Discriminating foliar adhered from metabolised Pb when monitoring vegetation exposed to windborne contamination

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Discriminating foliar adhered from metabolised Pb when monitoring vegetation exposed to windborne contamination

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Abstract

Monitoring heavy metals in vegetation near mining or industrial sites is crucial for detecting plant contamination; requiring discrimination between metals adhered to foliar surfaces from the internal concentrations. We investigated key factors that might contribute to lead (Pb) accumulation in leaves of local vegetation near a Pb mine: (i) distance from the pollutant source, (ii) morphological characteristics of leaf surfaces, (iii) their susceptibility to Pb loss by washing, and (iv) the effect of contrasting washing reagents in Pb removal. Native plant species were sampled at three field locations, possessing different leaf surface morphologies: glabrous (smooth), resinous (waxy) and hirsute (hairy). After washing with Citranox, EDTA or deionised water, Pb contents were assessed by ICP-OES and SEM-EDX. We observed an order of Pb (and other metals) retention from hirsute > resinous > glabrous, and found: i) greater Pb accumulation in leaves near the mine due to particulate matter (PM) deposition; ii) hirsute leaves retain the highest PM-Pb; iii) higher Pb removal (10-fold) by Citranox and EDTA compared to water; and iv) hirsute leaves retained considerable PM-Pb underneath trichomes despite washing, leading to Pb overestimation. Therefore, for accurate Pb monitoring, washed glabrous leaves are best indicated due to their negligible PM retention.

Keywords: air pollution; heavy metals; lead; leaf morphology; particulate matter, soil, aeolian, dust particles

Environmental Implication

Mining can be a source of environmental pollution, arising from tailings, mine drainage and dust spread by wind. Lead (Pb) is a toxic metal that contaminates soil, water, and often the air near mining sites, a significant pathway for human exposure. Monitoring metal pollution in vegetation is crucial for detecting Pb contamination sources. We show how foliar Pb deposition increases closer to the mine, accumulating particularly in hairy leaves (trichomes), confounding estimates of internal Pb (metabolised) with surficial Pb (particulate matter). Our results highlight that simple washing procedures with Citranox and sampling of smooth-surfaced leaves avoids overestimation of foliar Pb caused by dust retention.

Introduction

Metal and metalloid mining is a crucial activity that provides resources for the current industrial society, producing millions of tons of heavy metals annually, especially lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), manganese (Mn), barium (Ba) and chromium (Cr) (Alloway et al., 2013; Tibbett, 2024), with a market capital value of almost a trillion US dollars (Macklin et al., 2023). Yet, mining is also one the biggest sources of heavy metal pollution to the environment, including water, soil and air contamination (Nriagu 1996; Kabata-Pendias and Mukherjee, 2007; Gonzalez-Chavez et al. 2023; Li et al., 2023). Pollution resulting from mining activities can stem mainly from mine drainage, tailings, spreading of minerals during transportation or dust deposition spread by wind (Liao et al., 2008). It has been recently estimated that at least 23 million people are exposed to dangerous metal concentrations as a result of mining waste and tailings from both inactive and active mines (Macklin et al., 2023). Heavy metals also pose a risk when they accumulate in soils and plants, disrupting ecosystem functioning and potentially entering the food chain, in a critical pathway of biomagnification, as consumers ingest contaminated food sources (Tibbett et al., 2021).

Monitoring heavy metal levels in vegetation is an important practice to detect contamination in the environment, indicating potential exposure pathways and health risks to humans and animals in the affected area. Multiple factors may influence the accumulation of heavy metals in plants, including edaphic conditions, proximity to pollution source, and plant specific characteristics, such as physiology and morphology (Cuevas et al., 2023). Metal accumulation in plant leaves is generally associated with soil contamination, where ions move into the soil solution, becoming available for root uptake and eventual translocation to shoots (Tibbett et al., 2021). However, airborne particulate matter (PM) is also a common source of pollution, especially in dry environments, urban or near mining sites where dust is frequently emitted and dispersed (Gao et al., 2021; Augustsson et al., 2023; González-Chávez et al., 2023; Gajbhiye et al., 2024). In particular, Pb is an element with high emission rates into the atmosphere (Kabata-Pendias and Mukherjee, 2007), for which topsoil and vegetation are major sinks for PM deposition, especially around active mining sites (Ghaderian et al., 2007; Yang and Cattle, 2017; González-Chávez et al., 2023; Ma et al., 2023). While baseline levels of Pb in soils are around 17 mg kg^{-1} (Steinnes, 2013), topsoils near a Pb smelter were shown to have up to 540 mg kg^{-1} Pb, more than 10 times higher than reference areas (Douay et al., 2008). Humans are also exposed to Pb via atmospheric PM, such as reported by Zheng et al. (2021), in which Pb-bearing dust was a key health issue in Mount Isa, a city close to a Pb mine in Queensland, Australia, with ingestion of surface contaminated fruits and vegetables being a major exposure route.

Considering that Pb has low mobility inside plants, generally accumulating more in roots than in leaves (Souza et al., 2012; Castro-Bedriñana et al., 2018), monitoring foliar Pb can be highly influenced by PM deposition when near mining or processing facilities, as internal concentrations may be modest. Furthermore, leaf morphology can also play a major role in PM retention, as dust interception depends on leaf structure, phyllotaxy, presence of trichomes, number of stomata, presence of wax and size of petioles (Prajapati and Tripathi, 2008; Prigioniero et al., 2023). In particular, hirsute (hairy) or waxy leaves are especially prone to PM and metal surface accumulation (Prajapati and Tripathi, 2008; Sanchez-Lopez et al., 2015; Gonzalez-Chavez et al. 2023). However, hirsute leaves do not always retain more PM than glabrous (smooth) leaves, for example, Tiwari et al. (2023) showed that while bigger particles may retain more in rough or trichome-rich leaf surfaces, smooth leaves or with smaller trichomes, retain can smaller particles (respirable range) more efficiently.

Sampling is a crucial step in monitoring metal contamination and, while sampling methodologies and analytical techniques are often explored (Haghighizadeh et al., 2024), less attention has been given to sample preparation, such as washing methods. Heavy metal removal from leaves after washing has been observed by using water (deionised or distilled), diluted acids and detergents such as EDTA, HCl and Alcanox®, to varying results, depending on the metal in question (Porter, 1986; Oliva and Valdes, 2004; Peryea, 2005; Ram et al., 2014; Nawab et al., 2015; Barrios Arlanzon et al., 2019). For example, Oliva and Valdes (2004), observed no significant reduction of Pb after washing leaves with distilled water only, while Sanchez-Lopez et al. (2015) found Pb removal of 50-70% in leaves washed with Extran 2% (detergent) and HCl (10%), depending on the plant species.

Therefore, careful washing of leaves is an important practice to discriminate between internal and surface adhered contamination. Elevated heavy metal concentrations in plant leaves might indicate: a) soil and/water contamination, if metals are metabolised (internally) in plant tissues from root uptake and translocation to shoots; b) atmospheric deposition, when high metal contents are found mostly on the external surface of the leaves; or c) both cases, in which contamination arises from multiple pathways, including direct foliar absorption. However, to better inform intervention and remediation actions and avoid under- or over-estimation of metal concentrations, metabolised Pb should be differentiated from PM deposition as part of the monitoring process. Metabolised Pb will have phytotoxic implications, such as enzyme inactivation, and other systemic nutritional and metabolic disturbances (Zulfiqar et al., 2019), that are quite different from the general PM deposition effects, which affect primarily the leaf surface, increasing shading, decreasing photosynthesis or gas-exchange and altering leaf morphology (Rai, 2016).

Here we have investigated three straightforward washing procedures to distinguish internal and surface adhered Pb in different plant species with contrasting leaf surface morphologies, sampled at multiple locations near a lead mine which was a source of PM. We hypothesized that: i) Pb deposition would be higher in plants closer to the mine as a result of higher aeolian PM, compared to farther sites; ii) individuals with hirsute or resinous leaves would present higher Pb concentrations due to surficial PM entrapment by trichomes or wax, respectively, and iii) foliar characteristics (glabrous, resinous, hirsute) and trichomes affect Pb removal efficiency by different washing solutions; iv) aqueous Citranox^{TM} and EDTA solutions (chelating agents) are more effective in Pb removal than deionised water. These were tested by sampling leaves of different plant species presenting contrasting leaf surface morphologies, at different distances to the Pb source, and assessing Pb concentrations (and other metals) using ICP-OES and confirming our results with visualisation under electron microscopy (SEM).

2. Material and Methods

2.1 The site selection

Three sites were selected around a lead carbonate mine ($PbCO_3$), about 35 km west of Wiluna (Western Australia), an area with a long history of mining activities, especially for gold extraction. The climate in the region is arid and it receives c. 180 mm of rainfall per year on average, most of which falls in summer and autumn months, with wind direction predominantly from the Northeast, based on 1,734 observations from 1975-2016 (The Bureau of Meteorology, 2024).

As this study aims to discriminate leaf surface Pb (due to PM deposition) from internal foliar Pb concentrations to better inform monitoring schemes, we selected these locations based on their distances from the mining areas and tailing deposits. The three sites (Fig. 1; Table S1) were categorised based on their distance from the Pb source, which we hypothesised to present different likelihoods of Pb-contaminated PM accumulating on leaf surfaces of the surrounding vegetation: 1) "Site 0" was located within a Pb-concentrate tailing storage area, 2) "Site 1", located at 1.1 km from the tailing storage, and "Site 2", located approximately 7.2 km away from both Site 0 and Site 1 (Fig. S1), but in the vicinity of another Pb ore body allowing some limited Pb deposition. Considering the predominant wind direction (NE), Site 2 is expected to be downwind in relation to the mining site (Site 0).

Figure 1. Sampling locations, "Site 0" located within a Pb-concentrate tailing storage area, "Site 1", located at 1.1 km from the tailing storage, and "Site 2", located approximately 7 km away from both Sites 0 and 1. Yellow arrow indicates the mining site.

2.2 Sampling

Leaf sampling occurred 10 months after mining activities in the area ceased for care and maintenance, in February 2012, with an atypical rainfall of < 10 mm compared to the average in that month of c. 40 mm. Individual plants that were targeted for sampling were growing within 30 m of the marked GPS location (Fig. 1, Fig S1).

Samples were taken at breast height (1.3 to 1.5 m) for all species and involved the excision of a small branch (with pruning shears), comprising a sufficient number of mature leaves to allow replication of washing treatments; two individual plants were selected to represent each of three species common to the locality (Table S2). Sampling of small branches rather than individual leaves retained freshness of individual leaves prior to analysis. Care was taken to avoid sampling damaged growth. Upon excision, each sample was immediately placed in a re-sealable polyethylene bag and placed into an ice box to maintain sample freshness. All samples were kept in darkness at approximately 4^{o}C until processed in the laboratory; a period of not more than six days.

The same species were targeted for sampling at each site, with similar surface characteristics that were hypothesised to influence dust retention, categorised here as Leaf Types: *Acacia pruinocarpa* Tindale (Fabaceae), representing the glabrous (smooth) type, *Acacia aneura* F.Muell. ex Benth., representing the resinous (waxy) type, and *Solanum lasiophyllum* Dunal (Solanaceae), as the hirsute (hairy, with non-glandular stellate trichomes) leaf type (Fig. 2). We should note that some individuals of *S. lasiophyllum* were shorter than the Acacia species, where samples were taken from the top, at around 1 meter tall.

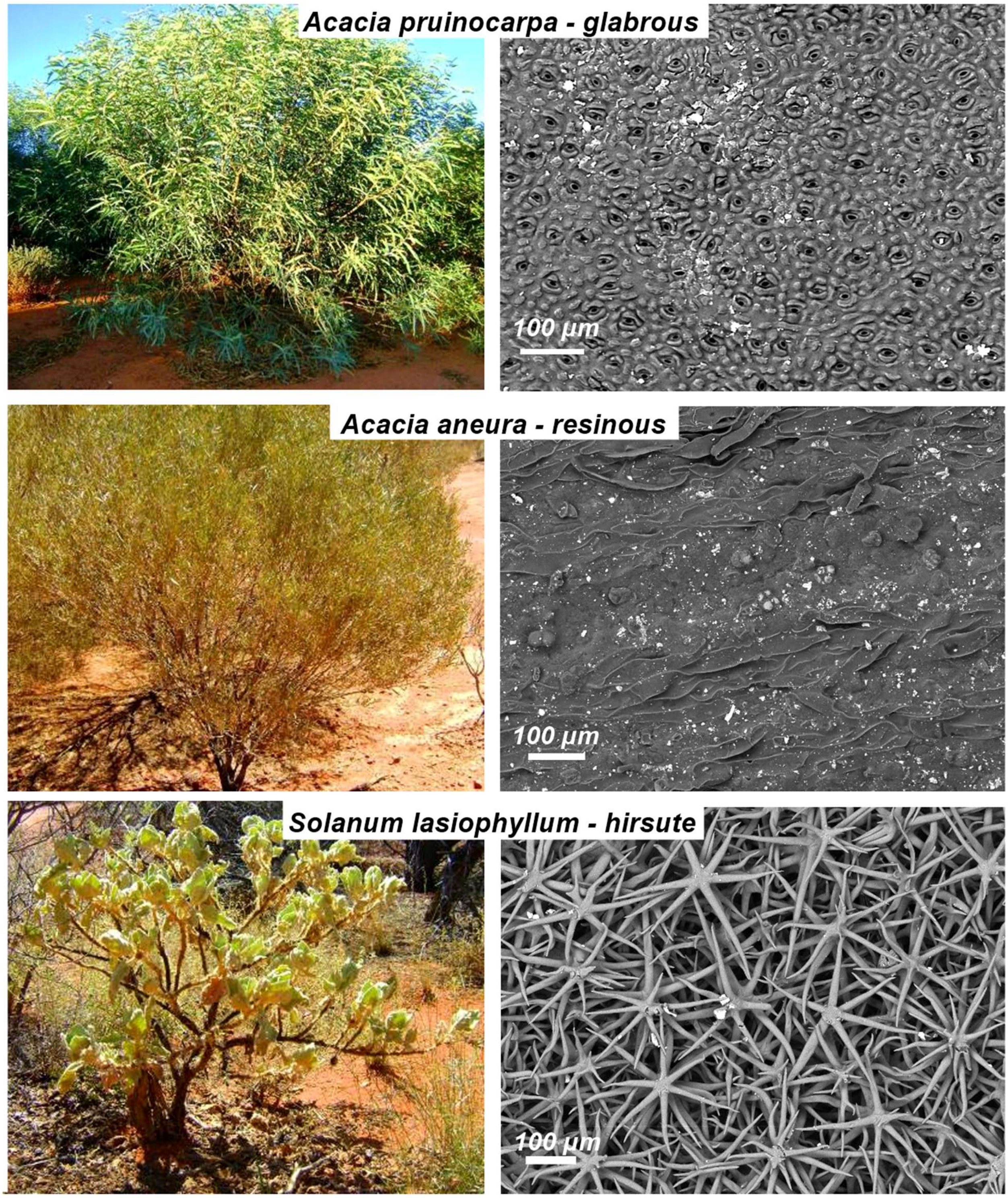

Figure 2. Images of the three selected species (left) from the field site and their respective scanning electron microscopy (SEM) images (right). Bright spots correspond to heavy mineral particles (scale: 100 µm). These images illustrate the three foliar types assessed in this study with glabrous (smooth), resinous (waxy) and hirsute (hairy) surfaces.

2.3 Washing treatments

All mature leaves were removed from the branches of the two sampled individuals (representing each species) and were pooled to comprise one composite sample. Each composite sample was thoroughly mixed and divided into two subsample groups, then again mixed and divided to obtain four homogenised subsamples of roughly equal mass (Table S2).

Each subsample was subjected to one of four washing treatments: 1) leaves washed in 0.01 mol L^{-1} ethylene di-amine tetra-acetic acid (EDTA); 2) leaves washed in 1% Citranox™ (a commercial blend of surfactants and weak organic acids); 3) leaves washed in purified de-ionised (milli-Q) water, and 4) leaves left unwashed. Subsamples designated for washing were manually shaken for one minute in 100 mL of solution in a closed container (c. 4 Hz). Owing to their larger size, leaves of *A. pruinocarpa* were washed by the same method, but in 200 mL of solution. After these treatments, a 10 mL aliquot of the washing solutions were taken for analysis. Washed leaves were then rinsed in running de-ionised water and patted dry with paper towels.

2.4 Sample preparation and analyses

After washing procedures, a few leaves per subsample were selected at random with tweezers and set aside for scanning electron microscopy. All remaining leaves were then dried in a ventilated oven at 65°C to a constant weight (c. 72 hours). Leaf subsamples were then homogenised by milling into a fine powder using a Sunbeam EM0400 multi-grinder. For each subsample approximately 0.15 g of ground leaf material was used for leaf digestion using HNO_3 (3 mL) and $HClO_4$ (1 mL) (Zasoski and Burau, 1977). For quality control, we included two blanks (no samples added) and an in-house reference material (*Medicago sativa*); our plant elemental analysis is certified by ASPAC (Australasia Soil and Plant Analysis Council) for several elements, including Pb. Digested extracts were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) using a Perkin Elmer Optima 5300 DV optical emission spectrometer. The limit of detection for Pb was of 0.44 mg kg^{-1}; due to the discrepancy in Pb concentration in our reference material in relation to our analysed samples, we refrained from providing recovery rates.

Considering that a known mass of leaf material (g) for each sample was washed in a known volume of solution, it was possible to determine the surface Pb concentration per unit of leaf mass that was removed during washing in mg kg^{-1}, instead of mg L^{-1}. Therefore, we determined the Pb present in the washing solution (Surface Pb, mg kg^{-1}), the Pb concentration in digested leaves (Foliar Pb, mg kg^{-1}), and the digested foliar Al and Fe concentrations (mg kg^{-1}).

2.5 Scanning Electron Microscopy of leaf surfaces

From each leaf sample that was designated for microscopic examination, a small section (3-5 mm^2) of leaf tissue was excised from an area adjacent to the mid rib and halfway between the leaf base and tip. Each of these was affixed to a 12.6 mm, pin type scanning electron microscope (SEM) mount with double sided carbon tape. To enhance conductivity of particularly thick and/or hairy samples, conductive carbon paint was applied to sample edges. All samples were then coated in graphite carbon to a thickness of approximately 20 nm. Samples were examined under a Tescan Vega3 SEM fitted with an Oxford Instruments X-Max 50 silicon drift, energy dispersive x-ray microanalysis system (EDS).

In order to verify if Pb-bearing particles were still present in the hirsute leaves after washing (beneath trichomes), images were also taken from Citranox-treated leaves after trichome removal, for EDX (Energy Dispersive X-ray Spectrometry) readings. For this, leaf surfaces were carefully scraped with a flat edged scalpel blade, cleaned with ethanol between samples. The X-ray spectra were obtained at a working distance of 15 mm and voltage of ≤ 15 KeV, from targeted grains of dust or surface soil materials identified in backscattered electron images, and AZtec software was used to identify elements from spectral patterns. All SEM imaging and analyses were conducted at the Centre for Microscopy, Characterisation & Analysis at The University of Western Australia.

2.6 Data Analysis

All variables were transformed by $x^{1/4}$ to attain a normal distribution, and before multivariate analyses, data was z-scored normalised to render the variables in the same scale (Legendre and Legendre, 2012; Dago et al., 2021). To better summarise the data and observe particular patterns, a Principal Component Analysis (PCA) was carried out in the transformed data set using seven variables: Surface Pb, Foliar Pb, Al, Cu, Fe, Zn, and delta Pb, which is the difference in foliar Pb concentrations between unwashed and washed samples. The multivariate variability in these samples was captured by a Euclidean distance matrix, which was further used for a Three-Way PERMANOVA (Permutational Multivariate Analysis of Variance), with 9999 permutations for main effects and pairwise tests (Anderson, 2017), at 5% significance level. Factors compared were: Site (0, 1, 2), Wash type (EDTA, Citranox, water) and Leaf type (glabrous, resinous, hirsute).

Univariate PERMANOVA (9999 permutations, $\alpha = 0.05$) was carried out to detect differences between factors for each variable, separately, (9999 permutations, $\alpha = 0.05$). All statistical analyses were performed using the software Primer-e v7 with the PERMANOVA+ add-on (Anderson et al., 2008; Clarke and Gorley, 2015).

3. Results

3.1 Multivariate analyses show hirsute leaves association with Pb, Al and Fe

The PCA of seven metal variables was able to explain 89.7% of the data variability (Fig. 3), and showed a clear separation between leaf types, in which hirsute leaves from Sites 0 and 1 were associated with both foliar Pb and the surface Pb removed in washing, while hirsute leaves from Site 2 were associated with higher amounts of other metals (Al, Zn, Cu and Fe). All glabrous leaves were negatively correlated with all metal concentrations, regardless of sampling sites. Interestingly, resinous leaves had an intermediate pattern, comparable to the glabrous leaves when sampled from Sites 2 and 1, but closer to hirsute leaves when sampled at Site 0, bearing higher Pb concentrations.

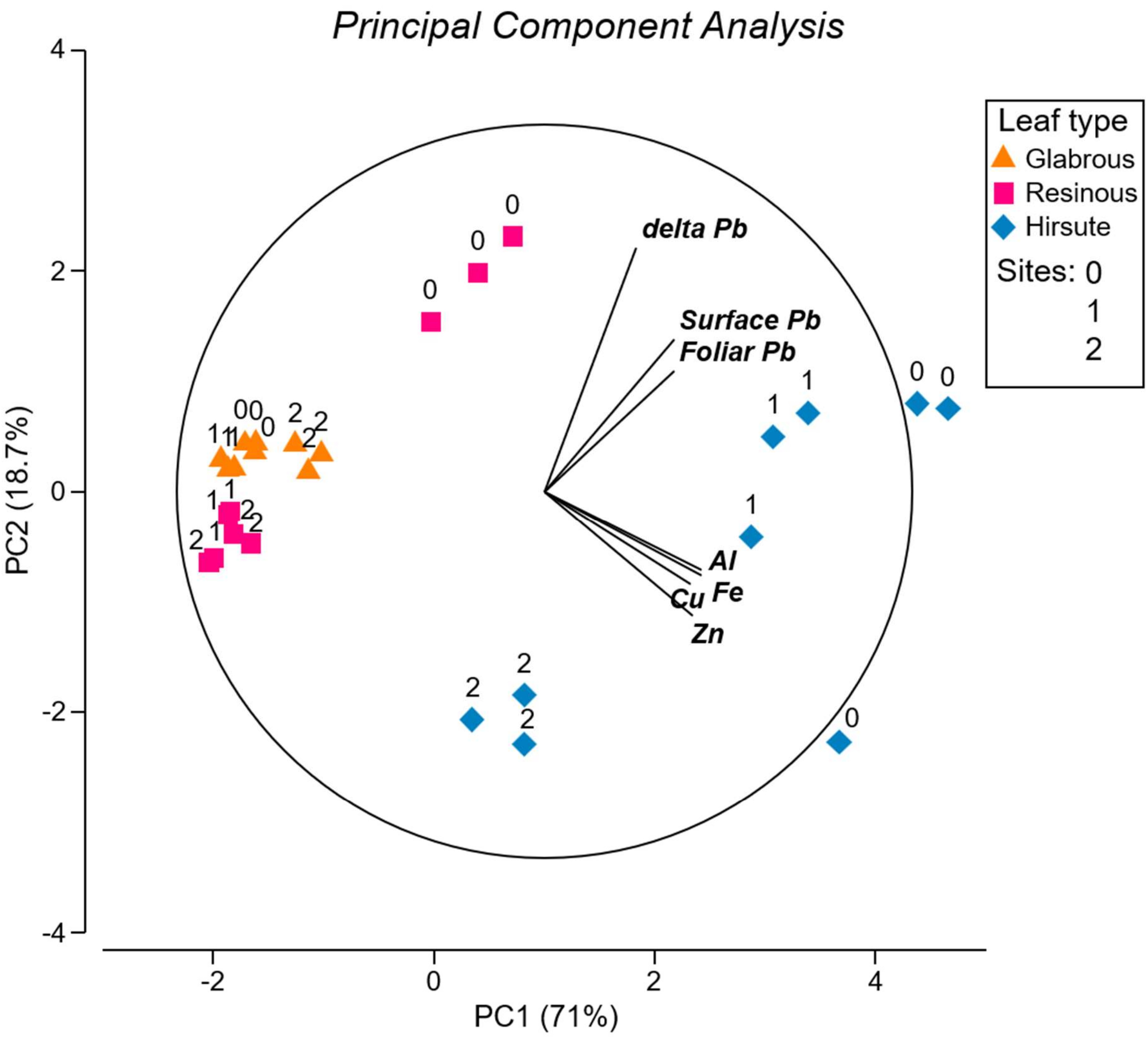

Figure 3. Principal component analysis using seven variables from leaves sampled in three different Sites: 0, 1 and 2; three leaf types: glabrous (smooth), resinous (waxy), and hirsute (hairy), under different washing treatments. Surface Pb: Pb concentration in the washing solution; Foliar Pb, Al, Cu, Fe and Zn: internal concentrations in washed leaves, delta Pb (the difference in foliar Pb concentrations between unwashed and washed samples): foliar $Pb_{unwashed}$ – foliar Pb_{washed} samples).

Site 0 was mostly correlated to the Pb variables, but only for the hirsute and resinous leaf types (Fig. 3). Site 1 was also correlated to Surface and Foliar Pb, but only for hirsute leaves, while most resinous samples and all glabrous samples (from all sites) were negatively correlated to all metal concentrations. PERMANOVA detected significant multivariate differences among all leaf morphologies, and the three site locations ($p < 0.01$).

3.2 EDTA and Citranox are more efficient in Pb removal

When analysing each variable individually, it is clear that leaves from Site 0 had overall more surface Pb removed by washing than Sites 1 and 2, of 30 mg kg^{-1} on average (Fig. 4a), and a similar pattern was seen in the Foliar Pb concentrations, reaching levels of around 120 mg kg^{-1} Pb (Fig. 4b). Leaves from the Site 2 presented overall much lower Pb concentrations, with only 1 mg kg^{-1} of surface Pb removal, and around 5 mg kg^{-1} of internal foliar Pb (Figs. 4a, 4b).

Both EDTA and Citranox solutions presented great efficiency in removing surface Pb, with washed-off Pb concentrations being 18-fold and 13-fold higher than in water treatments, respectively (Fig. 4c). Naturally, these different washing solutions did not influence internal foliar Pb (Fig. 4d).

3.3 High Pb accumulation and removal from hirsute leaves

Leaf surface morphology had a significant impact on surface Pb removal, in the order of hirsute > resinous > glabrous (Fig. 4e), with only 0.8 mg kg^{-1} Pb being removed from the latter. As for the internal Pb concentrations, no differences were detected between glabrous and resinous leaves, but both were significantly lower than in hirsute leaves (92 mg kg^{-1}) (Fig. 4f). Washed hirsute and resinous leaves presented almost half of the foliar Pb detected in unwashed leaves, while it had little difference on glabrous types (Fig. 4f).

Considering total Pb as the internal foliar Pb + washed-off Pb concentrations, we observed that EDTA and Citranox could remove on average 50% of the total Pb by washing off dust particles from hirsute and resinous leaves, with EDTA removing up to 83% (Table S3). In glabrous leaves, however, these solutions have little effect, removing only 16% of the total Pb, and in some cases as little as 3 and 5%, for Citranox and EDTA, respectively.

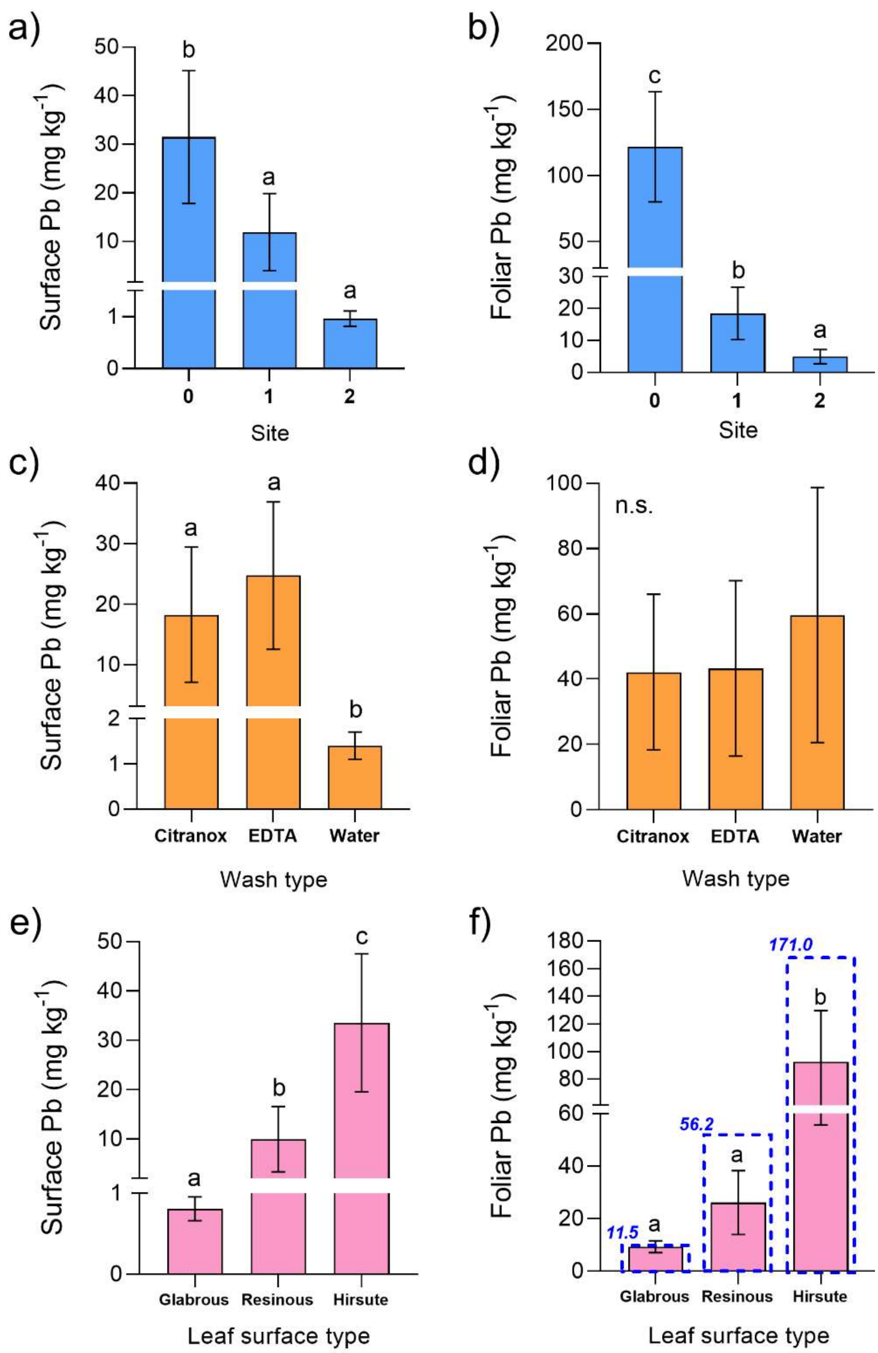

Figure 4. Pb concentration (a, c, e) from leaf surfaces after washing with EDTA, Citranox or water, from three different sampling sites (0, 1, 2) and leaf morphological types: glabrous (smooth), resinous (waxy), and hirsute (hairy). Foliar Pb concentrations (b, d, f) were determined in all samples by acid digestion. Displayed values are averages (n = 9) with standard error. Different letters correspond to significant pairwise differences, detected by PERMANOVA ($p < 0.05$). Values displayed with the dotted bars in f) correspond to mean values from unwashed samples.

3.4 Foliar Fe, Al and other metals

Although we did not assess Fe and Al concentrations in the washing solutions, these elements were determined in the digested samples after washing. Leaves from the site closest to the mine (Site 0), presented higher Fe and Al concentrations than the other sampling sites, on average 718 and 510 mg kg^{-1}, respectively (Figs. 5a, 5b). Interestingly, unlike with foliar Pb or Al (Fig. 5d), washing with EDTA significantly increased Fe concentrations within leaves (Fig. 5c).

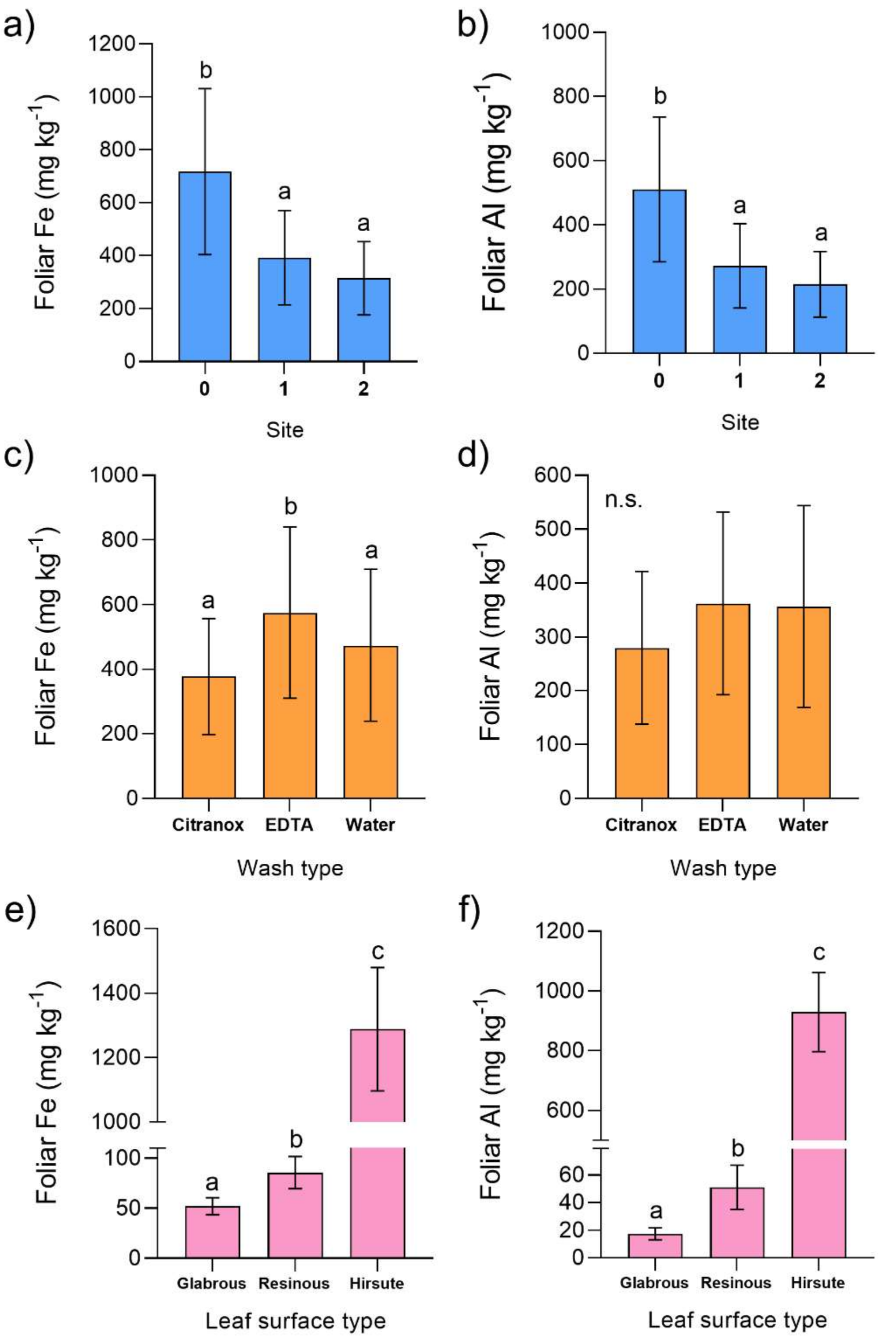

Figure 5. Foliar Fe (a, c, e) and Al (b, d, f) from different leaf types (glabrous, resinous, hirsute), sampled at three different sites (0, 1, 2), after washing with EDTA, Citranox or de-ionised water. Displayed values are averages (n = 9) with standard error. Different letters correspond to significant pairwise differences, detected by PERMANOVA ($p < 0.05$).

As with Pb, Hirsute leaves also presented the largest Fe and Al levels, of around 1,290 and 930 mg kg^{-1}, respectively, with their concentrations following a similar pattern of hirsute > resinous > glabrous (Figs. 5e, 5f). Hirsute leaves also had higher Cu and Zn levels and resinous leaves presented the highest Mn concentration, 10 times higher than glabrous and 2.5 times than hirsute leaves (Table 1).

Table 1. Internal foliar metal concentrations from different leaf types (glabrous, resinous, hirsute), sampled at three different sites, after washing with EDTA, Citranox or de-ionised water[1]. Values are the average ± standard errors (n = 9).

Factor	Cu	Mn	Mo	Zn
	mg kg^{-1}			
Site	n.s.	***	n.s.	**
0	11.7 ± 4	144 ± 34 **a**	0.94 ± 0.1	55.4 ± 23 **b**
1	10.4 ± 3	46.6 ± 10 **b**	0.82 ± 0.1	42.5 ± 17 **a**
2	8.0 ± 1	205 ± 66 **a**	0.88 ± 0.1	33.6 ± 12 **a**
Wash type	n.s.	n.s.	n.s.	n.s.
EDTA	9.0 ± 2	133 ± 50	0.84 ± 0.1	36.0 ± 13
Citranox	10.2 ± 3	136 ± 52	0.95 ± 0.1	43.7 ± 17
Water	10.8 ± 3	127 ± 45	0.85 ± 0.1	51.8 ± 22
Leaf type	***	***	n.s.	***
Glabrous	6.1 ± 0.5 **b**	29.1 ± 3 **a**	0.93 ± 0.1	7.5 ± 0.2 **a**
Resinous	4.3 ± 0.2 **a**	261 ± 57 **c**	0.82 ± 0.1	13.9 ± 0.4 **b**
Hirsute	19.6 ± 2.5 **c**	105 ± 21 **b**	0.90 ± 0.1	110 ± 13 **c**

[1] - Asterisks symbolise significant effects detected for each factor: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ or n.s. $p \geq 0.05$. Different letters represent significant pairwise differences between metal concentrations within each factor (PERMANOVA, $p < 0.05$).

3.5 Particulate matter accumulation underneath trichomes

After careful removal of trichomes in a sample from Site 0 (high Pb) that had been washed with Citranox, it was clear that many PM remained adhered to the leaf surface, as shown under SEM (Fig. 6). EDX spectra taken at different spots showed the presence of high Pb-bearing particles, as well as other elements such as Al, Fe, silicon (Si) and titanium (Ti).

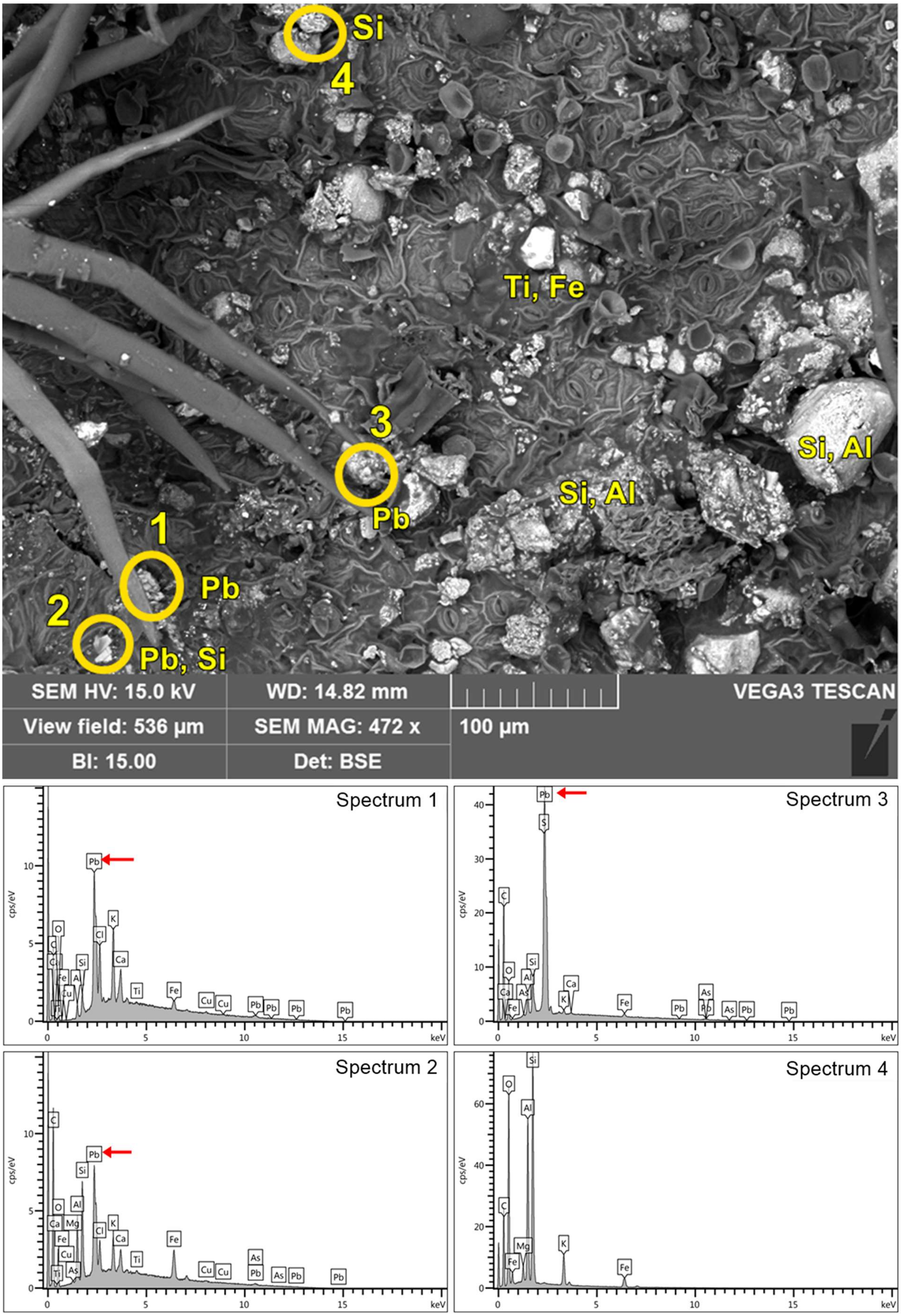

Figure 6. Backscattered electron micrograph of a hirsute leaf surface (*S. lasiophyllum* from Site 0) after Citranox washing and trichome removal. Circles indicate particulate matter of various sizes and compositions, from which EDX spectra were taken (1-4, displayed below, red arrows indicate Pb spectra). Other metals detected at high levels are also displayed: Si, Al, Fe and Ti.

3.6 Foliar Pb concentrations after exclusion of hirsute leaves

Considering the high Pb retention found in hirsute leaves despite washing procedures, we analysed the data for foliar Pb excluding all hirsute samples (Fig. 7). It becomes evident that hirsute samples led to an overestimation of internal Pb in samples from Sites 0 and 1 (Fig. 4). The latter, which previously presented 20 mg kg^{-1} Pb (Fig. 4b), had an almost 10-fold decrease after exclusion of hirsute samples, to around 2.5 mg kg^{-1} (Fig. 7), which was statistically similar to levels found in Site 2 (7 km away from the mine) (PERMANOVA, $p > 0.05$). With only glabrous and resinous samples, we found no significant differences between efficacy of washing solutions in Pb removal (Fig. S6), therefore the efficacy of washing solutions also depend on leaf morphology.

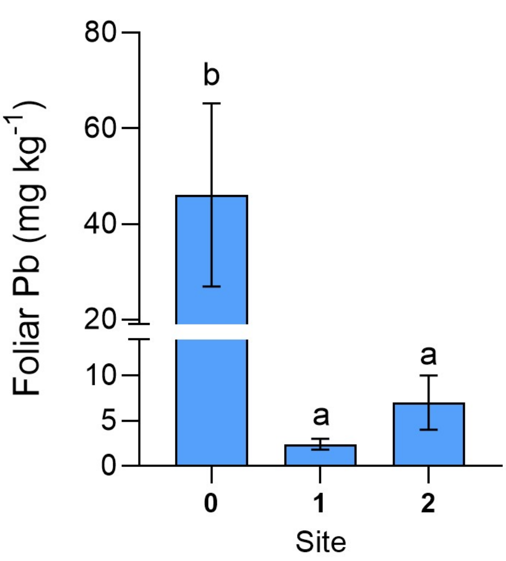

Figure 7. Foliar Pb concentration determined by acid digestion in sample collected at three different distances from the Pb source. Hirsute leaves were excluded from this analysis. Displayed values are averages (n = 6) with standard error. Different letters correspond to significant pairwise differences, detected by PERMANOVA ($p < 0.05$).

4. Discussion

4.1 Is atmospheric Pb deposition related to distance from the mine?

Higher Pb accumulation and Pb removal by washing were found in sites closer to the Pb source, especially in the hirsute leaves from *S. lasiophyllum* (Sites 0 and 1) and in the resinous *A. aneura* (Site 0). This confirms our initial hypothesis that distance to the mining site is an important factor in Pb PM accumulation, where Sites 0 and 1 accumulated high and medium levels of surficial Pb, respectively, implicating the mine as the primary source of airborne Pb. Atmospheric deposition of Pb is often the result of dust and PM emitted from Pb mines and tailings (Kabata-Pendias and Mukherjee, 2007; Liao et al., 2008; Zheng et al., 2021), as was the case in the current study. In the Australian mining town of Broken Hill, high topsoil concentrations of Pb were found to be emitted from the Pb mined orebody in a concentric manner at up to 0.8 km from the source, and isotopic analysis indicated such topsoil enrichment was mostly due to the dispersion of Pb-rich dust and PM, and not as a result of other sources, such as petrol or paint flakes (Yang and Cattle, 2017). According to Raji and Palamuleni (2023), agricultural products within 1 km of a gold mine could be detrimental to human health due to high levels of metals. In this work, the Pb concentrations found in washed leaves reached around 100 mg kg^{-1}, which are similar to values reported for vegetation close to a Pb/Zn mine in China, of 187 mg kg^{-1} (Hu and Ding, 2009), or close to a highly populated city in India, around 120 mg kg^{-1} (Gajbhiye et al., 2022). These are much higher than reported some urban European cities, found to be up to 15 mg kg^{-1} (Rodushkin et al., 2016; Pallavicini et al., 2018).

The distance effects on PM deposition observed in our data were greatly influenced by leaf morphology; and after removing hirsute samples from the analyses, we found very low foliar Pb concentrations in Site 1, similar to the values found in the farthest Site 2 (Fig. 7). Therefore, if glabrous leaves are targeted and adequately washed, a distance of 1 km could mitigate PM entrapment, allowing for more accurate discrimination between surface adhered and internal Pb, when monitoring vegetation near mining sites.

4.2 To what extent do leaf surface morphologies and trichomes affect Pb entrapment?

We show here that hirsute leaves tend to be more prone to general PM retention and consequently heavy metal retention, including Pb particles. Trichomes increase the leaf area by several fold, providing an additional surface for particulate retention (Corada et al., 2021), especially in leaves with stellate trichomes (Prigioniero et al., 2023), which are exactly the type found in *S. lasiophyllum*. Both the amount of surface Pb removed by washing and the internal Pb detected after acid digestion were significantly higher in hirsute leaves when compared to glabrous and resinous types. This pattern was followed for Cu, Zn, Fe and Al (Figs. 5e, 5f; Table 1) in and on the leaves. Sanchez-Lopez et al. (2015) reported elevated concentrations of Pb, Zn and Cu in unwashed hirsute leaves from individuals close to mine tailings and proposed their use as 'Phytobarriers' to prevent dust dispersion and limit the spread of heavy metals by wind.

Resinous leaves from *A. aneura* also presented higher superficial Pb concentrations than the glabrous type, but not as high as in hirsute leaves (Fig. 4e), suggesting an intermediate surface retention capacity. Higher PM retention in waxy or resinous leaves have also been indicated previously, as oil production and wax deposition is conductive of particle retention (Dzierzanowski et al., 2011; Li et al., 2024), although a systematic review has classified wax as having low effectiveness in trapping PMs in urban areas (Corada et al., 2021). Thus, glabrous leaves appear to be the most adequate foliar morphological type when monitoring internal Pb concentrations in vegetation close to mines or mine tailings, i.e. within 1 km radius, to avoid overestimation due to PM retention, although 100% PM removal is not guaranteed despite washing (Fig. S2). We should note that even after washing, Pb concentrations inside glabrous leaves may not stem solely from soil uptake, as Pb can be absorbed directly from PM adhered to the leaf surface (e.g. via open stomata) and only more refined techniques such as isotopic discrimination analyses will be able to provide a more informative picture into Pb pollution sources in the environment (Bi et al., 2018; Gao et al., 2021; Ma et al., 2023). Glabrous morphology is still preferable to hirsute types in monitoring and washing leaf field samples, as Gao et al. (2022) have recently demonstrated that trichomes can also enhance Pb absorption from airborne PM, possibly introducing another artefact during washing procedures.

4.3 Does leaf morphology affect washing efficacy?

Washing leaves decreased Pb concentration to various degrees, depending mostly on leaf morphological type and solution used (Fig. 4). As initially hypothesised, solutions based on chelating agents, i.e. EDTA and Citranox were more effective in surface Pb removal than deionised water, without influencing internal Pb concentrations. These results are in accordance with what was observed for Cu removal in different plant species, where Alconox or EDTA treatments had a higher removal rate in comparison to water, also less pronounced in glabrous leaves (Faucon et al., 2007). Here, the average Pb removal from glabrous leaves was from 13-19%, while those were of 22 to 63% for resinous, and from 30 to 49% in hirsute leaves (Table S3). This indicates that glabrous leaves are less susceptible to overestimation of accumulated Pb and able to provide more accurate representation of possible contamination, such as via soil, water and even direct absorption from PM in foliar surfaces (Fig. S2). Similarly, Barrios Arlanzon et al. (2019) observed different Pb concentrations in hirsute leaves of different plant species after various washing procedures, also finding higher Pb accumulation in the species with more hairy and sticky leaves.

Our results, along with the aforementioned studies, indicate that the efficacy of different washing solutions depend on leaf morphological types and that water washing, in particular, may not be enough to remove most metals from leaf surfaces (Adhikari and Struwig, 2024), as previously suggested by Ataabadi et al. (2012), especially from trichome-rich leaves. Indeed, running water was only able to remove around 66% of PM from leafy vegetables in urban environments and decrease its Pb contents (Augustsson et al., 2023), nonetheless, many other studies tend to only use water as the washing protocol, especially as to mimic household practices for vegetable washing (Ram et al., 2014; Xiong et al., 2014; Sharma et al., 2020; Gajbhiye et al 2022; Wu et al., 2022).

However, surface leaf morphology is often not a considered factor when water is selected for the washing procedure (Hu and Ding 2009; Serbula et al., 2012; Zalud et al., 2012; Xiong et al., 2019; Wu et al., 2022; Li et al., 2023; Raji and Palamuleni, 2023). For instance, Nawab et al. (2015), used water washing (deionised) while monitoring metal accumulation in the vegetation near a mining area, where samples of 23 plant species were collected, but the presence of trichomes or wax was not a considered factor. Similarly, leaf samples were washed with solely with water when monitoring metal pollution in dry riverbeds, including the species *Dittrichia viscosa* (Cuevas et al., 2023) known for its sticky leaves. In contrast, in the study by Gajbhiye et al. (2022), leaf morphology was a major factor when assessing PM retention in plants, yet only water washing was implemented.

Another reason for careful selection of washing reagents is that some of them may enhance cuticle permeability and even fix metals onto the leaf surface (Porter, 1986; Oliva and Valdes, 2004), also leading to metal overestimation. Such permeability alteration is possibly the reason we observed higher internal Fe in leaves treated with EDTA, as Fe-EDTA is a common fertiliser applied to crop leaves to correct Fe-deficiency, allowing foliar absorption (Rodríguez-Lucena et al., 2010). Considering the similarities between Citranox and EDTA in Pb removal, the former might be a better alternative as to avoid the infiltration of Fe into leaves.

Despite higher Pb removal from hirsute foliar surfaces in comparison to other leaf types, these leaves still presented larger internal Pb concentrations, as well as higher internal Fe, Al, Cu and Zn. Such results could be due to: 1) *S. lasiophyllum* may have an intrinsic capacity for metal accumulation, considering that other *Solanum* species have been considered for heavy metal phytoremediation, such as *S. nigrum* and *S. viarum* (Dou et al., 2022; Shukla et al., 2023); 2) its trichomes may enhance Pb foliar absorption (Gao et al., 2022) or act as a detoxifying sink for metals, as accumulation in the base of trichomes is a common mechanism for heavy metal tolerance in many hirsute leaves (Blamey et al., 2015; Ricachenevsky et al., 2021; Shukla et al., 2023); or 3) the stellate trichomes from hirsute *S. lasiophylluum* are a physical barrier that hinders the washing process, leading to overestimation of metals even after washing. Imaging via SEM shows that the latter is more likely, as many metallic particles remained adhered onto the surface of Citranox-washed leaves, beneath the trichomes, including Pb, Fe and Al-bearing minerals. It appears that airborne dust particles that become trapped in hirsute leaves eventually become deposited at the leaf surface. This probably occurs through the shaking motion of leaves as they are buffeted by breezes, or perhaps also through dynamic pressure differentials that would occur between the boundary layer of still, moist air at the leaf surface that is produced by the leaf hairs and the higher pressure, ambient moving air above the leaf hairs. We speculate that this difference in dynamic air pressure may result in the formation of eddies or drafts between the hairs, pushing trapped dust down to the leaf surface where the air is still. The PM size trapped in hirsute leaves are mostly over 10 μm, in line with the results reported by Tiwari et al. (2023), indicating that trichome-rich leaves are prone to retaining larger particles than smoother leaves.

Interestingly, the resinous leaves of *A. aneura* presented the highest Mn concentrations. Such elevated Mn may of course stem from residual dust remaining after washing, but because this was the only element found in a higher level compared to hirsute leaves, it seems that this may be a characteristic of *A. aneura*, as Mn was shown to be involved in deposition of cuticular waxes in resinous leaves (Hebbern et al., 2009; Alejandro et al., 2017). Thus, the mechanisms by which PM is effectively removed or retained deserves further investigation, considering PM sizes, metal type and the physical and chemical barriers found in foliar surfaces.

Conclusions

We show here that particulate matter deposition of Pb is a considerable fraction of the total foliar Pb near mining sites, depending on leaf morphological characteristics and the distance to the pollution source. This superficial Pb can be removed after simple washing procedures to different extents, mostly by EDTA and Citranox, and less so by deionised water, especially in hirsute leaves. Nevertheless, regardless of washing procedures, it is clear that leaf surface morphology is a crucial factor when monitoring Pb pollution in the environment, especially near mining sites and mine tailings, where higher PM dispersion and deposition are likely to occur. As shown here and in the literature, metal removal due to washing will depend on its chemical nature and interactions with metals, as well as the physical barriers from diverse foliar morphologies, with resinous and hirsute leaves being more prone to metal retention from air contamination. Therefore, in order to better assess internal Pb in plants and monitor the possible pathway of soil contamination, glabrous leaves appear to be a more reliable choice to avoid overestimation, provided they are not from a metal hyperaccumulator species. Although more costly, only isotopic studies will be able to determine Pb sources and discriminate between internal Pb due to soil uptake and Pb arising from direct foliar absorption.

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Supplementary files

Title: Discriminating foliar adhered from metabolised Pb when monitoring vegetation exposed to windborne contamination

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Table S1: Sampling sites selected for this study, GPS coordinates and likelihood of Pb accumulation in leaf surfaces.

Sites	GPS Coordinates (decimal degrees)	Elevation	Likelihood of surface Pb
Site 0	26°31'20.46"S 119°56'35.88"E	573 m	High
Site 1	26°31'13.88"S 119°57'18.91"E	548 m	Moderate
Site 2	26°35'16.00"S 119°55'43.39"E	556 m	Low

Table S2. Mean weights of composite foliage samples (± standard error) from two individuals of each plant species from each site (n = 4).

Site	Plant species	Leaf type	Mean sample dry weight (g)
0	*Acacia pruinocarpa* Tindale	glabrous	2.9 ± 0.14
	Acacia aneura Benth.	resinous	1.7 ± 0.11
	Solanum lasiophyllum Poir.	hirsute	0.8 ± 0.08
1	*Acacia pruinocarpa* Tindale	glabrous	8.5 ± 0.27
	Acacia aneura Benth.	resinous	3.2 ± 0.17
	Solanum lasiophyllum Poir.	hirsute	1.2 ± 0.06
2	*Acacia pruinocarpa* Tindale	glabrous	6.9 ± 0.15
	Acacia aneura Benth.	resinous	2.7 ± 0.14
	Solanum lasiophyllum Poir.	hirsute	1.3 ± 0.03

Table S3. Pb removed from leaf surfaces (across all sites) after washing with EDTA, Citranox or Milli-Q water. Values are percentages in relation to the total Pb concentration: surface Pb + internal foliar Pb (n = 3).

Leaf type	Wash type	Mean removal	Minimum removed	Maximum removed
			%	
Glabrous	EDTA	13	5	21
	Citranox	19	3	39
	Water	14	4	28
Resinous	EDTA	63	43	83
	Citranox	39	19	37
	Water	22	3	42
Hirsute	EDTA	49	27	61
	Citranox	47	32	66
	Water	30	1	85

Hirsute = Resinous > Glabrous (PERMANOVA, $p < 0.05$)
EDTA = Citranox > Water (PERMANOVA, $p < 0.05$)
Removal percentage was not significantly different across sites (PERMANOVA, $p = 0.15$)

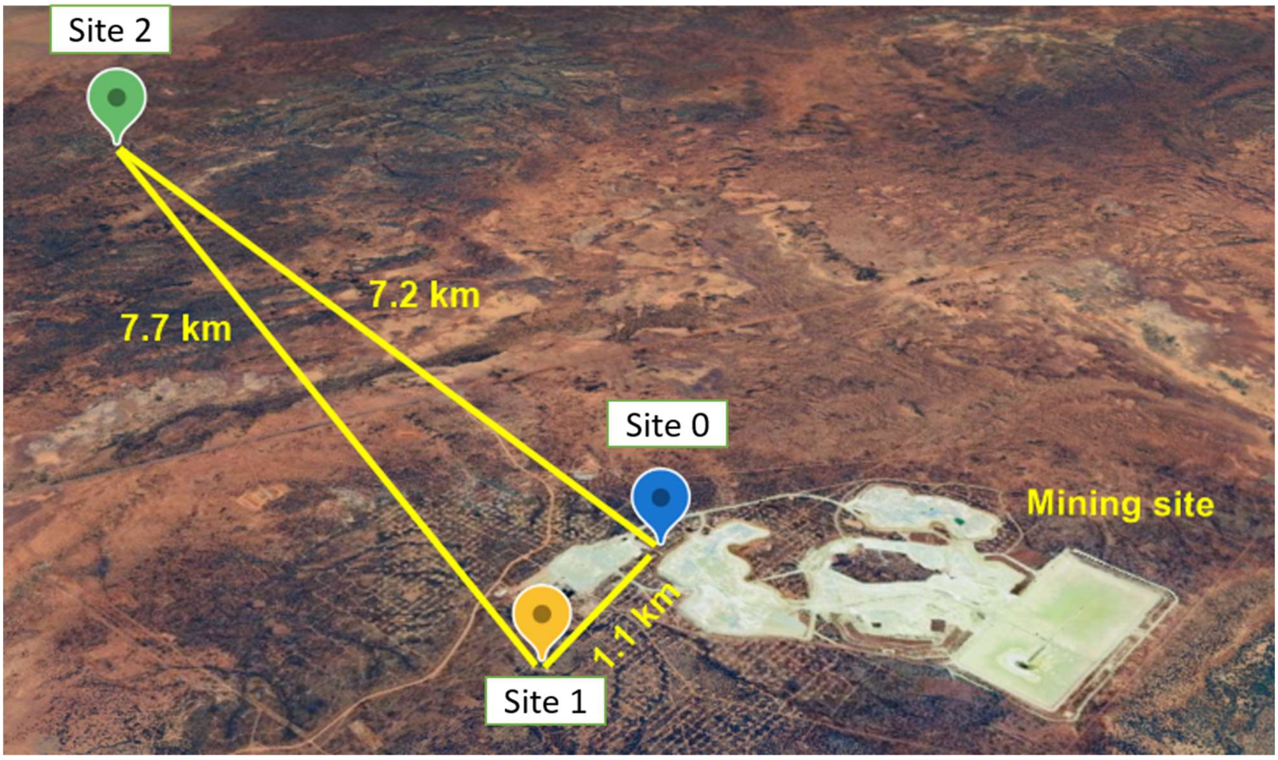

Figure S1. Aerial view showing the location of the three sampling sites, with different distances from the Pb-source (tailings from a mining site).

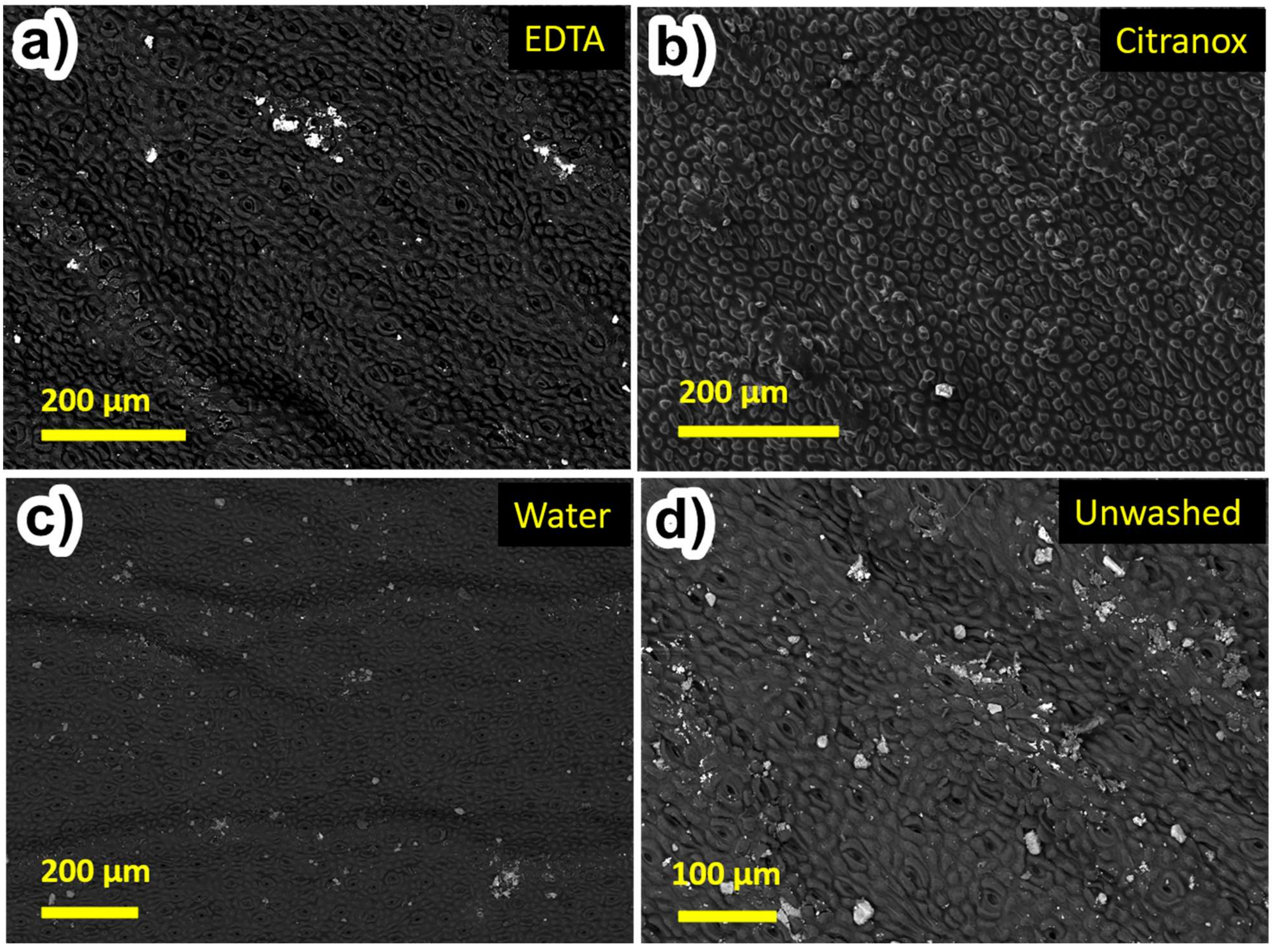

Figure S2. Backscattered electron micrographs of the glabrous leaf surfaces of washed and unwashed samples of *Acacia pruinocarpa* from Site 0: EDTA (a), Citranox (b), Milli-Q water (c) and unwashed (d). Surface dust particles show up as bright structures against the darker leaf surface, indicating the presence of heavier elements.

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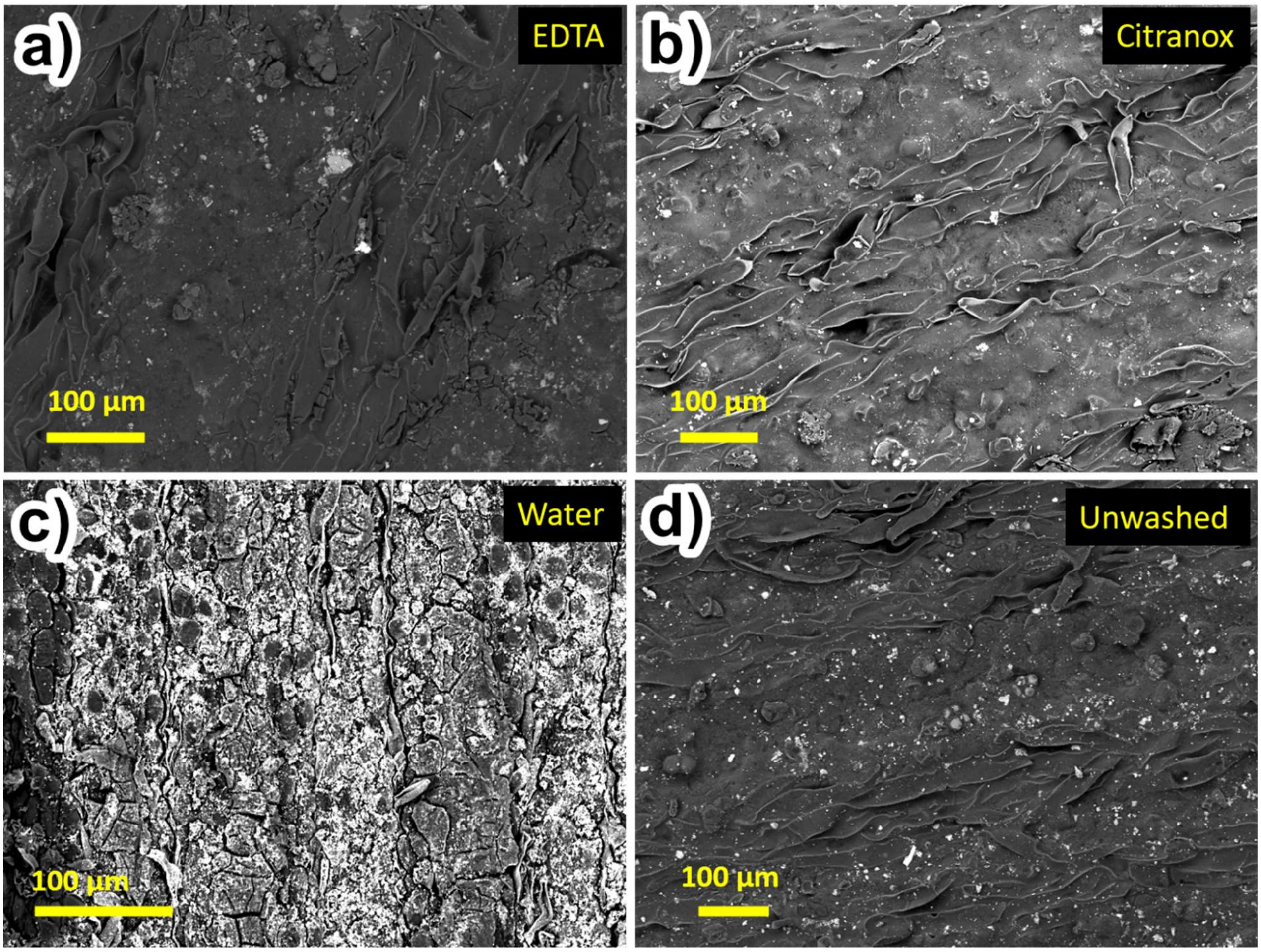

Figure S3. Backscattered electron micrographs of the resinous leaf surfaces of washed and unwashed samples of *Acacia aneura* from Site 0: EDTA (a), Citranox (b), Milli-Q water (c) and unwashed (d). Surface dust particles show up as bright structures against the darker leaf surface, indicating the presence of heavier elements.

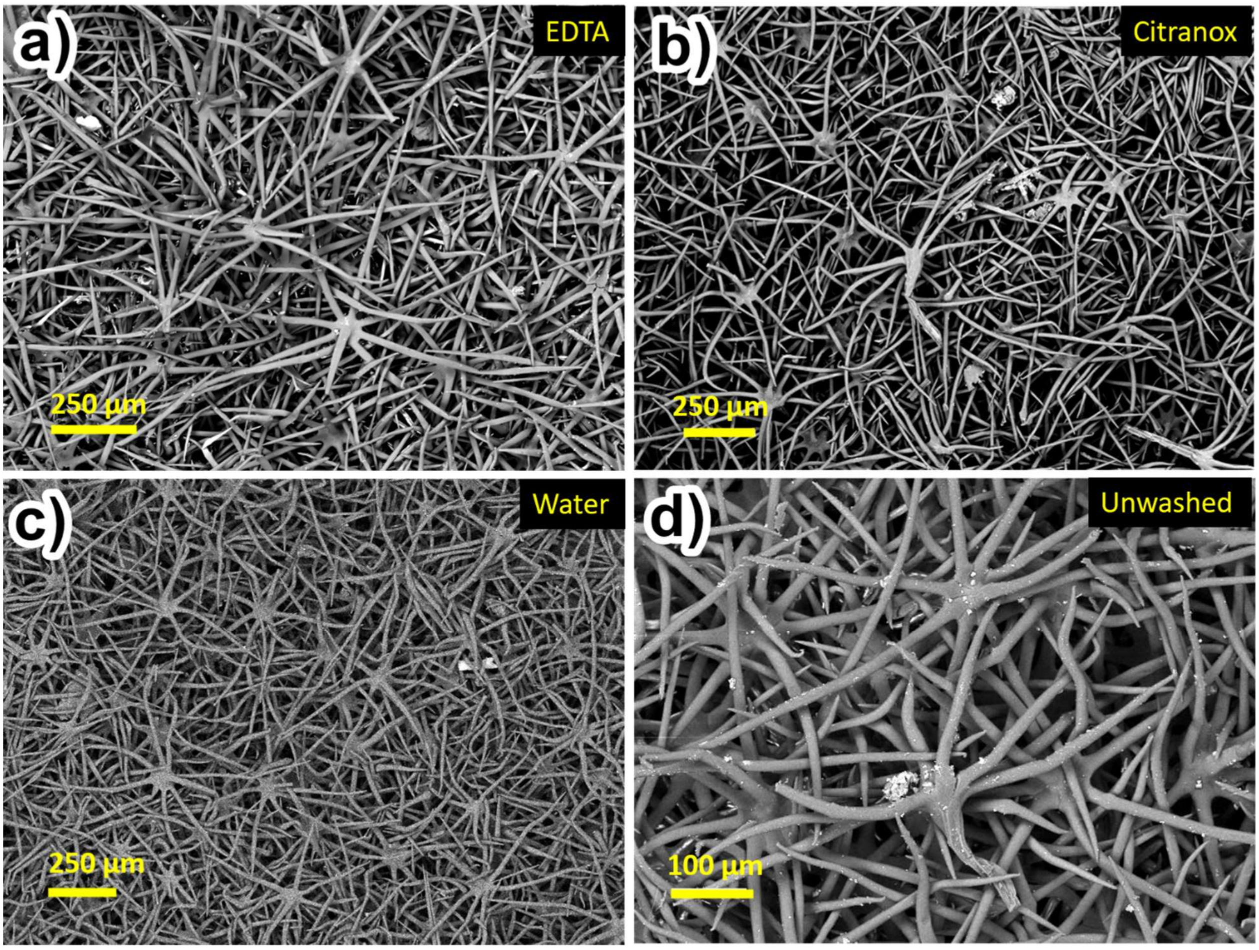

Figure S4. Backscattered electron micrographs of the hirsute leaf surfaces of washed and unwashed samples of *Solanum lasiophyllum* (hirsute) from Site 0: EDTA (a), Citranox (b), Milli-Q water (c) and unwashed (d). Surface dust particles show up as bright structures against the darker leaf surface, indicating the presence of heavier elements.

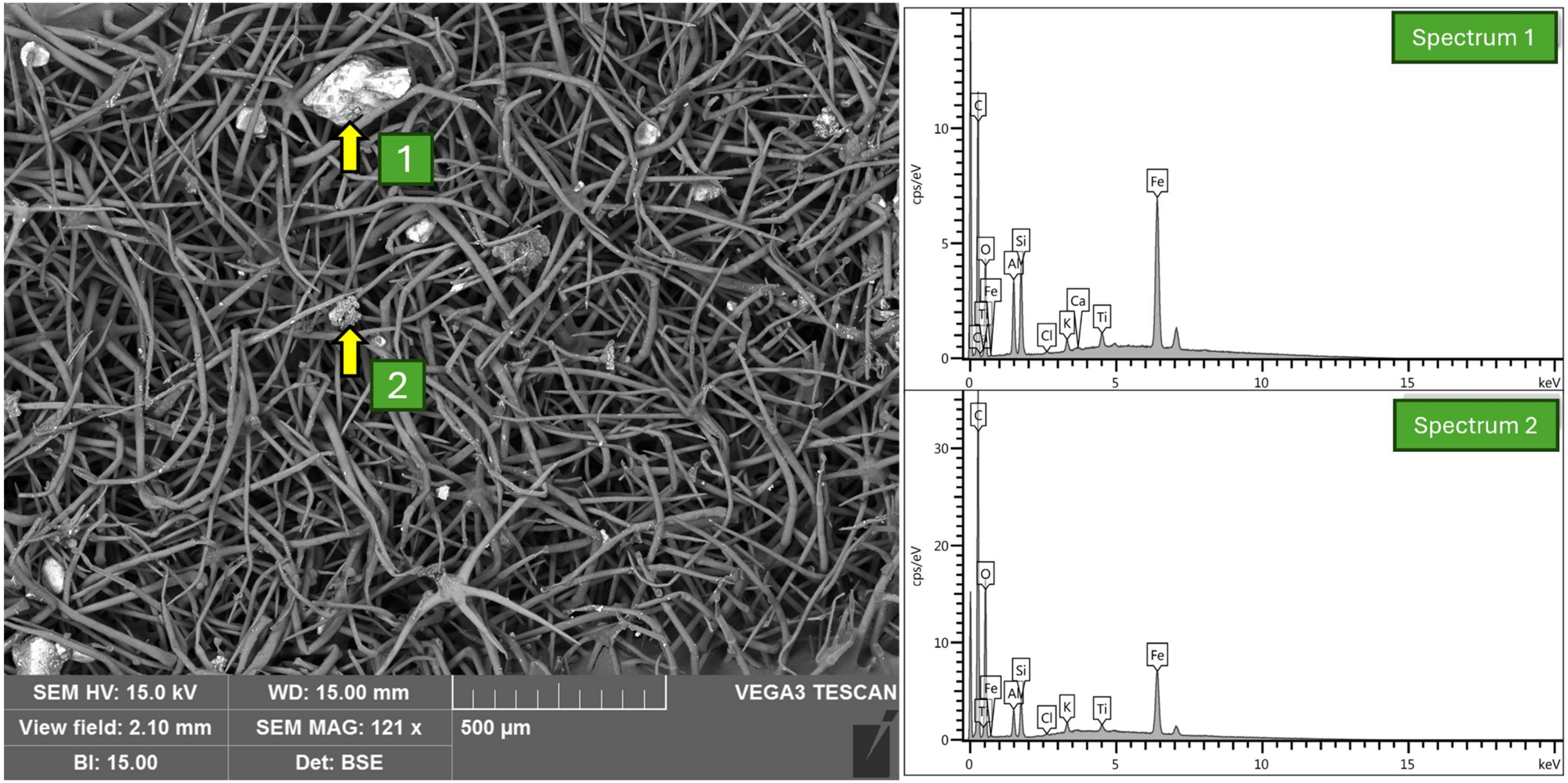

Figure S5. Backscattered electron micrograph of an unwashed hirsute leaf surface (*S. lasiophyllum* from Site 2), yellow arrows indicate particulate matter of various sizes and compositions, from which EDX spectra were taken. No Pb was found in these particles. Over 20 spectra were taken from multiple points and particles, and no considerable Pb was detected.

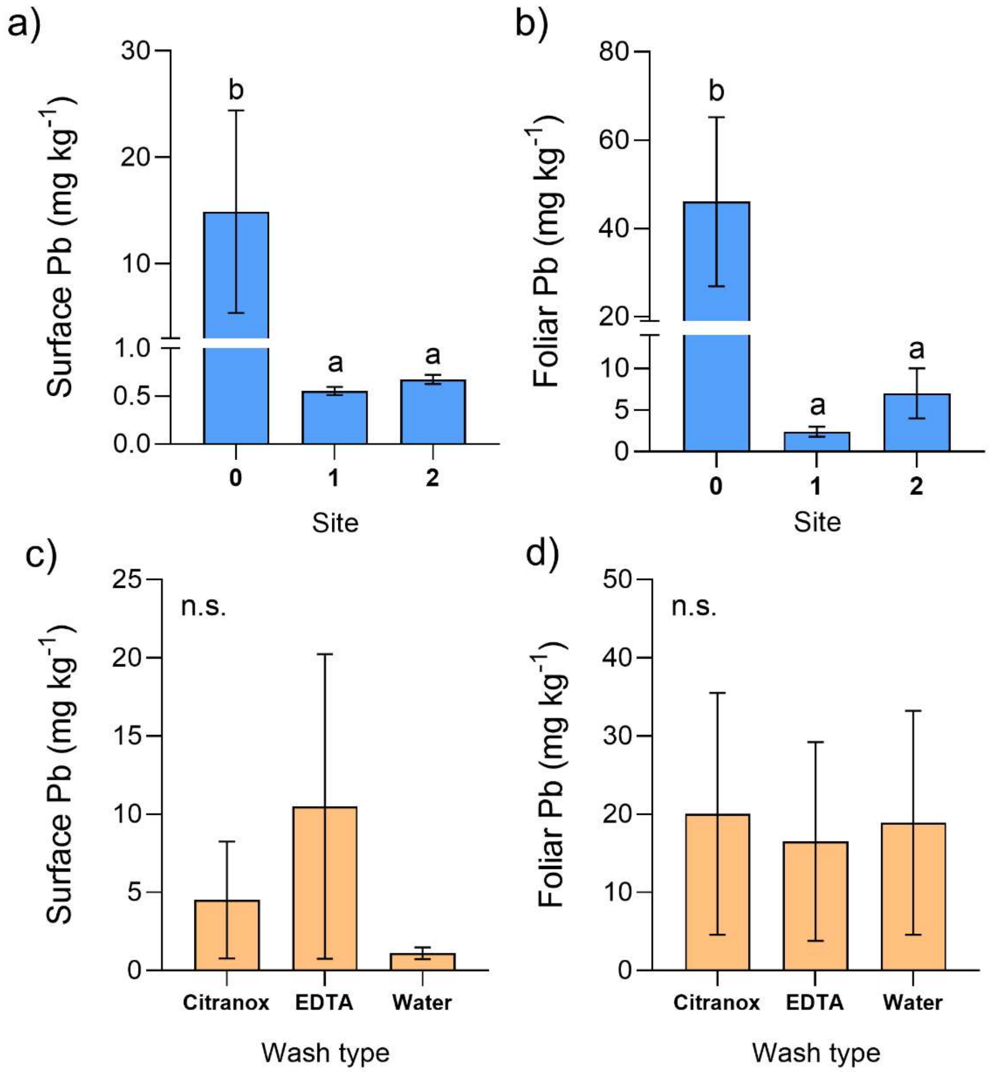

Figure S6. Surface Pb (a, c) from leaf surfaces after washing with EDTA, Citranox or water, from three different sampling sites (0km, 1km, 7km), after the exclusion of hirsute samples. Foliar Pb concentrations (b, d) determined by acid digestion, after exclusion of hirsute samples. Different letters correspond to significant pairwise differences, detected by PERMANOVA ($p < 0.05$).