site from branches facing towards the road side. Steel trays and fine plastic hair brushes were used to collect road dust from the edge of road. Soil samples from each site were collected from the surface at a depth of 40 cm by the scraper plate method (Gajbhiye et al. 2016c). Considering the average depth of the plant fine roots, the soil-surface was dug to the same depth. Four soil samples (10 grams each) were collected from each site from the same profile. All four samples were mixed to create a single uniform sample that represented the soil of that particular site. All samples were kept in air-tight Ziploc polyethylene bags and brought to the laboratory. The sampled dusted leaves were well stirred in a 200 ml glass beaker with 100 ml micro-distilled water for 10 minutes. In this suspension, foliar dust particles sticking to the surfaces of the leaves were separated. To obtain dust, the suspension was vaporized at 50 ° C on a hot plate. The washed leaves were oven dried for 24 hours at 50°C. After drying, the leaves were crushed with a mortar and pestle and passed through the sieve to obtain a fine powder. Road dust and soil samples were also kept at room temperature for 2 days, after which they were ground with a mortar and pestle and collected into separate beakers by weighing 0.5 grams of each. All samples were digested separately into aqua-regia solution (3HNO₃: HCl); sample preparation for metal analysis was performed according to the method of Gajbhiye et al. (2016c).

Analysis of metals

After sample preparation, heavy metal concentrations were determined using flame atomic absorption spectroscopy (AA 7000, Shimadzu, Japan). Standard solutions of the target metals were obtained from Inorganic Ventures (USA) (Fe: AAFc-1, 1000± 10 µg/ml, 2% (v/v) HNO₃ traceable to NIST-SRM 31269, Pb: AAPb-1, 1000 ± 10 µg/ml, 0.5% HNO₃ traceable to NIST-SRM 3128, Cd: AACd-1, 1000 ± 10 µg/ml, 3% (v/v) HNO₃ traceable to NIST-SRM 3108, and Cu: AACu-1, 1000 ± 10 µg/mL, 3% (v/v) HNO₃ traceable to NIST-SRM 3114). Five-point calibration curves were obtained by serial dilution of metal standard solutions and used to quantify the concentrations of the four metals (Pb, Cd, Cu, and Fe) in different samples

after correction for the blank value. The concentrations of each metal ($\mu\text{g g}^{-1}$) were analyzed in triplicate. The detection limits (DL: $\mu\text{g g}^{-1}$) of the four target metals were $0.04 \mu\text{g g}^{-1}$ (Fe), $0.08 \mu\text{g g}^{-1}$ (Pb), $0.006 \mu\text{g g}^{-1}$ (Cd), and $0.009 \mu\text{g g}^{-1}$ (Cu). The precision, when expressed in terms of relative standard deviation (RSD %), was below 5% for all metals. The average recovery of different metals in triplicate analyses from randomly selected fortified samples (soil) was as follows: Fe ($88.8 \pm 3.80\%$), Pb ($104.0 \pm 2.84\%$), Cd ($98.5 \pm 3.98\%$), and Cu ($113.5 \pm 4.30\%$).

Statistical analysis

The statistical analyses were carried out using the statistical package for the social science, version 22 (SPSS-22, IBM, Chicago, USA). One-way Analysis of variance (ANOVA) was applied to analyze the significant differences in concentrations of target toxic metals measured in different sample types. The level of significance (p value) was set at different level (< 0.05 and < 0.001). The correlation coefficient was used to determine different significance level ($p < 0.05$ to $p < 0.01$) between leaf vs. foliar dust, road dust, and soil samples.

Enrichment factor (EF)

The EF assesses the relative contribution of manmade sources to toxic metal levels in collected samples. The EF was calculated using the formula

$$EF = \frac{(S_{(E)}/S_{(R)})_{\text{leaf}}}{(C_{(E)}/C_{(R)})_{\text{crust}}}$$

where $S_{(E)}$ is the concentration of a target metal $_{(E)}$ in the examined environmental sample, $S_{(R)}$ is the concentration of the reference metal in the examined environmental sample, $C_{(E)}$ is the concentration of a target metal $_{(E)}$ in the crust, and $C_{(R)}$ is the concentration of the reference metal in the crust (Taylor and McLennan 1995). The $C_{(E)}$ values for Pb, Cd, and Cu were 20, 0.098 (98 ppb), and 25 ppm, respectively. Fe was used as the reference metal ($C_{(R)}$) with a crustal value of 35000 ppm (3.5 wt. %) (Taylor and McLennan 1995). Based on EF values, the following categories were used for pollution levels and source (natural or anthropogenic): $EF < 2$ = low enrichment, $2 \leq EF < 5$ = moderate enrichment, $5 \leq EF < 20$ = significant enrichment (low anthropogenic emission), $20 \leq EF < 40$ = very high enrichment (moderate anthropogenic emission), and $EF \geq 40$ = extremely high enrichment (high anthropogenic emission) (Buat-Menard and Chesselet 1979; Aničić et al. 2011).

Scanning electron microscopy

Fresh leaves were thoroughly washed with ionized water, blotted dry, and then cut into square sections (about 0.5 cm wide $\times$ 0.5 cm long) using razor blades. The leaves were cut from the margins and the mid-rib area. The leaf samples were kept in a mixture of 2.5% glutaraldehyde solution overnight to enable prefixation, after which the samples were kept in 2% osmium tetroxide for post-fixation for one hour. The samples were washed twice with phosphate buffer solution for 15 min and then passed through a series of acetone solutions (30%, 50%, 70%, 95%, and 100%) for dehydration. Drying was performed in a critical point drier (CPD) using CO_2 as a carrier gas. An aluminum stub with an adhesive surface was used to mount the leaf samples. The stub was coated with 40-60 nm gold using a Denton Vacuum. SEM analysis of samples was carried out with an FEI NOVA NANO SEM-450 scanning electron microscope equipped with different detectors.

Results And Discussion

Concentrations of metals in different matrices

The concentrations of the four target metals in the four sample types (matrices) are shown in Fig. 1. Site 6 (*S. siamea*) showed the highest concentrations of all metals in foliar dust (Table S1). The maximum concentration of Fe in foliar dust in the present study was more than 10 times higher than that observed in a previous study in Bilaspur, whereas it was

nearly 2 times higher for Pb (Gajbhiye et al. 2016c). The maximum concentration of Cd in foliar dust was comparable with that observed in Miskolc (industrial city), Hungary ($4 \pm 1 \text{ mg kg}^{-1}$: *Platanus x acerifolia*) (Simon et al. 2016). This comparative study was performed with samples from high traffic and active urban sites and showed traffic-related sources for these toxic metals.

The maximum concentration of Pb in *S. siamea* in this study was comparable with a previous study ($16.0 \pm 0.80 \text{ } \mu\text{g g}^{-1}$: *S. siamea*) (Gajbhiye et al. 2016c). However, it was more than 3 times higher than the concentration reported in Mashhad, Iran ($4.5 \pm 1.9 \text{ } \mu\text{g g}^{-1}$: *Platanus orientalis*) and 2 times higher than that reported in Córdoba, Argentina ($6.89 \text{ } \mu\text{g g}^{-1}$: *Tillandsia capillaris*) (Pourkhabbaz et al. 2010; Abril et al. 2014). For Cd, the maximum concentration ($0.75 \pm 0.09 \text{ } \mu\text{g g}^{-1}$: *A. scholaris*) was comparable with that reported in Okayama, Japan ($0.82 \pm 0.13 \text{ } \mu\text{g g}^{-1}$: *Rhododendron Pulchrum*: S7) (Suzuki et al. 2009). However, it was nearly 75 times higher than that reported in Shanghai, China ($0.109 \pm 0.060 \text{ } \mu\text{g g}^{-1}$: *Pittosporum tobira*) (Liang et al. 2017).

In road dust, the maximum concentrations for Pb ($79.8 \pm 1.47 \text{ } \mu\text{g g}^{-1}$) and Cu ($171 \pm 2.65 \text{ } \mu\text{g g}^{-1}$) were found at site 4 (*A. scholaris*). However, the maximum concentrations were 2 times lower and comparable to those for Pb ($153 \pm 52 \text{ } \mu\text{g g}^{-1}$) and Cu ($182 \pm 14 \text{ } \mu\text{g g}^{-1}$), respectively, in highway road dust in Ulsan, Republic of Korea. The maximum concentration of Cd ($2.72 \pm 0.72 \text{ } \mu\text{g g}^{-1}$) was found at site 6 (*S. siamea*); this concentration was comparable to those reported in Hong Kong ($3.77 \text{ } \mu\text{g g}^{-1}$) and Ulsan, Republic of Korea ($3.44 \pm 1.66 \text{ } \mu\text{g g}^{-1}$) (Li et al. 2001; Duong and Lee 2011). In contrast, the maximum concentration was around 4 times higher than that reported in Beijing, China ($0.72 \pm 0.74 \text{ } \mu\text{g g}^{-1}$) (Wei et al. 2015).

In soil, the maximum concentrations of Fe ($36942 \pm 2.08 \text{ } \mu\text{g g}^{-1}$), Pb ($11.6 \pm 0.97 \text{ } \mu\text{g g}^{-1}$), and Cd ($0.21 \pm 0.04 \text{ } \mu\text{g g}^{-1}$) were found at site 2 (*M. indica*). These concentrations were much lower than those reported in London (Pb = $294 \text{ } \mu\text{g g}^{-1}$, Cd = $1.0 \text{ } \mu\text{g g}^{-1}$), Shanghai (Pb = $70.69 \text{ } \mu\text{g g}^{-1}$, Cd = $0.52 \text{ } \mu\text{g g}^{-1}$), and Hong Kong (Pb = $93.4 \text{ } \mu\text{g g}^{-1}$, Cd = $2.18 \text{ } \mu\text{g g}^{-1}$) (Thornton 1991; Li et al. 2001; Shi et al. 2008). However, the maximum concentration of Cu ($25.4 \pm 2.37 \text{ } \mu\text{g g}^{-1}$: Site 6) was comparable with that reported in Hong Kong ($24.8 \text{ } \mu\text{g g}^{-1}$).

Source apportionment of metals and relationships between the different matrices

The results of ANOVA showed the significant differences ($p < 0.05$ and 0.001 level) among concentration of metals in road dust, leaf, and foliar dust sample within study sites (Fig. 1). A correlation analysis was conducted between metal concentrations within different samples to determine their sources of origin. In foliar dust, significant correlations were found between Pb vs. Cd ($r = 0.93$, $p < 0.01$), Pb vs. Cu ($r = 0.85$, $p < 0.05$), and Cd vs. Cu ($r = 0.89$, $p < 0.01$) [SM: Table S2a (i)]. Similar results were found in leaf and road dust samples [SM: Tables S2a (ii) and (iii)]. However, in soil, the correlations were not significant, implying negligible anthropogenic influence. A correlation analysis was also conducted between leaf and other samples (foliar dust, road dust, and soil) (SM: Table S2b). All metals (Fe, Pb, Cd, and Cu) showed significant correlations ($p < 0.05$, 0.01) between leaf vs. foliar dust and leaf vs. road dust.

Enrichment Factor (EF)

To identify the sources of toxic metals in the four samples (foliar dust, leaves, road dust, and soil) and thus determine whether the toxic metals are derived from anthropogenic activity or natural sources, the EFs were calculated (SM: Table S3).

In foliar dust, the EFs for Pb, Cd, and Cu showed moderate to very high enrichment, which suggests anthropogenic origin. In leaf samples, the EFs of Pb and Cu showed similar levels of enrichment (significant enrichment). This finding was comparable with the EF value of *Tilia* spp. and *Aesculus hippocastanum* leaves ($\text{EF} > 10$) isolated from an urban area in

Belgrade, Serbia (Tomašević and Aničić 2010). However, for Cd, the EFs ranged from 22.2 (*B. monosperma*) to 81.1 (*A. scholaris*). With the exception of site 3 and site 5, all other sites showed extremely high enrichment ($EF \geq 40$), with maximum enrichment at site 4, followed by site 6, site 2, and site 1. A similar trend was observed for the EFs of mosses isolated in Zlatibor, Serbia (Pb = 25.2 and Cd = 22.5) and Italy (Pb = 19.1 ± 12.5 , Cd = 22.4 ± 12.3), which reflects anthropogenic origin (Gerdol et al. 2000; Dragovic and Mihailovic 2009). The high EF value of Cd in the *S. siamea* leaf samples is comparable with a previous study (Gajbhiye et al. 2016c). In road dust, Pb and Cu showed moderate to significant EFs. The EFs of Cd ranged from 9.20 (*B. monosperma*) to 25.7 (*S. siamea*), indicating significant to very high enrichment. A similar pattern of Pb and Cu EFs in road dust was observed by Fujiwara et al. (2011), which was mainly associated with traffic emission and vehicular part corrosion. The road dust EFs of Pb and Cu were comparable with the road dust EFs from Baoji, China, which indicated an anthropogenic source of origin (Lu et al. 2009). In soil samples, all selected metals had an EF of 2 or below, suggesting that they originated from natural sources or were background concentrations.

SEM-based micro-morphological study

The micro-morphological parameters considered for each plant are listed in Table 1.

Table 1
Comparative micro-morphological study of the leaves of six plant species.

S. No.	Tree species	Family	Leaf shape	Epicuticular wax		Stomata		Trichome	
				Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
1	<i>F. religiosa</i>	Moraceae	Ovate	Absent	Present	Absent	Present	Absent	Absent
2	<i>M. indica</i>	Anacardiaceae	Oval-lanceolate	Absent	Present	Absent	Present	Absent	Absent
3	<i>B. monosperma</i>	Fabaceae	Ovate	Absent	Absent	Absent	Absent	Present	Present
4	<i>A. scholaris</i>	Apocynaceae	Oblong - lanceolate	Present	Present	Absent	Absent	Present	Present
5	<i>A. indica</i>	Meliaceae	Lanceolate	Absent	Present	Absent	Present	Present	Absent
6	<i>S. siamea</i>	Fabaceae	Ovate	Present	Present	Present	Present	Present	Present

F. religiosa

In *F. religiosa*, emergence was visible at the adaxial surface of the mid-rib portion of the leaves, which can facilitate deposition of PM (Figs. 2a, b, and c). On the abaxial surface, epicuticular waxy platelets were found. Waxy platelets were arranged in rosette-like structures on which PM was deposited in a range of 3.244 to 4.169 μm (Figs. 2d, e). Moreover, stomata were found on the abaxial surface. These stomata were covered by PM and had a mean opening of $1.719 \mu\text{m} \times 904.6 \text{ nm}$ (Fig. 2f).

M. indica

The adaxial surface of *M. indica* leaves shows rough morphological variations due to sparse arrangement of the cuticular layer (Jamil et al. 2009). This roughness permits the deposition of PM of different sizes (929.8 nm to 14.08 μm , Figs. 3a and b). For instance, fine PM (0.1 to 2.5 μm) was deposited on the surface (Fig. 3c). The abaxial surface was found to be rougher, presumably due to the presence of the resin secretor gland, stomata, and epicuticular wax with deposited PM (Figs. 3d and e). The abaxial surface of *M. indica* has been reported to have a granular form of epicuticular wax surrounding the stomata, a wrinkled cuticular surface, and reticulate-shaped glands, all of which encourage the retention

and accumulation of PM (Panes and Zamora 1991; Liu et al. 2012). As such, the stomata were able to uptake fine PM with a diameter below 1000 nm (e.g., 931.4 nm and 840.2 nm) (Fig. 3f).

B. monosperma

Both surfaces of *B. monosperma* leaves were covered at high density with large trichomes with similar surface morphology types (513.4 μm and 355.5 μm) (Figs. 4a and c). This morphology facilitated deposition of large particles up to $173.4 \times 55.76 \mu\text{m}$ (Fig. 4b) on the adaxial surface and below 17.32 μm on the abaxial surface (Fig. 4d).

A. scholaris

The leaves of *A. scholaris* have projection-like structures on both surfaces near the milk secretor glands with epicuticular wax (*Apocynaceae*). The presence of trichomes and appendages facilitated high deposition and accumulation of PM in the fine ($\text{PM}_{2.5}$) to respirable (PM_{10}) range on the adaxial surface (1.41 to 4.40 μm) (Figs. 5a and b). However, the significant deposition of PM damaged the leaf surface, leading to accumulation of PM inside the leaf (Fig. 5c). In addition, trichomes were found in higher density on the abaxial surface than on the adaxial surface (Figs. 5d and e), and fine PM deposits (0.54 μm to 3.21 μm) were observed on the rough surface of the groove in the mid-rib portion of the leaf (Fig. 5f). The waxy layer covering the surface of the gland facilitated entrapment of PM (Figs. 5g, h). The deposited PM aggregated and accumulated on the abaxial surface, thus forming a deposition layer over the surface (Fig. 5i).

A. indica

The leaves of *A. indica* also showed different micro-morphological characteristics when compared to other plants. On the adaxial surface, trichomes were present only on the midrib portion (Figs. 6a and b). The morphological features of the trichome surface facilitated the accumulation of PM on the surface (Fig. 6c). The presence of stomata and the raised surface of the epidermal cell wall make the abaxial surface rougher; this surface showed more PM deposition than the adaxial surface (Figs. 6d and e). Moreover, the abaxial surface had stomata that facilitated PM accumulation through stomatal pores (Fig. 6e). Granulated epicuticular wax and the raised epidermal cell wall structure also have been shown to enable PM accumulation on both surfaces (e.g., Pal et al. 2002).

S. siamea

On the adaxial surface of *S. siamea* leaves, dust-loaded epidermis, irregular patches of an epicuticular waxy layer, and dense trichomes were observed (Figs. 7a, b). Grooves were found at the surface of the mid-rib area; these grooves were covered with a wax lining that facilitates the deposition of small PM (Fig. 7c). The mean length of the observed trichomes was 60.76 μm , with deposition of PM with sizes up to the fine range (712.2 nm to 10.32 μm) (Fig. 7d). Moreover, PM was deposited in and around stomatal openings on the adaxial surfaces (Fig. 7e). Hence, PM-bound toxic metals can potentially accumulate in stomata on the leaf surface (Gajbhiye et al. 2016c). Similarly, the abaxial surfaces showed high trichome density, epicuticular wax, and stomata that facilitate deposition and increase the efficiency of PM accumulation (Figs. 7f, g, and h). Fine PM (e.g., 152.8 nm to 714.5 nm) was deposited over the stomata and readily accumulated in the internal stomatal pores ($7.823 \times 2.127 \mu\text{m}$) (Figs. 7i and j).

As described in section 3.1, the concentration levels of target toxic metals varied considerably across the different sample types such as foliar dust, road dust, and leaves. Comparison of the data with those of previous studies indicated that the samples were significantly enriched with the target metals. The lack of correlation between leaf vs. soil samples indicates that metals can accumulate in leaves from foliar dust or road dust resuspension, suggesting the significance of metals of airborne origin (Gajbhiye et al. 2016c). Likewise, the EF results demonstrated that the Pb, Cd, and Cu in the foliar dust, leaf, and road dust samples arose from anthropogenic activities. The EFs of the various samples can be ranked as follows:

(leaf > foliar dust > road dust > soil). Thus, the metals in the leaves came mainly from airborne sources (e.g., traffic sources and other urban sources) and not from soil.

Leaf surface roughness is determined by grooves and bulges of arranged epidermis cells, the presence of epicuticular wax, secretor glands, veins, and number/density of stomata and trichomes on both surfaces (Pal et al. 2002; Wang et al. 2013; Simon et al. 2014; Gajbhiye et al. 2016c). Epicuticular wax was observed on surfaces of *S. siamea* and *A. scholaris* leaves, indicating that these leaves can accumulate PM (e.g., Wang et al. 2015). *S. siamea* showed more diverse leaf surface micro-morphological features (epicuticular wax, stomata, and trichomes) compared to other plant species (Figs. 7a-j, Table 1). Moreover, the amount of retained PM has been shown to be directly proportional to the numbers of stomata and trichomes on both leaf surfaces (Liu et al. 2012; Chen et al. 2017). However, the particle type and particle diameter class and assessment scale should all be considered in order to accurately evaluate the comprehensive particle retention abilities of an urban tree species (Liu et al. 2018). Hence, based on the micro-morphological plant leaf features and the accumulation pattern of PM (or PM-bound toxic metals), the six plants are ranked based on their accumulation potential as follows: *S. siamea* > *A. scholaris* > *M. indica* > *F. religiosa* > *A. indica* > *B. monosperma*.

Conclusions

This study was carried out to investigate the possibility of effectively monitoring and controlling airborne PM pollution (along with PM-bound toxic metals of Pb, Cd, and Cu) in urban environments by evaluating the characteristics of urban tree leaves. EF analysis of the different sample concentrations revealed that the metals mainly originated from airborne sources (traffic and industrial emissions). SEM analysis of the plant leaf surfaces (*F. religiosa*, *M. indica*, *B. monosperma*, *A. scholaris*, *A. indica*, and *S. siamea*) revealed that surface roughness is influenced by the presence of micro-morphological appendages such as stomata, trichomes, epicuticular wax, and the epidermal cell wall. Each plant leaf has distinct characteristics that facilitate the retention and accumulation of PM in different size ranges. Moreover, stomata play an important role in the absorption of PM/bound toxic metals. Based on SEM and statistical analyses, the leaves of *S. siamea* showed the highest metal accumulation potential. Metal accumulation was also significant in *A. scholaris* and other plants. Based on our findings, these plants can be used for PM (PM-bound toxic metals) phytomonitoring and management in urban environments.

Declarations

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Statements and Declarations

No potential conflict of interest was reported by the author(s).

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Figures

Figure 1

Metal concentrations in the four samples at the six study sites ($\mu\text{g g}^{-1}$, $n = 3$). The differences in metal concentrations in different sample types were analysed by one-way ANOVA.

Figure 2

Scanning electron micrographs of *F. religiosa* leaf surfaces showing PM deposition and accumulation: (a), (b), and (c) adaxial leaf surface; (d), (e), and (f) abaxial leaf surface.

Figure 3

Scanning electron micrographs of *M. indica* leaf surfaces showing PM deposition and accumulation. (a), (b), and (c) adaxial surface; (d) and (e) abaxial surface. (f) PM accumulation inside stomata on the abaxial surface.

Figure 4

Scanning electron micrographs of *B. monosperma* leaf surfaces showing PM deposition and accumulation. (a) and (b) adaxial surface; (c) and (d) abaxial surface.

Figure 5

Scanning electron micrographs of *A. scholaris* leaf surfaces showing PM deposition and accumulation. (a), (b), and (c) adaxial surface; (d), (e), (f), (g), (h), and (i) abaxial surface.

Figure 6

Scanning electron micrographs of *A. indica* leaf surfaces showing PM deposition and accumulation. (a), (b), and (c) adaxial surface; (d), (e), and (f) abaxial surface.

Figure 7

Scanning electron micrographs of *S. siamea* leaf surfaces showing PM deposition and accumulation. (a), (b), (c), (d), and (e) adaxial surface; (f), (g), (h), (i), and (j) abaxial surface.

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