Thallium accumulation and distribution in *Silene latifolia* (Caryophyllaceae) grown in hydroponics

Amelia Corzo Remigio · Philip Nti Nkrumah · Filip Poščić · Mansour Edraki · Alan J. M. Baker · Antony van der Ent

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**Abstract**

*Purpose* Thallium (Tl) is one of the most toxic elements known and its contamination is an emerging environmental issue associated with base metal (zinc-lead) mining wastes. This study investigated the nature of Tl tolerance and accumulation in *Silene latifolia*, which has so far only been reported from field-collected samples.

*Methods* *Silene latifolia* was grown in hydroponics at different Tl concentrations (0, 2.5, 5, 30 and 60 μM Tl). Elemental analysis with Inductively coupled plasma atomic emission spectroscopy (ICP-AES) and laboratory-based micro-X-ray fluorescence spectroscopy (μ-XRF) were used to determine Tl accumulation and distribution in hydrated organs and tissues.

*Results* This study revealed unusually high Tl concentrations in the shoots of *S. latifolia*, reaching up to 35,700 μg Tl g⁻¹ in young leaves. The species proved to have exceptionally high levels of Tl tolerance and had a positive growth response when exposed to Tl dose rates of up to 5 μM. Laboratory-based μXRF analysis revealed that Tl is localized mainly at the base of the midrib and in the veins of leaves. This distribution differs greatly from that in other known Tl hyperaccumulators.

*Conclusions* Our findings show that *S. latifolia* is among the strongest known Tl hyperaccumulators in the world. The species has ostensibly evolved mechanisms to survive excessive concentrations of Tl accumulated in its leaves, whilst maintaining lower Tl concentrations in the roots. This trait is of fundamental importance for developing future phytoextraction technologies using this species to remediate Tl-contaminated mine wastes.

**Keywords** Elemental distribution · Hyperaccumulator · Metal uptake · Phytoextraction · *Silene latifolia* · Thallium

**Introduction**

Thallium (Tl) is one of the most toxic elements known to mankind (Lennartson 2015) and recently, it has been
considered as an ‘emerging pollutant’ (Antoniadis et al. 2019; Ma et al. 2022; Wang et al. 2021). Thallium toxicity is even greater than that of other well-known inorganic poisons such as arsenic (As), lead (Pb) and cadmium (Cd) (Liu et al. 2020; Viraraghavan and Srinivasan 2011), and the element is listed as one of the US Environmental Protection Agency’s 13 Priority Pollutants (Keith and Tellier 1979). Thallium can exist in two different oxidation states (+1 and +3), although in the environment thallous (+1) is predominant (Lennartson 2015; Ma et al. 2022). Thallium toxicity in animals and humans is a consequence of its chemical similarity to K⁺ (Mullins and Moore 1960). Even small doses of Tl can be lethal to humans (8 mg kg⁻¹ body weight) (Moeschlin 1980). Thallium is readily taken up from the soil by plants (including some common vegetables), and transported internally using K⁺ pathways (Scheckel et al. 2004). These properties make Tl readily available to biota, leading to bioaccumulation in crops such as Sonchus oleraceus and Zea mays (Antoniadis et al. 2019; Dmowski et al. 2014; Wang et al. 2021).

Thallium is generally present in nature at low concentrations although mining and processing activities release this element into the environment (Karbowska 2016). The background Tl concentration in the Earth’s crust ranges from 0.85 to 1 μg g⁻¹, with increasing concentrations in acidic igneous and clay-rich sedimentary rocks (Kabata-Pendias and Mukherjee 2007). Geochemically anomalous areas, such as in the vicinity of base metal mines [notably Pb and zinc (Zn)], can contain elevated Tl concentrations (>5 μg g⁻¹) (Lis et al. 2003; Liu et al. 2020; Tremel et al. 1997). Some tailings materials originating from Zn-Pb mines have high Tl concentrations, e.g. up to 40 μg Tl g⁻¹ at the mines sites near Saint-Laurent-Le-Minier and Ganges in Southern France (Leblanc et al. 1999), and 424 μg Tl g⁻¹ (average) at the Raibl (Cave del Predil) mine site in Italy (Fellet et al. 2012). Some subsoils in the Swiss Jura Mountains (at the Erzmatt site) which have developed from localized areas of mineralized carbonate rock contain up to 6000 μg Tl g⁻¹ (Voegelin et al. 2015).

Hyperaccumulator plants can attain exceptionally high concentrations of potentially toxic metals and metalloids in their shoots without developing toxicity symptoms (Baker 1981; van der Ent et al. 2013). These plants are models for fundamental research in ecophysiology and biogeochemistry, and applied research such as their use in ‘green technologies’, e.g., phytoextraction to clean-up soils, and phytomining to extract valuable metal(loid)s from unconventional resources for economic purposes (Corzo Remigio et al. 2020; Nkrumah et al. 2019; van der Ent et al. 2015). A notional threshold for Tl hyperaccumulation has been set at 100 μg Tl g⁻¹ in above-ground tissues (dry weight) (Reeves et al. 2018; van der Ent et al. 2013). Thallium hyperaccumulators include two species from the Brassicaceae family: Biscutella laevigata with up to 32,700 μg Tl g⁻¹ in its leaves (Fellet et al. 2012), and Iberis linifolia (synonym: Iberis intermedia) with up to 4000 μg Tl g⁻¹ in its leaves (LaCoste et al. 1999). Silene laetifolia (S. latifolia subsp. alba, synonym: Melandrium album) is another Tl hyperaccumulator which belongs to the Caryophyllaceae family. The populations of this species from the Saint-Laurent-le-Minier area can accumulate up to 1500 μg Tl g⁻¹ in leaves from soils containing up to 32 μg g⁻¹ total Tl (Escarré et al. 2011). Furthermore, three species from the Violaceae family, Viola allcharaensis, V. arsenica and V. macedonica, have also been reported to accumulate 2190 μg Tl g⁻¹, 9090 μg Tl g⁻¹ and 4290 μg Tl g⁻¹ in their leaves, respectively, when growing in soils with 13.7–2140 μg g⁻¹ Tl at the former As-Sb-Tl Allchar Mine in the Republic of North Macedonia (Bačeva et al. 2014).

The Caryophyllaceae family is known for numerous metallophytes that occur on copper (Cu) and Zn-Pb metalliferous gossans and mine wastes around the world (Baker and Brooks 1989). The genus Silene in particular has species with high tolerance to Cu, Zn and Cd, of which the European widespread species S. vulgaris is the best studied taxon (Schat et al. 2000). Other metallophyte species include S. paradoxa in Europe (Arnetoli et al. 2008), S. burchelli and S. cobalticola in Central Africa from Cu-cobalt (Co) metalliferous soils (Baker and Brooks 1989; Malaisse et al. 1983). Silene paradoxa behaves as an excluder by restricting the translocation of different metals, such as Cd, Cu, nickel (Ni) and Zn, from the root-to-shoot, displaying metal resistance at the cellular level (Colzi et al. 2014). In laboratory experiments, S. cobalticola was confirmed to be more tolerant to Cu and cobalt (Co) compared to S. burchelli (Baker et al. 1983). Silene vulgaris limits Cu translocation from its roots (~2000 μg Cu g⁻¹) to shoots (4–215 μg Cu g⁻¹) by efficiently binding the Cu in root cells (Song et al. 2004). Silene vulgaris is also tolerant to other metals such as chromium (Cr) (Pradas-del-Real
et al. 2013), Cd and Zn (Ciarkowska and Hanus-Fajerska 2008; Ernst and Nelissen 2000). The resistance to Cu in S. vulgaris has been explained by an enhanced Cu efflux from the roots due to the increased SvMT2b gene expression, and its resistance to Zn by an enhanced tonoplast transport of Zn in the roots (Verkleij et al. 2001).

Silene latifolia is native to Europe and Western Asia and naturalized around the world (Barluenga et al. 2011; Castillo et al. 2014; Mikhaylova et al. 2021). Silene latifolia is well adapted to polluted areas along with other plant species in Southern Italy. It can accumulate up to 102.5 μg Tl g⁻¹ in its shoots when growing in soils with 9.40 μg Tl g⁻¹ (Visconti et al. 2018). This species thus has potential to extract Tl from polluted soils using phytoextraction (Corzo Remigio et al. 2020). Thallium phytoextraction could give an economic return, given that Tl metal is valuable, worth US$ 7500 ha⁻¹ and an average of 800 μg Tl g⁻¹ in its biomass, it may yield ~7.5 kg of Tl ha⁻¹ yr⁻¹ (Visconti et al. 1999). Brassica juncea can produce 15 t ha⁻¹ and with an average of 500 μg Tl g⁻¹ may yield ~7.5 kg of Tl ha⁻¹ yr⁻¹ (Rader et al. 2019). However, these potential Tl yields (and monetary returns) remain to be tested and proven under ‘real-life’ conditions.

To date, there are only data available for S. latifolia from Tl-polluted soils in the field (Duri et al. 2020; Escarré et al. 2011). No experimental trials have yet been conducted under controlled conditions to assess Tl accumulation and tolerance in S. latifolia. The present study was therefore devised to assess Tl (hyper)accumulation in S. latifolia grown hydroponically, and to determine the in situ distribution of Tl and other elements in hydrated plant organs.

Materials and methods

Hydroponics dosing treatments Seeds of S. latifolia originating from the ancient Zn-Pb mines near Saint-Laurent-le-Minier (43°55’54.8"N, 3°39’48.5”E) mining region in Gard, Southern France were germinated on Gelzan gel in 2 mL Eppendorf tubes (made up with 0.5-strength Hoagland’s solution). The seeds were then vernalized for one week at 3 °C, and acclimatized for four days at 26 °C, making a total of 11 days of germination. The seedlings were then transferred to hydroponic cultures after the cotyledons had fully emerged. The hydroponics experiment was conducted in a temperature-controlled room with five containers (11×30×40 cm; capacity ~12 L). The nutrient solution was based on 0.5-strength modified Hoagland’s formulation: K (3 mM as KNO₃), Ca (2 mM as Ca(NO₃)₂·4H₂O), P (1 mM as NH₄H₂PO₄), Mg (0.5 mM as MgSO₄·7H₂O), Fe (40 μM as Fe(K)-HBED), Cl (1 μM as KCl), B (25 μM as H₃BO₃), Mn (2 μM as MnSO₄·4H₂O), Zn (2 μM as ZnSO₄·7H₂O), Cu (0.1 μM CuSO₄·5 H₂O), Mo (0.1 μM as Na₂MoO₄·2 H₂O) and 2 mM MES (2-(N-morpholino)ethanesulfonic acid) buffer adjusted to pH 5.5 with KOH (van der Zee et al. 2021). When plants were sufficiently large (after 15 days), the nutrient solution was spiked with Tl (as TlNO₃) to yield five treatment levels: 0 (control), 2.5, 5, 30, and 60 μM Tl (corresponding to 0, 0.5, 1, 6, and 12 mg L⁻¹ Tl, respectively). The solutions were aerated with air-stone diffusers at the base of each container. All plants were harvested after 16 days in the Tl treatment solutions.

Plant growth conditions Six seedlings for each treatment unit were transplanted to 3 cm circular retainer baskets with a foam holder to allow immersion in the nutrient solution and also to protect roots from the light. The experimental plants were grown on for 31 days in a growth cabinet with a 12/12-h light/dark cycle, using high-intensity photosynthetically active radiation (PAR) LED lights (Valoya, model B200, Finland) at a photosynthetic photon flux density of 350 μmol m⁻² s⁻¹ and a 26/20 °C day/night temperature regime. At harvest, plants were separated into young leaves, old leaves, and roots. The plant samples were rinsed three times with deionized water and were subsequently dried at 40 °C for 120 h in a drying oven.

Chemical analysis of plant samples Oven-dried samples were homogenized, weighed (~100 mg), and pre-digested using 2 mL of HNO₃
(70%, Ajax-Finechem, Univar) for 24 h. The samples were then digested on a heated block (Thermo Scientific™ Touch Screen Dry Bath/Block Heater) for 1 h at 70 °C followed by 1 h at 125 °C, cooled, and brought to volume (10 mL) with ultrapure water (Milli-Q, Merck). These digests were analyzed by inductively-coupled plasma atomic emission spectroscopy (ICP-AES, Thermo Scientific iCAP7400) for major and minor elements, as described earlier (Corzo Remigio et al. 2021).

Scanning electron microscopy with energy-dispersive X-ray spectroscopy Small leaflet fragments were excised from the plant with a razor blade and immediately shock-frozen by pressing quickly against a solid block of stainless steel with a high thermal mass (2 kg) that was cooled by liquid nitrogen (-196 °C). Subsequently, the whole block (with samples in thermal contact) was moved into the vacuum chamber of a lyophilizer (Thermoline) and immediately pumped down. Vacuum (0.004 millibar) was achieved <5 min which ensured thermal insulation, and ensured that the metal block remained cold and very slowly warmed to the set temperature of the lyophilizer (-85 °C). After 24 h the freeze-drying was progressed in increments of 5 °C, and for another 24 h to room temperature (a total of 48 h). The samples were then sealed in a box with silica gel, mounted on stubs, sputter-coated with carbon and analyzed using scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM–EDS, Hitachi SU3500), as described previously (Corzo Remigio et al. 2021).

Laboratory micro-X-ray fluorescence elemental mapping The μ-XRF analysis was undertaken at the University of Queensland (UQ) Facility on fresh, hydrated leaves as described previously (Corzo Remigio et al. 2021). In summary, leaves were mounted between two layers of thin film immediately after excision from the plant in order to limit dehydration. The μ-XRF analysis used a 100 ms per pixel dwell time and took approximately 24 h for each scan. There were no visible signs of specimen degradation (e.g., wilting or discoloration) at the end of the scans. The XRF data were exported into ImageJ as TIFFs and visualized with the built-in ‘Fire’ LUT (Schneider et al. 2012).

Statistical analyses The assumption of data normality was assessed using the Shapiro–Wilk normality test, and homogeneity of variances tested with Levene’s test. However, biomass data and Tl concentrations in S. latifolia samples failed both assumptions and therefore non-parametric tests were conducted. The significance of differences in dry biomass subjected to different Tl concentrations in the nutrient solutions was assessed by a Kruskal–Wallis test. The significance of differences in Tl concentrations in the different parts of S. latifolia organs (roots, old leaves, and young leaves) resulting from the independent treatments were assessed with the non-parametric Scheirer-Ray-Hare test, equivalent to a parametric two-way ANOVA, followed by Dunn’s pairwise post hoc tests. Spearman’s rank correlation coefficient was used to estimate the association between Tl treatments and Tl concentrations in different plant organs. Statistical analyses were performed using R version 4.0.2 with RStudio 1.3.959 at a significance level of \( p < 0.05 \), or significance as indicated.

Results

Plant growth performance in the thallium dosing regimen Silene latifolia dry biomass yield in the different Tl treatments is shown in Table 1 and Fig. 1. A Kruskal–Wallis test showed that Tl treatment levels did not have a significant effect on biomass (\( H_{(4)} = 8.83, \ p = 0.07 \)). However, the highest biomass was obtained in the 5 μM Tl treatment with 4820 mg (median) per plant, whilst the lowest was in the 60 μM Tl treatment (1950 mg per plant). The standard deviation of the biomass was relatively higher in the highest Tl treatment (Table 1) as some individuals (one in each of the 30 and 60 μM Tl treatments) showed signs of toxicity (necrotic leaves) with lower biomass.

Accumulation of thallium in roots and shoots Thallium concentrations in S. latifolia across the different Tl treatments are given in Table 1 and Fig. 2. A Scheirer-Ray-Hare test showed a significant effect of Tl treatments (nutrient solution) for Tl uptake, \( H_{(4, 72)} = 62.167, \ p < 0.001 \), and also statistically significant accumulation of Tl in different tissues (old leaves, young leaves and roots), \( H_{(4, 72)} = 13.344, \ p = 0.001 \). Thallium concentrations in shoots and roots increased with solution Tl concentrations (Fig. 2). Three significantly different groupings could be detected (\( p < 0.05 \), Dunn’s test) (Fig. 2).
The group with the highest Tl concentration included the 30 and 60 μM Tl treatments, and the group with the lowest the 2.5 and 10 μM Tl treatments; the group with an undetectable Tl concentration was the control (0 μM Tl) treatment (Table 1 and Fig. 2). The highest accumulation of Tl occurred in young leaves in the 30 μM Tl treatment with 35 700 μg Tl g⁻¹ (median ± SE: 12 900 ± 4140), whereas the lowest concentrations were in the roots in the 2.5 μM Tl treatment (median ± SE: 40.8 ± 17.6 μg Tl g⁻¹) (Table 1). A post hoc test showed significant difference in Tl concentrations between leaves and roots (p < 0.05), with leaf concentrations one to two orders of magnitude higher than in the roots (Table 1). Spearman’s rank correlation revealed a strong significant relationship between Tl in the nutrient solution and the Tl concentrations in leaves and roots (rₛ(88) = 0.763, p < 0.001). The translocation factors (TFs) in S. latifolia (based on the quotient of the shoot and root Tl concentrations in Table 1) are all >1 and up to 42, typical of a hyperaccumulator (Lorestani et al. 2012).

### Table 1 Effects of Tl exposure in solution on Tl uptake and growth of leaves (young and old), and roots of Silene latifolia grown in hydroponics

| Treatment | 0 μM Tl | 2.5 μM Tl | 5 μM Tl | 30 μM Tl | 60 μM Tl |
|-----------|---------|-----------|---------|----------|----------|
| [Tl] in young leaves (μg g⁻¹) | < LOD | 696 ± 333 | 705 ± 466 | 12,900 ± 4140 | 16,700 ± 2810 |
| [Tl] in old leaves (μg g⁻¹) | < LOD | 504 ± 140 | 561 ± 322 | 13,800 ± 3370 | 9870 ± 4440 |
| [Tl] in roots (μg g⁻¹) | < LOD | 40.8 ± 17.6 | 131 ± 15.9 | 329 ± 200 | 694 ± 158 |
| Total biomass (mg) | 3760 ± 459 | 4000 ± 529 | 4820 ± 528 | 2850 ± 658 | 1950 ± 792 |

Values are medians ± standard error (n = 6). LOD is the limit of detection (0.176 μg Tl g⁻¹)

The SEM-EDS analysis of magnified images of S. latifolia shows its morphology (Figs. 5 and 6) and the composition of major constituents (Table 2). Brighter areas in the BSE mode show the presence of Tl, and so the point analysis was deliberately focused on these areas. Trichomes of S. latifolia are cylindrical and swollen at the base and have elongated and acuminate tips (Fig. 5a and c). Points 1 and 2 were focused in the trichome of a young leaflet, where K (24.5 and 23.7 wt%) is one of the major constituents after O (60.4 and 63.5 wt%). The same trichome had Tl concentrations of 13.2 and 11.1 wt%, respectively. Points 3 and 4, focused on epidermal cells, with relatively higher concentrations of K (28.3 and 28.9 wt%) and Tl (20.4 and 16.3 wt%), followed by P (1.2 and 1.3 wt%) and Mg (0.6 and 0.8 wt%). Point 5 focused on the base of the trichome of the old leaflet; Tl was below the limit of detection here, whereas K was highly enriched (64.2 wt%). Point 6 was focused on the vein, where major constituents were O (51.6 wt%) and K (40.2 wt%) and Tl was a minor constituent with 6.7 wt%. Points 7 and 8 were focused on epidermal cells, where the major constituent was O (45.4 wt% and 50.4 wt%) and K (43.1 wt% and 39.5 wt%), and Tl was a minor constituent with 9.6 wt% and 8.1 wt%, respectively. It can be seen from the BSE-EDS elemental maps that Tl distribution appears to mirror that of K, although being present at lower concentrations. The trichome itself does not appear to contain appreciable Tl, although a low concentration is visible in the basal area (Fig. 6).
Fig. 1 Biomass graphs and images of *Silene latifolia* subjected to different Tl concentrations in the nutrient media (0, 2.5, 5, 30 and 60 μM Tl). For each treatment, median values (line inside the box), 25–75% inter-quartile range boxes and whiskers are displayed. The terminals of whiskers represent the lowest datum point still within 1.5 times inter-quartile range of the lower quartile, and the highest datum point still within 1.5 times inter-quartile range of the upper quartile. Outliers, data outside the whiskers, are shown as black circles. Data were analyzed by the non-parametric Kruskal–Wallis test (*p* > 0.05, ns = not significant). The dashed line represents the average biomass across all of the treatments.
Furthermore, Tl is not present in the stomata (Fig. 6). Subcellular analysis based on the SEM images was not considered as there is a high likelihood of metal redistribution at this level during the freeze-drying process (Siegele et al. 2007).

**Discussion**

Our hydroponic study confirms Tl hyperaccumulation in *S. latifolia* and reveals for the first time the distribution of Tl in its plant organs. Plant biomass increased in the 0–5 μM Tl treatment levels before decreasing in the 30–60 μM Tl treatments (Fig. 1), suggesting a stimulatory effect as observed for other toxic elements at low concentrations — a phenomenon known as hormesis (Agathokleous et al. 2019; Jalal et al. 2021; Salinitro et al. 2021). This has also been found in the Tl hyperaccumulator *B. laevigata* (Corzo Remigio et al. 2022). However, with exposure to higher Tl concentrations, some individuals developed necrotic older leaves. In nature, *S. latifolia* plants have been found to have highly variable Tl concentrations (up to 1500 μg Tl g⁻¹) that do not relate well with soil Tl concentrations (range 0–32 μg Tl g⁻¹), suggesting possible genetic variability in Tl uptake among individuals (Escarré et al. 2011). In our study, the experimental plants had variable Tl concentrations even within the same treatment, *e.g.*, the highest Tl concentration was 35,700 μg g⁻¹ in young leaves exposed to 30 μM Tl, and the median ± SE was 12,900 ± 4140 μg Tl g⁻¹ (Fig. 2; Table 1). Similarly, *B. laevigata* subsp. *laevigata*, from the Raibl/Cave del Predil (Italy) population was also reported to attain up to 32,700 μg Tl g⁻¹, growing in mine soils with an average Tl concentration of 400 μg Tl g⁻¹ (Fellet et al. 2012).
The bioconcentration factor (BCF, the quotient of metal concentration in shoots:soil) of Tl hyperaccumulators is one of the highest reported compared to other non-essential metal(loid)s (Robinson and Anderson 2018); e.g. B. laevigata has a BCF of 27.3 (Fellet et al. 2012). In addition, Tl hyperaccumulators have high translocation factors. These traits make Tl phytoextraction applicable for a wide range of Tl-contaminated soils (Corzo Remigio et al. 2020). The potential Tl yields and market value of Tl make Tl phytoextraction potentially an economically attractive proposition. We estimate that S. latifolia can attain Tl yields of 5.88 kg Tl ha\(^{-1}\) yr\(^{-1}\) (based on the calculation of Tl concentration in plants \(\times\) whole plant biomass), considering 16 plants m\(^{-2}\) and 0.46 t of biomass with 12,900 μg Tl g\(^{-1}\) from the 30 μM Tl treatment, suggestive of significant economic returns.

Thallium distribution in hydrated leaves of S. latifolia (Figs. 3 and 4) differs from that of B. laevigata (Corzo Remigio et al. 2022). In young leaves, Tl is predominantly localized in the mid-rib and the surrounding vascular bundles, whereas in B. laevigata Tl is localized at the foliar margins. In old leaves of S. latifolia, Tl is highest at the base and in the tip, whereas in B. laevigata it is across the surface and margins. Thallium enrichment in the basal and apical regions of old leaves of S. latifolia (Fig. 3) is similar to Mn distribution in the Mn hyperaccumulator Gossia fragrantissima, where the metal storage pattern is probably associated with transpiration (Abubakari et al. 2021). The SEM–EDS analysis of freeze-dried leaves...
foliar fragments of *S. latifolia* revealed an uneven distribution of Tl across the epidermal cells (Figs. 5 and 6), which has been reported previously for other hyperaccumulators and suggested as a protection mechanism for photosynthetically-active mesophyll cells (Leitenmaier and Küpper 2013). Trichomes in *S. latifolia* were present on both the abaxial and adaxial surfaces (Figs. 3 and 4), and are abundant as expected from observations of leaves from European populations (Blair and Wolfe 2004). Similar to the *B. laevigata* population from Saint-Laurent-le-Minier (Corzo Remigio et al. 2022), Tl was not present in

**Table 2** Energy Dispersive X-Ray Spectroscopy (EDS) point analyses in leaflets fragments of *Silene latifolia* from the 60 μM Tl treatment. The spots indicate the location of the point analysis as shown in Fig. 5

| Specimen            | Spot | Element (wt %) | Tl     | O       | K       | P       | Mg      |
|---------------------|------|----------------|--------|---------|---------|---------|---------|
| *Silene latifolia*  |      |                |        |         |         |         |         |
| Young leaflet       |      |                |        |         |         |         |         |
| (Figs. 5a and 5b)   | 1    | 13.2 ± 0.3     | 60.4 ± 0.4 | 24.5 ± 0.2 | 1.1 ± 0.1 | 0.9 ± 0.1 |
|                     | 2    | 11.1 ± 0.3     | 63.5 ± 0.3 | 23.7 ± 0.2 | 0.9 ± 0.1 | 0.7 ± 0.1 |
|                     | 3    | 20.4 ± 0.3     | 48.8 ± 0.4 | 28.3 ± 0.2 | 1.2 ± 0.1 | 0.6 ± 0.1 |
|                     | 4    | 16 ± 0.3       | 53 ± 0.4   | 28.9 ± 0.2 | 1.3 ± 0.1 | 0.8 ± 0.1 |
| Old leaflet         |      |                |        |         |         |         |         |
| (Figs. 5c and 5d)   | 5    | <LOD           | 32.3 ± 0.4 | 64.2 ± 0.4 | 1.5 ± 0.1 | 1.4 ± 0.1 |
|                     | 6    | 6.7 ± 0.2      | 51.6 ± 0.3 | 40.2 ± 0.3 | 0.9 ± 0.1 | 0.4 ± 0.1 |
|                     | 7    | 9.6 ± 0.3      | 45.4 ± 0.4 | 43.1 ± 0.3 | 1.5 ± 0.1 | 0.4 ± 0.1 |
|                     | 8    | 8.1 ± 0.3      | 50.4 ± 0.3 | 39.5 ± 0.3 | 1.3 ± 0.1 | 0.6 ± 0.1 |

Values are concentrations in weight percent (wt%) ± error of analysis (σ uncertainty). LOD denotes the limit of detection (0.1 wt%).
the trichomes themselves, but in the trichome bases (Fig. 6). Trichomes typically protect plants against physiological constraints, such as water loss/absorption and extreme temperatures, and from herbivory by making leaves unpalatable and/or excreting substances such as toxins (Werker 2000). Metal-enriched trichome bases have also been observed in other hyperaccumulators, e.g., Mn in Odontarrhena calcidica (synonym: Alyssum murale) (Broadhurst et al. 2009; McNear and Küpper 2014). To date, however, there remains no clear explanation of the role of trichomes in metal compartmentalization (Hopewell et al. 2021; McNear and Küpper 2014).

This study has shown that S. latifolia accumulates Tl in response to increasing Tl concentrations in a hydroponic system. In a similar Tl dosing trial with Sinapis alba exposed to 2 mg Tl L⁻¹, the highest Tl in shoots was 87.2 μg g⁻¹ (Holubík et al. 2021), whereas in our experiment at 5 μM Tl (corresponding to 1 mg Tl L⁻¹) S. latifolia attains 705 ± 466 μg Tl g⁻¹ in young leaves, and even more Tl is taken up in the higher Tl treatments, thereby showing that it is demonstrably highly Tl tolerant.

Conclusions

This study assessed S. latifolia tolerance and hyper-accumulation of Tl, an element of interest due to its extreme toxicity and high economic value. Silene
latifolia possesses a strong capacity to accumulate Tl in its shoots, similar to B. laevigata, which implies that it has evolved ecophysiological mechanisms enabling it to survive in Tl-polluted environments. However, it is notable that the foliar Tl distribution in S. latifolia is different from that of B. laevigata. Further detailed analysis using synchrotron X-ray fluorescence microscopy is required to elucidate the (sub)cellular distribution of Tl in these two species, which could then provide more insights into the mechanisms underlying their Tl tolerance and hyperaccumulation. From both the ecophysiological and phytoextraction perspective, more research is required to evaluate the responses of S. latifolia to Tl-enriched substrates at the population level. As S. latifolia has been introduced to regions other than its native habitats in Europe and Western Asia (Mikhaylova et al. 2021), phytoextraction applications deserve full assessment of the (ecological) implications of any introduction into non-native areas. Considering the chemical similarity of Tl and K, and the relatively high Tl bioconcentration factors compared to other metals, it is very likely that there are other Tl (hyper)accumulators yet to be discovered. It would surely be worthwhile to search for more Tl hyperaccumulators in the field and by performing large-scale screening of herbarium collections using novel non-destructive methods based on portable X-ray fluorescence spectroscopy (pXRF) (Purwadi et al. 2021).

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Fig. 6 Scanning electron microscopy—backscattered electron (SEM-BSE) coupled with energy dispersive X-ray spectroscopy (EDS) mapping of an old leaflet fraction of a freeze-dried Silene latifolia specimen, displaying BSE image, oxygen map, potassium map, and thallium map.
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**Author contributions** ACR, PNN and AVDE designed and conducted the experiment. ACR undertook the chemical analysis of the samples. ACR, PNN and AVDE performed data processing and analysis. AJMB, FP and ME provided critical insights to shape the research outcomes. All authors contributed to the writing of the manuscript.

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**Data availability** The data generated during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Competing interests** The authors declare no conflicts of interest relevant to the content of this article.

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