

Research Paper

Cellular-level imaging reveals distinct thallium detoxification strategies in *Silene latifolia* accessionsGaia Regini^{a,1} , Ksenija Jakovljević^{b,1} , Marco Dainelli^a , Dennis Brueckner^c , Cristina Gonnelli^{a,*} , Antony van der Ent^d ^a Department of Biology, Università degli Studi di Firenze, via Micheli 1, Florence 50121, Italy^b Department of Ecology, Institute for Biological Research Siniša Stanković, National Institute of the Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, Belgrade 11108, Serbia^c Deutsches Elektronen-Synchrotron, Notkestrasse 85, Hamburg 22607, Germany^d Laboratory of Genetics, Wageningen University and Research, Droevendaalsesteeg 1, Wageningen 6708 PB, the Netherlands

ARTICLE INFO

Keywords:

Thallium

Hyperaccumulation

Localization

Leaf epidermis

ABSTRACT

The facultative thallium (Tl) hyperaccumulator *Silene latifolia* can attain the highest known concentrations of Tl of any plant species. This trait is, however, differentiated at the population level with only two known Tl-tolerant and hyperaccumulating accessions occurring at metalliferous sites. This study aimed to reveal the internal distribution of Tl in plant organs and tissues. *Silene latifolia* subsp. *alba* from Tl-hyperaccumulating and Tl-sensitive accessions were grown hydroponically under different Tl treatments and analyzed to determine Tl accumulation. Plant organs were investigated using synchrotron X-ray fluorescence microtomography to determine cellular-level distribution of Tl and other elements. The results reveal markedly different Tl distribution patterns, with mainly vacuolar localization in the epidermis of the hyperaccumulating population and a more even distribution across various cell types in the sensitive population. The differences were found even at the same applied concentrations (5 μM), being more pronounced in the Tl-hyperaccumulating plants at the highest treatment (100 μM of Tl). Elemental analysis showed Tl concentrations $> 100 \mu\text{g g}^{-1}$ in both accessions under all treatments, reaching up to $6910 \mu\text{g g}^{-1}$ in Tl-hyperaccumulating plants treated with 100 μM of Tl. Compartmentalization in the vacuoles of epidermal cells proved to be a key mechanism for Tl hyperaccumulation in the tolerant accession, acting in both detoxification and offers protection against herbivory. In the sensitive accession, the localization of Tl in the mesophyll explains the toxic effect. Differences in Tl localization are of significant importance for deciphering the molecular and genetic bases of this extraordinary phenomenon.

1. Introduction

Metal hyperaccumulation is one of the most fascinating traits plants have evolved, restricted to a small number of species globally and generally viewed to have evolved as a defence mechanism against pathogens and herbivores (Baker and Brooks, 1989; Poschenrieder et al., 2006). Hyperaccumulators attain extremely high concentrations of specific elements in their shoots, at levels higher than those in the substrate and far exceeding any physiological requirement (Baker, 1981; van der Ent et al., 2013). The hyperaccumulation phenomenon attracts wide interest for both fundamental studies and applied research in

phytotechnology aimed at the remediation/phytoextraction of metal-contaminated soils (Verbruggen et al., 2009; Kidd et al., 2018).

Of all the elements that can be hyperaccumulated, thallium (Tl) is among the rarest with only 11 taxa known to hyperaccumulate it, out of the over 700 known hyperaccumulator species known (Krämer, 2010; Reeves et al., 2018). Thallium is the most toxic element to biology, surpassing even arsenic (As) and mercury (Hg) in its toxicity to metazoans (Kazantzis, 2000; Lennartson, 2015; Kemnic and Coleman, 2024). The nominal Tl hyperaccumulation threshold in plants has been set at $100 \mu\text{g Tl g}^{-1}$ in leaf dry weight (d.w.) (van der Ent et al., 2013, 2021), as normal plants have concentrations $< 1 \mu\text{g Tl g}^{-1}$ d.w. (van der Ent et al.,

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Received 17 December 2025; Received in revised form 28 January 2026; Accepted 27 February 2026

Available online 28 February 2026

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2021). In recent years, Tl-hyperaccumulating plants have attracted research attention for advancing the understanding of the underlying molecular and physiological mechanisms (Regini et al., 2024, 2025) and because of the potential for Tl phytoextraction from contaminated soils and mine wastes (LaCoste et al., 1999; Robinson and Anderson, 2021). The potential for Tl phytomining has been tested using *Iberis intermedia* on Tl spiked soils (up to 3000 $\mu\text{g g}^{-1}$ Tl in the soil), with an estimated yield of 10 t ha^{-1} containing approximately 800 $\mu\text{g Tl g}^{-1}$ d.w. (Anderson et al., 1999; Leblanc et al., 1999), and using *Biscutella laevigata* and *Silene latifolia* on Zn-Pb mine tailings in a pioneering poly-metallic phytoextraction experiment (Nkrumah et al., 2022).

Thallium hyperaccumulation is known principally in several facultative hyperaccumulator species from Zn-Pb mines in Southern France (Ganges) and Northern Italy (Cave del Predil) (Escarré et al., 2011; Fellet et al., 2012) and from the As-Tl outcrop at Allchar in North Macedonia (Jakovljević et al., 2025a). In Tl-enriched environments the element can occur at concentrations $> 10 \mu\text{g Tl g}^{-1}$ (Kazantzis, 2000; Zhang et al., 2000), while most normal soils have Tl concentrations $< 1.7 \mu\text{g Tl g}^{-1}$. Therefore, it is likely that more Tl hyperaccumulators await discovery as Tl is rarely analyzed in plant samples, for example at numerous Zn-Pb mines or at the Lanmuchang Tl deposit in China (Zhang et al., 2000). In any case, Tl is emerging as a 'hidden' contaminant of increasing concern, as it is widespread and can easily enter the food chain (Xiao et al., 2004), in part because of its high bioconcentration factor in many brassicaceous crops allowing them to accumulate up to 8300 $\mu\text{g Tl g}^{-1}$ in controlled conditions (Corzo-Remigio et al., 2024).

Among the best-studied facultative hyperaccumulators known so far, *B. laevigata* (Brassicaceae) is Tl hyperaccumulating depending on the population and local environmental conditions (Pošćić et al., 2013, 2015), with Tl concentrations in field-collected plants reaching up to 32,700 $\mu\text{g Tl g}^{-1}$ d.w. (Fellet et al., 2012). Plants grown in hydroponic conditions, showed a maximum concentration of 16,100 $\mu\text{g Tl g}^{-1}$ d.w. in intermediate and young leaves that serve as the main accumulation sink, due to efficient phloem recycling from old leaves, and Tl localization in the blade and margins of the leaves (Corzo Remigio et al., 2022a). Thallium is localized in the vacuoles of epidermal cells, particularly beneath trichomes and in trichome basal cells (Corzo Remigio et al., 2022a), in form of solid crystalline deposits measuring 3–5 μm (Salinitro et al., 2024), as also reported in other Brassicaceae for edible use, like *Brassica oleracea* (Corzo-Remigio et al., 2024). Higher Tl concentrations in field-collected plants were reported by Jakovljević et al. (2023) in *V. arsenica*, with up to 58,900 $\mu\text{g Tl g}^{-1}$ d.w.; in the same study, a predominant localization of Tl in the leaf apices and sepals was found in *V. arsenica* and *V. allchariensis*, while in *V. tricolor* subsp. *macedonica* the metal accumulated mostly in the stems and petioles. The aforementioned species were collected at the Allchar site, a global anomaly that not only hosts a range of Tl-accumulating and hyper-accumulating plant species (Jakovljević et al., 2024, 2025a, b), but also holds the record for the highest Tl concentration ever documented in a plant, with 80,000 $\mu\text{g g}^{-1}$ d.w. in *S. latifolia* field-collected plants from the Allchar site (Jakovljević et al., 2025a).

Due to the extremely high metal concentrations, and its consequent potential for Tl phytoextraction on account of its rapid growth rate and relatively high biomass (Corzo Remigio et al., 2020, 2022a), *S. latifolia* Poir. subsp. *alba* (Mill.) Greuter & Burdet represents a new and ideal model system for studying Tl hyperaccumulation. This species is widely distributed in Europe, Western Asia, and Northern Africa (POWO, 2025), with Tl hyperaccumulation thus far recorded in two populations occurring on metalliferous soils: the population near Ganges/St Laurent le Minier (France) with up to 1489 $\mu\text{g g}^{-1}$ d.w. growing in nature (Escarré et al., 2011) and up to 35,700 $\mu\text{g Tl g}^{-1}$ d.w. in young leaves when grown in hydroponic culture (Corzo Remigio et al., 2022a), and the aforementioned population at the Allchar site (Jakovljević et al., 2025a). All other populations occurring on non-metalliferous soils are Tl sensitive. Previous studies (Regini et al., 2024, 2025) revealed certain Tl accumulation- and photosynthesis-related traits in this species, with Tl

localization remaining relatively unknown, except for the indication of enrichment at the base of the midrib and in the veins of leaf surfaces from the metalliferous population (Corzo Remigio et al., 2022b). Insight into the localization and chemical speciation of a hyperaccumulated element in plant tissues and cells, in this case Tl, is highly valuable for advancing understanding of the underlying tolerance and accumulation mechanisms (van der Ent et al., 2018).

Synchrotron X-ray fluorescence (XRF) microscopy and micro-tomography (XRF- μCT) are unique in their ability to analyze hydrated plant organs and tissues non-destructively. In this study we investigated metalliferous (Tl-hyperaccumulating) and non-metalliferous (Tl-sensitive) accessions of *S. latifolia* subsp. *alba* hydroponically exposed to Tl, using synchrotron XRF- μCT analysis, to elucidate the distribution of Tl at tissue and cellular levels. The outcomes will shed light on the mechanisms of Tl tolerance between the two contrasting accessions and explain Tl hyperaccumulation. This information is crucial for future molecular and genetic studies to unravel the physiology of this extraordinary rare phenomenon in the plant world.

2. Materials and methods

2.1. Plant culture conditions and dosing experiments

Silene latifolia subsp. *alba* seeds were collected in July 2023 from two populations in France: a Tl-sensitive population from a non-contaminated site near Barraux, France (45.433333° N, 5.983333° E), and Tl-hyperaccumulating population from a metalliferous site rich in Zn-Pb-Cd-Tl near Saint-Laurent-le-Minier, France (43.931944° N, 3.655277° E). These two accessions, Tl-hyperaccumulating and Tl-sensitive, have been extensively characterized by Regini et al. (2024). The dry seeds were surface-sterilized, rinsed with sterile distilled water and sown in square Petri plates (12 × 12 cm) containing a sterilized culture medium of 1% agar and modified half-strength Hoagland's solution (Hoagland and Arnon, 1950) in MilliQ water (Millipore), buffered with 2 mM 2-morpholinoethanesulphonic acid, and adjusted to pH 5.5 with KOH. The plates were kept in the dark at a day/night temperature of 24/16°C and, after 48 h, the radicle-emerged seeds were exposed to light. After 14 days, the plantlets were transferred to hydroponic cultures. Each plant was placed in a 50 mL sealed Falcon tube with modified half-strength Hoagland's solution of the same composition as described above. The plants were placed in a growth chamber with a 16 h (day) photoperiod, a day/night temperature of 24/16°C, an irradiance of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 60–65% relative humidity. After a 7-day pre-culture period, plants of equal size were selected for the treatments: both accessions were exposed to 2.5 and 5 μM Tl supplied as TlNO_3 (Sigma-Aldrich) in a background solution with the same composition as the pre-culture solution. The concentration was selected as the highest comparable value based on previous studies (Corzo Remigio et al., 2022b; Regini et al., 2024, 2025), as the Tl-sensitive population exhibited significant signs of stress when exposed to higher concentrations. In addition, the Tl-hyperaccumulating population was exposed to 10, 30, and 100 μM Tl. The exposure lasted 10 days, with the treatment renewed after 5 days by discarding the old solution and completely replacing it with a freshly prepared solution. For the XRF- μCT , air-dried seeds collected from a Tl-hyperaccumulating population were used without further treatment.

2.2. Synchrotron μXRF experiments

One sample per treatment from both the Tl-sensitive and Tl-hyperaccumulating populations of *S. latifolia* subsp. *alba* was used for the analysis. The X-ray fluorescence microscopy and microtomography (XRF- μCT) experiments were conducted at PETRA III (Deutsches Elektronen-Synchrotron, DESY), a 6 GeV synchrotron radiation source, specifically at the hard X-ray microprobe experiment on the undulator beamline P06 (Boesenberg et al., 2016). The P06 beamline is equipped

with a cryogenically cooled Si (111) double-crystal monochromator. An ion chamber upstream of the sample is used to monitor the incoming flux, while a 500- μm -thick Si PIPS diode with a 19-mm-diameter active area [PD300–500CB, Mirion Technologies (Canberra) GmbH, Germany] located downstream of the sample can be used to record the transmitted X-ray intensity in order to extract absorption data. A pair of Kirkpatrick-Baez mirrors was used to focus 16 keV X-ray beam down to about 440 nm $\times$ 300 nm (horizontal $\times$ vertical) for the tomographic measurements or 460 nm $\times$ 400 nm (horizontal $\times$ vertical) for the 2D measurements, resulting in a flux of about 8.0×10^9 photons s^{-1} or 7.6×10^9 photons s^{-1} on the sample, respectively. Detection of XRF during the tomography measurements was performed using a four-element Vortex silicon drift detector (Hitachi High-Tech, USA) and a 16-element Ardesia silicon drift detector (Politecnico di Milano), placed at 90° and 270°, respectively, with the forward direction of the X-ray beam defined as 0°. During the 2D measurements, the XRF signal was detected using a 16-element Ascanio silicon drift detector (Politecnico di Milano) placed in backscatter geometry (180°). All XRF detectors were used in combination with Xpress 3 pulse processors (Quantum Detectors, UK). The samples for tomographic μXRF measurements were inserted into Kapton capillaries and placed under a cryostream for analysis to reduce possible beam damage. The samples for 2D μXRF imaging were mounted on Kapton sticky tape with silicon adhesive (3 M™ Polyimide Film Tape 5413) stretched over a 3D printed ABS plastic frame. They were covered with Ultralene® Window Film, 4.0 μm thick (Cole-Parmer SamplePrep 3525).

2.3. Elemental analysis of plant and soil material samples

The *Silene* plants analyzed with synchrotron μXRF were dried on silica gel and ground to a fine powder (<200 μm) in an impact mill (IKA TubeMill 100 Control) and subsamples inserted into custom XRF sample holders and covered with 6.0 μm polypropylene thin film (Chemplex Industries Inc.) for XRF analysis. The XRF analysis of the plant powdered material was performed using a Z-Spec JP500 instrument (Z-Spec Inc.) in 'Plant' mode (Kahlon et al., 2024; Zhang et al., 2025). This instrument uses monochromatic X-ray fluorescence excitation at 17.48 keV to analyse elements from Z = 13 (Al) to Z = 40 (Zr) on the K-lines and up to Z = 92 (U) on the L-lines with optimum sensitivity for elements Cu-Se and Hg-Tl-Pb with limits of detection ranging from 0.009 to 0.025 $\mu\text{g g}^{-1}$. Two certified standards were included for the plant material analysis (NIST SRM 1570a Trace elements in spinach leaves and NIST SRM 1573a Tomato leaves).

2.4. Data processing and statistical analyses

Data acquisition was performed using a custom workflow (Garrevoet, 2025) and the XRF data were processed with non-linear least squares fitting as implemented in PyMCA (Solé et al., 2007). Energy calibration was achieved by measuring various thin film deposited foils with XRF emission lines that were well-spaced over the energy range (Micromatter Technologies Inc., Canada). For other elements, calibration was calculated using the foil measurement results in combination with parameters from *xraylib* (Schoonjans et al., 2011). The matrix used for modelling was hydrated plant material with the empirical formula $\text{C}_{7.3}\text{O}_{33}\text{H}_{59}\text{N}_{0.7}\text{S}_{0.8}$, a density of 0.8 g cm^{-3} and a thickness of 300 μm . This resulted in 32-bit.tiff files with pixel values corresponding to the area density of each element in $\mu\text{g cm}^{-2}$ converted to $\mu\text{g g}^{-1}$ values by: measured area density ($\mu\text{g cm}^{-2}$) / (thickness of the layer (cm) $\times$ reference volume density (g cm^{-3})) for 2D measurements, and measured volume density ($\mu\text{g cm}^{-3}$) / reference volume density (g cm^{-3}) for tomographic measurements. The figures were prepared in ImageJ (Schneider et al., 2012) by changing the lookup table (LUT) to 'Fire' and adjusting the pixel intensity range with the levels slider so that the background is scaled to black and the highest concentration values are scaled to the upper end of the LUT (nearly white). Concentration

scale bars were added using the 'calibration' tool, along with length scales. All tomographic datasets were reconstructed using a maximum-likelihood expectation-maximization (MLEM) algorithm (Bruyant, 2002).

3. Results

3.1. Tissue-level distribution of thallium in *Silene latifolia* subsp. *alba*

The spatial distribution of Tl in tissues of *S. latifolia* subsp. *alba* is shown in Figs. 1–5. For comparison, the distribution of elements such as K and Zn was included in some scans.

3.1.1. Distribution of thallium in roots

The localization of Tl differed in the roots of Tl-hyperaccumulating and Tl-sensitive accessions, with Tl preferentially associated with the distal part of the roots, mainly the meristematic area in Tl-hyperaccumulating plants (Fig. 1a), while in the Tl-sensitive accession, most Tl accumulated in the elongation area. At the same treatment concentration (5 μM), at the tissue level, Tl was particularly enriched in the root stele and endodermis of the Tl-sensitive accession (Fig. 1b). In these areas of the Tl-hyperaccumulating accession, Tl was strongly depleted, with preferential accumulation in the cortical parenchyma, especially in the outer layer of cells. High concentrations of Tl have not been observed in either accession.

3.1.2. Distribution of thallium in petioles and leaves

Concentrations of Tl in the plants increased with its concentration in the solution, with petioles and basal parts of the leaves being the sites of predominant enrichment (Figs. 2–4). Spatial analysis indicated differences in the distribution of Tl in the petioles of Tl-hyperaccumulating and Tl-sensitive accessions, with most Tl located in the epidermis. While some Tl enrichment was observed in the parenchyma under the lowest treatment applied to Tl-sensitive plants, in the Tl-hyperaccumulating accession Tl was found mainly in the epidermis at both 5 μM and 100 μM , with some Tl present in the central vascular bundle of plants treated with 5 μM . The epidermis, particularly on the adaxial side, was also the main site of Tl localization in the leaves, with a notably distinct distribution compared to the abaxial side at the highest applied Tl concentration in Tl-hyperaccumulating accession (100 μM Tl), and more Tl present in the distal parts of the leaf. In stomatal guard and subsidiary cells depletion of Tl was observed. Accumulation of Tl in the epidermis was also found in Tl-sensitive accession, mainly in the midrib area, with enrichment in the mesophyll tissues (particularly the palisade), and at the base of the trichomes (Fig. 4).

3.1.3. Distribution of thallium in seeds

Synchrotron XRF- μCT analysis of seeds from the Tl-hyperaccumulating accession (collected from the field, not cultured), reveals that Tl is mainly localized in the cotyledons, with the highest accumulation in the mesophyll, lower levels in the epidermis, and minor enrichment in the radicle. In the endosperm and testa, Tl was depleted, in contrast to Zn, which showed a more or less homogeneous distribution with the highest concentrations in the hilum, and K, continuously distributed in the seed coat and present at considerably lower concentrations in the inner structures (Fig. 5).

3.2. Thallium concentrations in plants

The *S. latifolia* subsp. *alba* plants used in X-ray fluorescence microscopy and microtomography experiments were analyzed for Tl concentrations and the results are shown in Table 1. Clear differences were observed between Tl-hyperaccumulating and Tl-sensitive plants, even when treated with the same Tl concentrations. The Tl concentrations in the plants were consistent with those in the solution, exceeding 100 $\mu\text{g g}^{-1}$ in plants from both populations and all treatments, with the

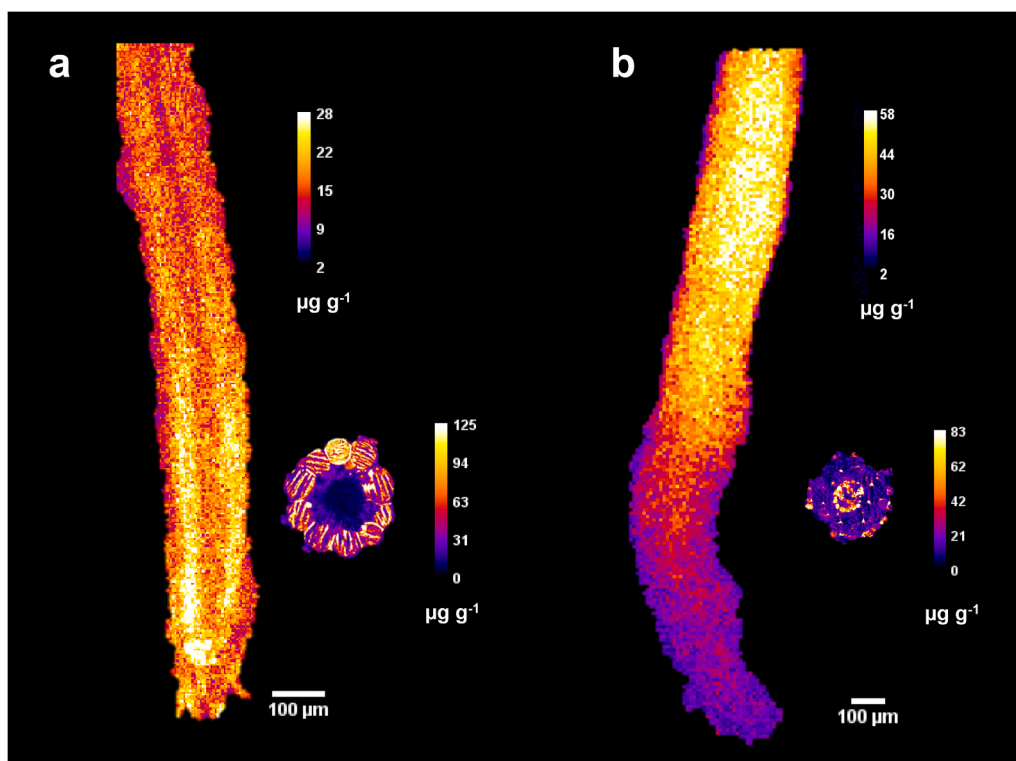


Fig. 1. Synchrotron-based μ XRF elemental maps of Tl in roots of Tl-hyperaccumulating (A) and Tl-sensitive (B) *Silene latifolia* subsp. *alba*, showing localization along the root and in root cross-section.

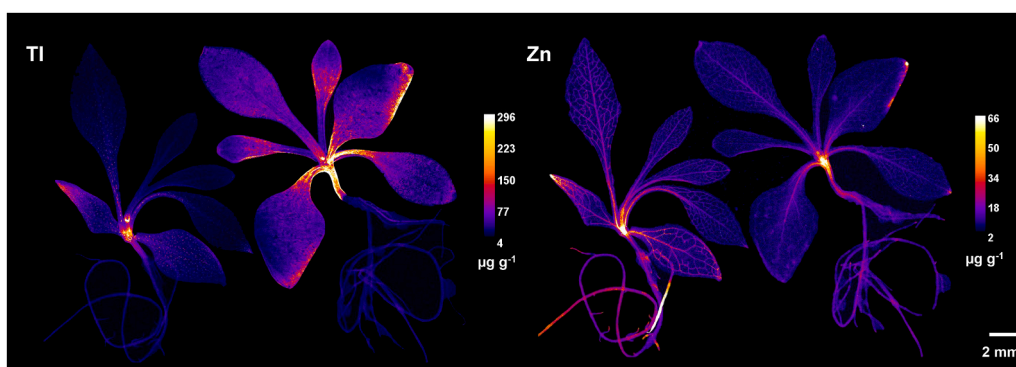


Fig. 2. Synchrotron-based μ XRF elemental maps of Tl and Zn in Tl-sensitive (left) and Tl-hyperaccumulating (right) *Silene latifolia* subsp. *alba*.

highest concentrations ($\sim 7000 \mu\text{g g}^{-1}$) found in Tl-hyperaccumulating accession treated with $100 \mu\text{M}$ Tl.

4. Discussion

The distribution pattern of Tl in plant tissues of *S. latifolia* subsp. *alba* differs strongly between the Tl-hyperaccumulating and Tl-sensitive accession. In the roots of the Tl-hyperaccumulating accession, Tl accumulates mainly in the meristematic zone. A similar localization at the root apex has been found in some Zn hyperaccumulators, which in this case was associated with elemental essentiality (Zhukovskaya et al., 2025). On the other hand, in Tl sensitive accession of *S. latifolia* subsp. *alba* higher Tl was found in the elongation area, whereas the meristem was highly Tl depleted. This opposite localization could result from the higher Tl susceptibility of this population, representing an process to protect sensitive meristematic cells, which contain the stem cell niche for all differentiated plant tissues and organs (Stahl and Simon, 2010). In the roots of the Tl-sensitive accession, Tl accumulates mainly in the

central cylinder, probably due to a translocation barrier mechanism that prevents high metal concentrations from reaching the shoots. Conversely, the marked Tl depletion in the inner cortex and stele of the roots in Tl-hyperaccumulating accession could be due to continual influx from the cortex into the tracheary cells and further up to the shoot, consistent with the hyperaccumulator phenotype (Verbruggen et al., 2009; Krämer, 2010). Remarkable Tl enrichment in hyperaccumulator plants has been found in the outer layer of the root cortex, which has a predominant role in turgor-dependent mechanical support (Lynch et al., 2022). Similar finding have been reported for *Viola tricolor* subsp. *macedonica*, a facultative metallophyte highly tolerant to high Tl concentrations (Jakovljević et al., 2025b), whereas in metalicolous accessions of *B. laevigata*, high concentrations of Tl were also found in vascular tissue (Salinitro et al., 2024).

Whereas in the Tl-sensitive accession, Tl was present at much lower concentrations and was more evenly distributed between the epidermis and mesophyll, in the petioles of the Tl-hyperaccumulating accession most of the Tl occurs in the epidermis. Moreover, this distinctive pattern

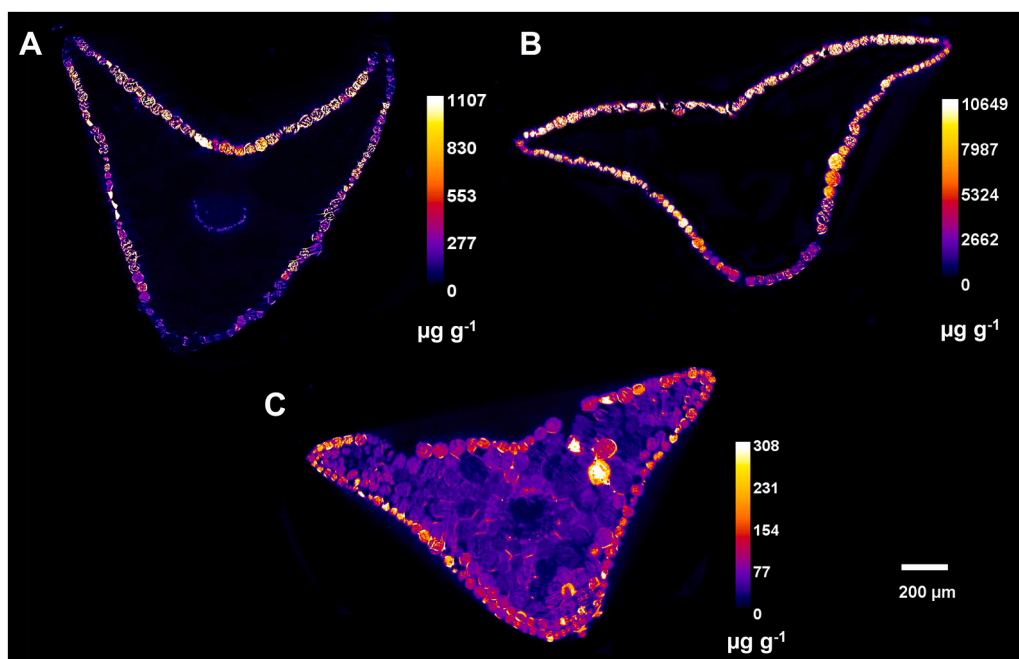


Fig. 3. Synchrotron-based μ XRF elemental maps of Tl in petioles of Tl-hyperaccumulating plants treated with 5 μ M (A) and 100 μ M (B) Tl and Tl-sensitive (C) *Silene latifolia* subsp. *alba* treated with 5 μ M Tl.

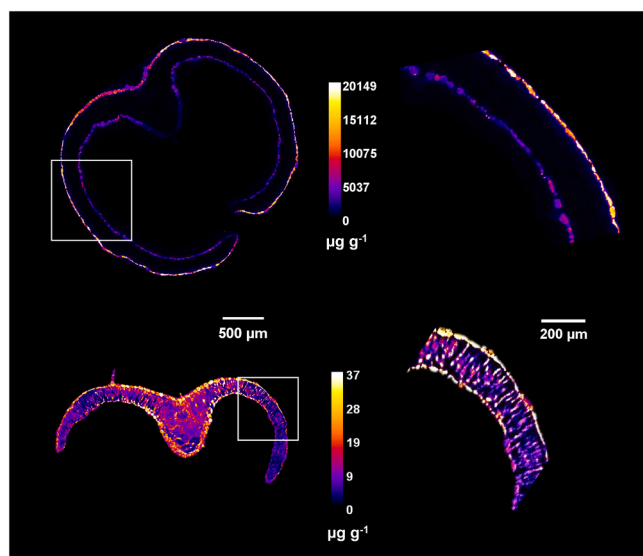


Fig. 4. Synchrotron-based μ XRF elemental maps of Tl in leaves of Tl-hyperaccumulating (upper part) and Tl-sensitive (lower part) *Silene latifolia* subsp. *alba*.

of Tl accumulation was observed at the same applied concentration (5 μ M), indicating different pathways for Tl in Tl-hyperaccumulating and Tl-sensitive accessions of *S. latifolia* subsp. *alba* which is particularly evident when the same concentration scale is used (Figure S1). Similarly, high Tl concentrations accumulated in the leaves of Tl-hyperaccumulating accession, mainly in the epidermis, with evident depletion in the stomatal complex. This confirms guard cell protection from Tl toxicity through an exclusion mechanism hypothesized by Regini et al. (2025) to explain the unaffected stomatal conductance of the hyperaccumulating population, despite the high Tl concentration in the leaves. Metal exclusion from guard cells to preserve stomatal function has also been reported in some Ni hyperaccumulators (Psaras et al., 2000). Compartmentalization in epidermal vacuoles in the leaves is one

of the most common strategies for tolerance of metals and metalloids, observed for various elements and in most hyperaccumulator species (Krämer, 2010; Leitenmaier and Küpper, 2013; van der Ent et al., 2019). The absence of Tl in the mesophyll of the Tl-hyperaccumulating accession could explain their ability to photosynthesize even at very high metal concentrations in the shoots, as reported by Regini et al. (2025). Conversely, in Tl-sensitive accession enrichment of Tl is also observed in the mesophyll, especially in the palisade tissue, potentially explaining its toxicity to the photosynthetic apparatus (Regini et al., 2025). Although mesophyll cells are common secondary compartment when elemental concentrations increase greatly, leading to the redistribution (Küpper et al., 2001), for hyperaccumulating species such as *Nocca praecox* this process was shown to be elemental-specific, with Ni and Zn localized mainly in the epidermis, and Cu, Cd, Mn, and Pb within the mesophyll (Vogel-Mikuš et al., 2008). Preferential accumulation in the epidermis was also found in the foliar tissues of *Sedum alfredii*, a hyperaccumulator of Zn, to a greater extent in the hyperaccumulating ecotype (Tian et al., 2009). In addition to the epidermal cells, metals and metalloids are sequestered at the base of the trichomes, metabolically inactive cells, thus preventing plant toxicity (Li et al., 2023). The same was found for *S. latifolia*, although with no significant enrichment of Tl, as previously shown by SEM-EDS analysis (Corzo Remigio et al., 2022b). The same mechanism of detoxification and allocation of excessive concentrations of Ni was shown in *Odontarrhena chalcidica* (synonym: *Alyssum murale*) (McNear et al., 2005).

Allocation of metals and metalloids in the endosperm can represent an additional adaptation strategy to potentially toxic elemental concentrations, being much higher in metalliculous populations, as found for Zn in the seeds of *B. laevigata*, thus protecting the embryo and reproduction (Babst-Kostecka et al., 2020). However, in *S. latifolia* subsp. *alba* most of the Tl was found in the cotyledons and partly in the radicle. In addition to indicating an efficient mechanism of Tl enrichment even at this stage, it may play a role in protecting plantlets from herbivory during germination. A similar result was found for Tl in *B. laevigata*, and for Cu in *N. praecox* and *A. thaliana*, whereas in *N. praecox* Zn was mainly localized in the cotyledons and epidermis (Vogel-Mikuš et al., 2007; Schnell Ramos et al., 2013; Salinitro et al., 2024). The localization of Tl in the epidermal cells of the

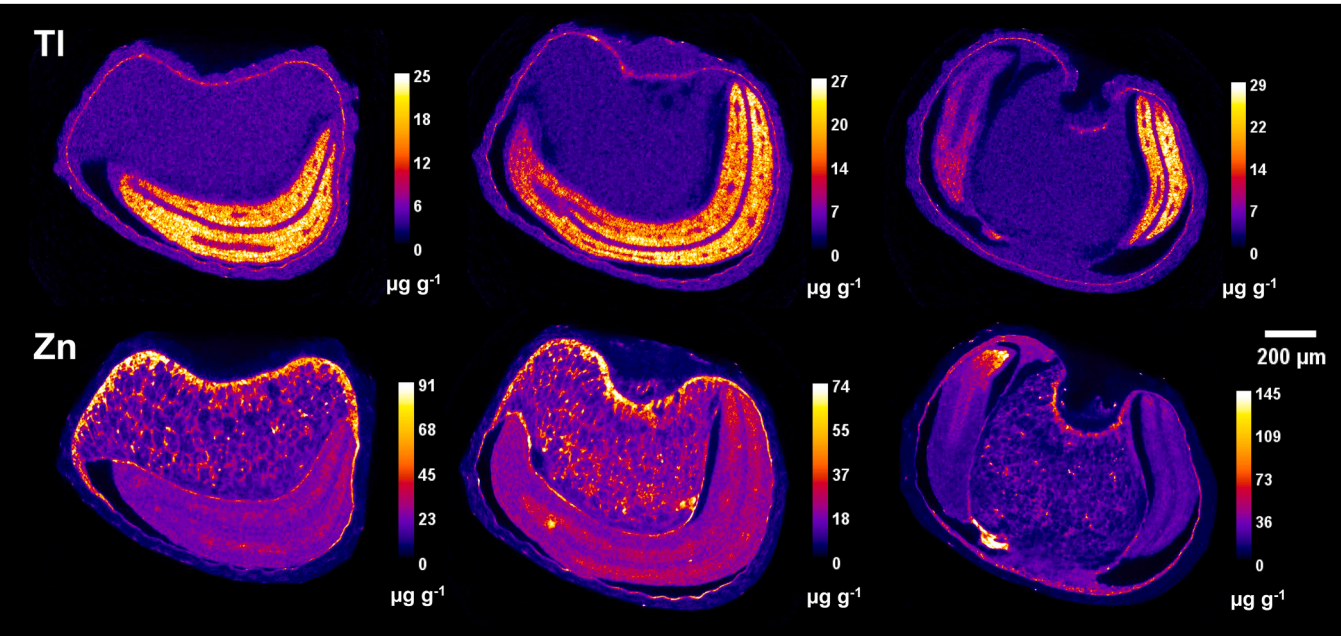


Fig. 5. Synchrotron-based μ XRF elemental maps of Tl and Zn in the seeds of Tl-hyperaccumulating populations of *Silene latifolia* subsp. *alba* growing in the nature.

Table 1

Thallium concentrations ($\mu\text{g g}^{-1}$) in Tl-hyperaccumulating and Tl-sensitive plants of *Silene latifolia* subsp. *alba* plants under different Tl treatments (2.5–100 μM), given as mean $\pm$ standard deviation.

Taxon	Sample	Tl
<i>Silene latifolia</i> subsp. <i>alba</i>	MET C	1.29 $\pm$ 0.15
<i>Silene latifolia</i> subsp. <i>alba</i>	MET 2.5	293 $\pm$ 20.2
<i>Silene latifolia</i> subsp. <i>alba</i>	MET 5	623 $\pm$ 65.7
<i>Silene latifolia</i> subsp. <i>alba</i>	MET 10	1100 $\pm$ 95.5
<i>Silene latifolia</i> subsp. <i>alba</i>	MET 30	2640 $\pm$ 12.2
<i>Silene latifolia</i> subsp. <i>alba</i>	MET 100	6910 $\pm$ 122
<i>Silene latifolia</i> subsp. <i>alba</i>	N MET C	2.14 $\pm$ 0.383
<i>Silene latifolia</i> subsp. <i>alba</i>	N MET 2.5	110 $\pm$ 3.52
<i>Silene latifolia</i> subsp. <i>alba</i>	N MET 5	226 $\pm$ 12.1

MET - Tl-hyperaccumulating plants; N MET - Tl-sensitive plants; C – control.
Figure captions

Tl-hyperaccumulating accession, combined with the very high toxicity of Tl, may confer pronounced herbivory protection and thus adaptative advantage for the evolution of this trait.

The differences in Tl accumulation between Tl-hyperaccumulating and Tl-sensitive accessions were confirmed by elemental analysis, with Tl concentrations in the plants increasing as their concentration in the solution increased. Under the same treatment hyperaccumulating plants had higher accumulation of Tl, with the highest concentration, 6910 $\mu\text{g g}^{-1}$, greatly exceeding the nominal hyperaccumulation threshold of 100 $\mu\text{g g}^{-1}$ (van der Ent et al., 2021).

Strongly associated with K^+ and Na^+ selective channels during uptake, Tl co-locates with K, interfering with K regulation and increasing oxidative stress, as was shown in for example *Triticum turgidum* (Renkema et al., 2015), *A. thaliana* (Chang et al., 2024), and *Diitrichia viscosa* (Espinosa et al., 2023). Although the hypothesized mechanism of co-location is associated with a number of (hyper)accumulators of Tl (Jakovljević et al., 2025a), in some species, such as *B. laevigata*, a hyperaccumulator of Tl, however, no significant correlation or inhibition between Tl and K was observed (Corzo Remigio et al., 2022a). The correlation in the distribution of Tl and K in *S. latifolia* subsp. *alba* was revealed to depend on plant organ and population characteristics (Figure S2). In the hyperaccumulating population, Tl and K appeared to co-localize in the outer layer of the root cortex, where both elements

reached their highest concentration, and probably contributed to the functioning of this tissue in root growth through turgor-driven expansion. In the internal part of the root, K appeared less depleted than Tl, suggesting the beginning of pathway diversification, with Tl more readily translocated to the shoots. The lack of co-localization in the Tl-sensitive accession could result from Tl toxicity causing K leakage from the outer cells, thus also partially explaining the reduced growth of this population in the presence of Tl. In the Tl-hyperaccumulating accession, the distribution of these two elements in the leaves appeared distinctly different, with the strict compartmentalization of Tl in the epidermis for detoxification, and K, essential for mesophyll functioning, especially for photosynthesis (Lu et al., 2025), being more evenly distributed. This resulted in a stable ionome and preserved photosynthetic activity under increasing Tl treatments (Regini et al., 2024). However, the sample size and the self-absorption of K limit the reliability of the results to the signal from the outer 50 micrometres, as deeper within the sample the signal is extinguished.

The differences in Tl distribution and varying levels of tolerance in *S. latifolia* subsp. *alba* populations suggest that distinct molecular components regulate its uptake, translocation and hyperaccumulation. Identifying the main genes responsible for Tl hyperaccumulation would not only enhance understanding of the mechanisms of elemental uptake and accumulation in sites with varying Tl loading, but could also improve phytoremediation and phytomining practice, making recovery from biomass economically feasible. In this study the chemical speciation of Tl was not determined, and whilst μ XRF excels at determining elemental localization (answering the question of "where"), it does not identify chemical species (answering the question of "what form"). However, it is possible with X-ray Absorption Spectroscopy (XAS) to elucidate the chemical speciation of Tl, as this could shed further light on the mechanisms of Tl handling in the plant. The current study provides a static "snapshot" of Tl localization and future experiments incorporating dose time-series would be valuable for revealing the dynamic processes of Tl uptake, translocation, and compartmentalization. Finally, next-level insights will come from genetics, including making crosses between the Tl-hyperaccumulating and Tl-sensitive accessions to obtain a segregating population. This could also involve transcriptomics to provide leads to genes involved with selective Tl uptake and translocation.

Author contributions

GR, CG, and AvdE conceived and planned the study. GR, MD and CG conducted the hydroponics dosing experiment. GR, CG, MD, KJ, DB and AvdE conducted the synchrotron experiment. DB performed the fitting of the XRF data and KJ processed the data for figure preparation. KJ and AvdE designed the figures. GR, CG, KJ and AvdE evaluated and interpreted the data. KJ, CG and AvdE drafted the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

CRedit authorship contribution statement

Gaia Regini: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Cristina Gonnelli:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Ksenija Jakovljević:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Antony van der Ent:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dennis Brueckner:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis, Data curation. **Marco Dainelli:** Writing – review & editing, Investigation, Formal analysis, Data curation.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Given her role as a member of the Editorial Advisory Board, Cristina Gonnelli had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to another journal editor. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We acknowledge DESY (Hamburg, Germany), a member of the Helmholtz Association HGF, for the provision of experimental facilities. Parts of this research were carried out at PETRA III. Data was collected using beamline P06 operated/provided by DESY Photon Science. Beamtime was allocated for proposal I-20250020 EC. This research was supported in part through the Maxwell computational resources operated at Deutsches Elektronen-Synchrotron DESY, Hamburg, Germany. We would like to thank Gerald Falkenberg and Jan Garrevot for assistance in using beamline P06. The authors acknowledge the support of National Biodiversity Future Centre to the University of Florence, Department of Biology, funded by the Italian Ministry of University and Research, PNRR, Missione 4 Componente 2, “Dalla ricerca all’impresa”, Investimento 1.4, Project CN00000033. KJ acknowledges the support of Ministry of Science Technological Development and Innovation of the Republic of Serbia (Grant No 451–03–136/2025–03/ 200007).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.envexpbot.2026.106340](https://doi.org/10.1016/j.envexpbot.2026.106340).

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